ICMI 11

ABSTRACT SUPPLEMENT

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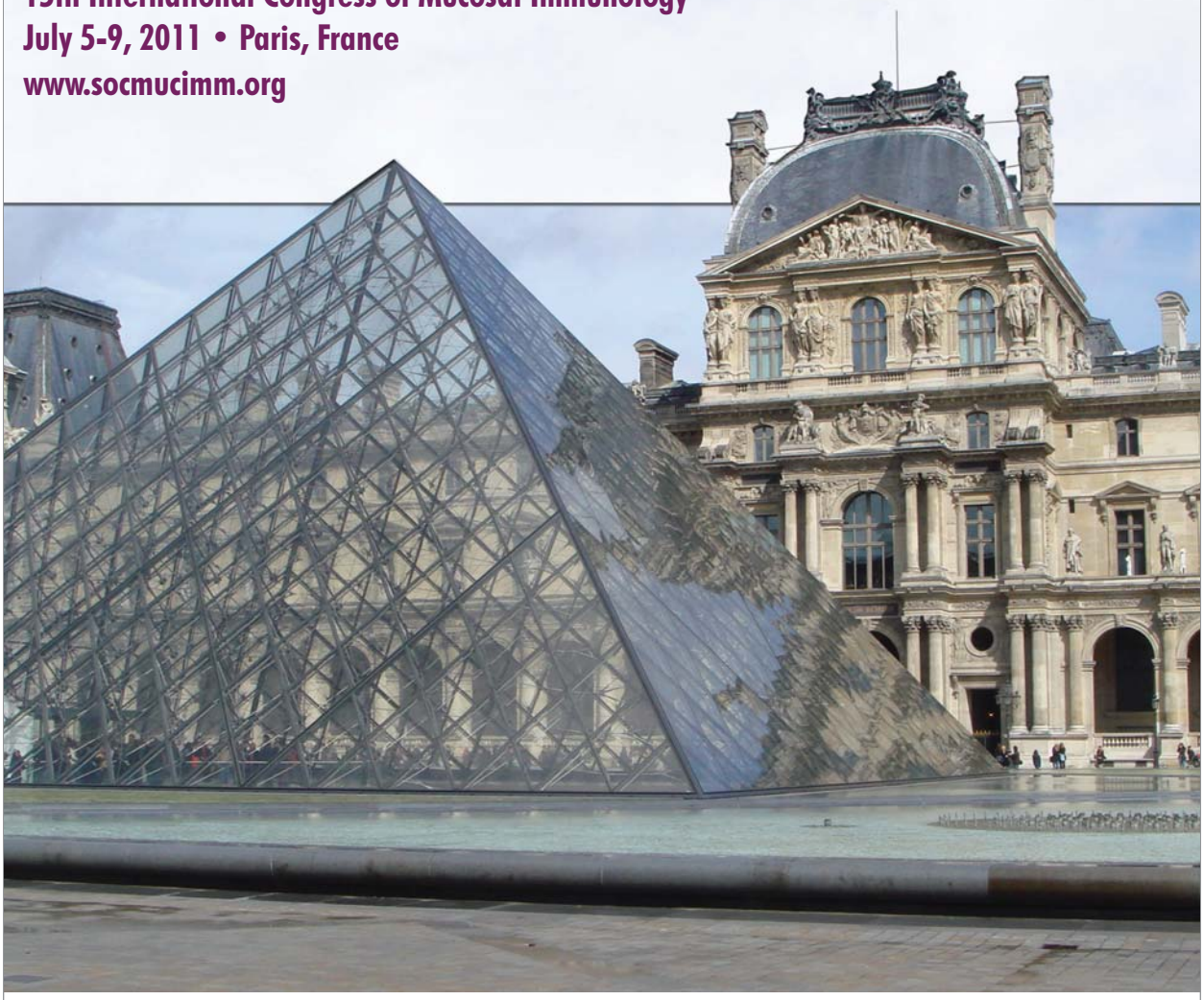

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Surface Barrier Immunology Study Group (SBARIS)

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Oral Presentations: Wednesday, July 6

Innate Immunity I (1400)
Wednesday, July 6, 14:30-16:00

OR.1. Bacterial Outer Membrane Vesicles Induce NOD1-dependent Autophagy in Epithelial Cells

Maria Kaparakis-Liaskos[1], Aaron Irving[1], Michael Gantier[1], Hitomi Mimuro[2], Dana Philpott[3], Chihiro Sasakawa[2], Richard Ferrero[1]. [1]Monash Institute of Medical Research, Clayton, VIC, Australia; [2]University of Tokyo, Tokyo, Japan; [3]University of Toronto, Toronto, ON, Canada

Gram negative bacterial peptidoglycan (PG) is specifically recognized by the host intracellular pathogen recognition molecule NOD1. We recently reported that all Gram negative mucosal pathogens deliver PG to cytosolic NOD1 in host epithelial cells via a novel mechanism involving outer membrane vesicles (OMVs). More importantly, we determined that NOD1 was essential for the development of OMV-specific innate and humoral immune responses *in vivo*. The aim of this study was to examine the intracellular processing of OMVs in order to further understand their role in bacterial pathogenesis and the development of OMV-specific immune responses. We identified that internalized bacterial OMVs induced aggregates of the autophagosome component LC3 in epithelial cells. LC3-GFP puncta did not form in OMV-stimulated macrophages, indicating that this response was cell-specific. Using siRNA NOD1 knockdown or NOD1 knockout cells, we showed that NOD1 was essential for the induction of OMV-induced autophagy. Furthermore, OMV-stimulation of LC3 or ATG5 knockdown cells resulted in reduced inflammatory responses when compared to control cells. Collectively, our findings suggest that NOD1 is essential for the induction of autophagy and inflammation in response to OMVs. Therefore we propose a cell-specific, NOD1 dependent mechanism for the generation of innate immune responses to pathogenic OMVs at mucosal surfaces.

OR.2. NLRP3 is Required for Protective Immunity to Respiratory Infection with Streptococcus Pneumoniae

Edel McNeela[1], Daniel Neill[2], Cathy Baxter[1], Aras Kadioglu[2], Ed Lavelle[1]. [1]Trinity College Dublin, Dublin, Ireland; [2]University of Leicester, Leicester, United Kingdom

The respiratory pathogen streptococcus pneumoniae is the main cause of bacterial pneumonia. The toxin pneumolysin (PLY) expressed by the bacterium is a key virulence factor and potential candidate for inclusion in pneumococcal subunit vaccines. In order to develop these novel vaccines it is important to understand how pneumococci and PLY interact with the host immune system. In mice, IL-17A and IFN-γ play important protective roles in immunity to pneumococcal infection. We found that intranasal infection of mice with PLY-deficient pneumococci induced significantly less IFN-γ and IL-17A in the lungs compared to infection with wild-type bacteria. PLY alone did not induce cytokine secretion by macrophages or dendritic cells (DC), but did synergize with TLR agonists to enhance secretion of the Th1 and Th17 cell polarizing cytokines IL-12, IL-23, IL-6, IL-1β, IL-1α and TNF-α. In addition, live pneumococci promoted IL-1β secretion by DC and this was dependent on PLY. The enhancement of IL-1β secretion by whole live pneumococci and by PLY in DC required NLRP3, identifying PLY as a novel NLRP3 inflammasome activator. Importantly, NLRP3 was required for protective immunity against respiratory infection with S. pneumoniae. This is the first study showing the importance of NLRP3 in protection against a gram-positive bacterium.

OR.3. Multifaceted Role of Serine Antiproteases Trappin-2 and Elafin in Defense Against HIV Mucosal Transmission

Anna Drannik, Xiao-Dan Yao, Bethany Henrick, Kakon Nag, Sumiti Jain, Kenneth Rosenthal. McMaster University, Hamilton, ON, Canada

Better understanding of protective mechanisms against mucosal HIV transmission is critical. Trappin-2 and elafin (Tr-2/E) have been identified as biomarkers of resistance to HIV infection in cervico-vaginal fluids (CVL) from resistant commercial sex workers (R-CSWs) in Kenya; yet their mode(s) of action are unclear. We showed that Tr-2/E reduced inflammatory mediators and expression of RIG-I and Mda5 in genital epithelial cells (ECs). Here, we attempted to characterize: anti-HIV activity of Tr-2/E in transcytosis model with human endometrial ECs; as well as CVLs and EC from R-CSWs and susceptible (S-CSWs). Our results demonstrated that: (1) Significantly higher Tr-2/E in CVLs were confirmed in R-CSWs versus S-CSWs; (2) Higher Tr-2/E in CVLs were associated with reduced mRNA of TLR-2, 4 and RIG-I in EC from R-CSWs compared to S-CSWs; (3) Antibody-depletion of Tr-2/E from CVLs and siRNA silencing of Tr-2/E significantly increased R5 HIV infectivity in TZM-bl assays; (4) Recombinant Tr-2 and E each independently inhibited HIV attachment and transcytosis across ECs *in vitro*; (5) Treatment of virus or cells with rTr-2/E significantly decreased attachment to ECs, suggesting direct and indirect mechanisms of antiviral activity. Collectively, our findings highlight the multifaceted role of Tr-2/E in defense against HIV mucosal transmission.

OR.4. TLR Expression Profile and Function in Gastrointestinal Stem Cells (GISC) and Non-transformed Epithelial Cell Lineages Derived from the Cultured GISCs from Each Segment of the Human GI Tract

David Harrison, Ansamma Joseph, Rajeswari Ananthnarayan, Asit Panja. AlfaGene Bioscience Inc., Somerset

TLRs recognize pathogen associated molecular patterns and activate the innate immune system against invading microbes. While site preference for many infectious organisms in the GI tract is well established, the expression profile and function of TLRs in various regions of the GI tract has not been delineated. We studied the expression of TLRs (1, 2, 4, 6, and 10) and their adaptor protein MyD88 in adult GISCs and epithelial lineages

derived from these GISCs. Differences in TLR expression and function among micro-environmentally different segments of the GI tract were examined by RT-PCR of RNA isolated from GISCs (positive for Oct4, Nanog and LIN28). The pluripotency of these cells was further demonstrated by the expression of markers specific to four epithelial lineage subtypes: chromogranin-A for enteroendocrine; MUC2 and trefoil factor 3 for goblet; sucrase isomaltase, alkaline phosphatase, and dipeptidyl-peptidase-4 for columnar; and lyzozyme, defensin, and MMP7 for Paneth epithelial cells. Our results demonstrate a unique expression profile of functional TLRs on both GISC and epithelial cells derived from these SCs from distinct regions of the GI tract. These findings will aid in understanding the mechanisms of region-specific innate immune response and help better develop new treatments for GI infectious diseases.

OR.5. NLRP6 is a Novel Regulator of Tissue Homeostasis in the Colon

Till Strowig[1], Eran Elinav[1], Andrew Kau[2], Jeffrey Gordon[2], Richard Flavell[1]. [1]Yale University, New Haven, CT; [2]Washington University, St. Louis, MO

In the intestine, the immune system and resident microflora maintain homeostasis to ensure the existence of a diverse, functionally indispensable flora. Germline-encoded receptors have important functions in distinguishing between the steady state and infections. The family of NLR proteins consists of intracellular proteins with many of them directly or indirectly involved in the recognition of cellular stress and pathogen-associated molecular patterns. Mutations in family members such as NOD2, NLRP3 and IPAF are associated with defects in controlling inflammation and tissue repair in the intestine upon pathogen infection and tissue injury. We have found that the previously little-characterized NLR protein, Nucleotide-binding oligomerization domain, Leucine rich-repeat and Pyrin domain-containing protein 6, NLRP6, is highly expressed in the intestine. Compared to wild-type mice, mice deficient in NLRP6 have an increased susceptibility to chemically-induced colitis which correlates with decreased levels of IL-18 in the serum and colon explants in NLRP6-deficient mice during disease pointing to the possibility that NLRP6 forms an inflammasome. Notably, a preliminary 16S rRNA analysis identified distinct changes in the composition of the gut flora that may contribute to the increased disease severity. We propose that NLRP6 acts as novel receptor contributing to tissue homeostasis in the colon.

OR.6. Antioxidant-dependent Subversion of Innate Immune Function by Francisella Tularensis

Chandra Bakshi[1], Meenakshi Malik[2]. [1]New York Medical College, Valhalla, NY; [2]Albany College of Pharmacy and Health Sciences, Albany, NY

Francisella tularensis (Ft) the causative agent of tularemia is amongst the most deadly agents of biological warfare and bioterrorism. One critical characteristic of Ft is its ability to dampen or subvert the host immune responses. Ft suppresses TLR2 mediated induction of pro-inflammatory cytokines TNF-alpha and IL-6. We have reported that antioxidants of Ft not only scavenge reactive oxygen (ROS) generated in response to the infection, but also interfere with redox-sensitive signaling pathways to suppress pro-inflammatory cytokines and macrophage activation. We further investigated how Ft antioxidants interfere with ROS-dependent activation of cytosolic NOD-like Receptor (NLR) signaling components that leads to diminished processing and secretion of IL-1beta and IL-18, and cell death. Markedly lower quantities of IL-1beta and IL-18 were induced in macrophages infected with Ft LVS or highly virulent Ft SchuS4 indicating the suppressive effects of these stains on NLRs and inflammasome activation. Inflammasome reconstitution experiments revealed that Ft suppresses NLRP3 mediated inflammasome and IL-1beta secretion which was associated with lower caspase-1 levels. However, loss of oxidant scavenging ability of Ft resulted in significantly elevated ROS-dependent IL-1beta production in macrophages infected with Ft antioxidant mutants. Additionally, the increased IL-1beta levels were associated with ROS-dependent activation of caspase-1. The loss of antioxidants of Ft also caused rapid cell death in infected macrophages. Collectively, these results demonstrate that antioxidant defenses of Ft maintain a reducing environment in macrophages thereby inhibit activation of ROS-responsive NLR; the NLRP3, to suppress generation of pro-inflammatory innate immune response.

IBD Cytokines (1401)
Wednesday, July 6, 14:30-16:00

OR.7. IL-33 Induces Selective Expansion of an IL-17-producing CD11b$^+$F4/80$^+$ Macrophage Population in Experimental IBD

Luca Pastorelli[1], Rekha Garg[1], Benedetta Mattioli[1], Carlo De Salvo[1], Maurizio Vecchi[2], Theresa Pizarro[1]. [1]Case Western Reserve University School of Medicine, Cleveland, OH; [2]University of Milan, Milan, Italy

Th17 immune responses play a major role in the pathogenesis of IBD. IL-33 stimulation of mesenteric lymph node cells (MLNs) from ileitis-prone SAMP1/YitFc (SAMP) mice potently induces IL-17 and confirms previous reports of this phenomenon in draining lymph node cells from inflamed, arthritic joints. The aim of this study was to investigate the role of a potential IL-33/IL-17 axis in SAMP mice. IL-33 was administered daily (one week) and ilea histologically-assessed for inflammation; qRT-PCR was performed for TLR expression. MLNs were evaluated by FACS or cultured/analyzed for cytokine profiles. IL-33 blockade was achieved by twice-weekly administration (4 weeks) of an ST2-Fc protein. MLNs from IL-33- vs. vehicle-treated mice produced increased IL-17, IL-22, and IL-12, with no significant difference in IL-23. Ileal TLR2, TLR5, and TLR9 was elevated in IL-33 treated mice, which consistently showed an expanded population of activated F4/80$^+$ macrophages. Specificity of this response was confirmed by IL-33 blockade, which markedly decreased ileitis and percentages of CD11b$^+$F4/80$^+$ and IL-17-producing CD11b$^+$ cells, but not CD4$^+$ T cells. These data suggest the presence of an IL-33/IL-17 axis, which may be involved in the pathogenesis of chronic intestinal inflammation by promoting expansion of a mucosal CD11b$^+$F4/80$^+$ IL-17-producing macrophage population in experimental IBD.

OR.8. An Essential Immunomodulatory Role for Type I Interferons in T Cell Mediated Colitis

Abhi Kole[1], Kazuya Kitamura[1], Aymeric Rivollier[1], JianPing He[1], Kevin Maloy[2], Brian Kelsall[1]. [1]National Institutes of Health, Bethesda, MD; [2]University of Oxford, Oxford, United Kingdom

Type I interferons (IFN-1) have both antiviral and immunomodulatory properties. We investigated the role of IFN-1 in intestinal immune homeostasis. IFN-1 was constitutively expressed by MHC IIhi lin- DCs and macrophages (MP) from the colons of WT, but not TRIF-/- mice. Transfer of CD4+CD45RBhi naïve T cells from WT or IFN-alpha receptor (IFNAR1)-deficient mice into WT RAG1-/- mice resulted in similar onset and severity of colitis. In contrast, WT T cells transferred into RAG1-/- x IFNAR1-/- mice developed more severe colitis with earlier onset (2 vs 8 weeks) compared to RAG1-/- hosts. IFNAR1 signaling on hematopoietic cells from RAG-/- hosts was required to delay colitis development. T cells transferred into RAG1-/- x IFNAR1-/- mice underwent faster proliferation in the mesenteric lymph nodes and disseminated to the colon earlier than those transferred into RAG1-/- mice. Furthermore, DCs and MPs from the MLNs and colons of IFNAR1-/- mice turned over more quickly and produced less IL-10, IL-1R antagonist, and IL-27 but equivalent IL-23 compared to cells from WT mice. These data show TLR-driven IFN-1 constitutively produced by intestinal DCs and MPs is essential for their production of anti-inflammatory cytokines, and the control of intestinal inflammation in this experimental model of colitis.

OR.9. Hematopoietic-derived IL-37 Protects Mice from Colitis by Down-regulating Local Production of Pro-inflammatory Cytokines

Eoin McNamee[1], Joanne Masterson[1], Paul Jedlicka[1], Martine McManus[1], Almut Grenz[1], Colm Collins[1], Philip Bufler[2], Charles Dinarello[1], Jesus Rivera-Nieves[1]. [1]University of Colorado, Denver, Aurora, CO; [2]Ludwig-Maximilians University, Munich, Germany

IL-37, a newly described member of the IL-1 cytokine family, functions as a fundamental inhibitor of innate immunity. We generated a transgenic mouse strain that expresses IL-37 (IL-37tg) and subjected the mice to dextran sulfate sodium (DSS)-induced colitis. IL-37 mRNA was not expressed in the resting state, but required disruption of the epithelial barrier, with a 6-7 fold increase ($P=0.02$) on days three and five of continuous DSS exposure. During the development of colitis, clinical scores of IL-37tg mice were reduced by fifty percent ($P<0.001$) and histological indices of colitis were one third of WT mice ($P<0.001$). The reduced inflammation was associated with decreased recruitment of leukocyte subsets into the colonic lamina propria. In addition, *ex vivo* colonic tissue production of IL-1β and TNF were decreased 5 and 13 fold, respectively, compared with WT mice ($P\leq0.005$); however, IL-10 increased 6-fold ($P<0.001$). Of note, the increase in IL-10 was not required for the anti-inflammatory effect of IL-37, as blockade of IL-10R did not reverse IL-37tg-mediated protection. Mechanistically, IL-37 originating only from infiltrating leukocytes was sufficient to exert profound anti-inflammatory effects, as WT mice reconstituted with IL-37tg bone marrow were protected from colitis. IL 37 thus emerges as key suppressor of intestinal inflammation.

OR.10. TSLP Promotes IL-3-independent Basophil Hematopoiesis and Type 2 Inflammation at Mucosal Sites

Mark Siracusa[1], Steven Saenz[1], Brian Kim[1], Michael Comeau[2], David Artis[1]. [1]University of Pennsylvania, Pennsylvania, PA; [2]Amgen, Inc., Seattle, WA

Polymorphisms in the gene encoding the epithelial-derived cytokine thymic stromal lymphopoietin (TSLP) are associated with the development of multiple allergic disorders in humans, suggesting that TSLP may play a role in the induction of allergic inflammation. Supporting genetic analyses, exaggerated epithelial cell-specific TSLP production is associated with asthma and food allergies in patients and studies in murine systems demonstrated that TSLP promotes Th2 cytokine-mediated immunity and inflammation at mucosal sites. However, the mechanisms through which TSLP promotes Th2 cytokine responses and type 2 inflammation remain poorly defined. Here we demonstrate that TSLP promotes systemic basophilia, that disruption of TSLP-TSLPR interactions results in defective basophil responses and susceptibility to the gastrointestinal nematode Trichuris and that TSLPR-sufficient basophils can restore Th2 cell-dependent immunity *in vivo*. Critically, TSLP acted directly on bone marrow-resident progenitors to selectively promote basophil differentiation independently of IL-3-IL-3R signaling, identifying a previously unrecognized pathway of basophil development and activation. Further, genome-wide transcriptional profiling of TSLP-induced basophils identified phenotypic characteristics that were distinct from classical basophils. Collectively, these studies indicate that expression of TSLP may promote type 2 inflammation at mucosal tissues by regulating bone marrow hematopoiesis and eliciting the population expansion of a functionally distinct population of basophils.

OR.11. Mucosal CD90+ Stromal Cell Phenotypes Switch from Immunosupressive (IL-6lowPD-L1+) to Inflammatory (IL-6high PD-L1low) Resulting in Th1/Th17 Responses During Crohn's Disease

Iryna Pinchuk[1], Ellen Beswick[2], Jamal Saada[1], Jennifer House[1], Gerhard Rogler[3], Victor Reyes[1], Don Powell[1]. [1]University of Texas Medical Branch, Galveston, TX; [2]University of New Mexico, Albuquerque, NM; [3]University of Zürich, Zürich, Switzerland

Background: Underlying mechanisms of dysregulation of CD4+ Th1/Th17 responses during Crohn's Disease (CD) are unclear. CD90+ myofibroblasts/fibroblasts (CMFs) are abundant cells in the colonic mucosa. CMFs isolated from normal mucosa (N-CMS) suppress IFN-γ, a Th1 cytokine via PD-L1-dependent mechanism. Decreased PD-L1 expression and enhanced production of IL-6, a cytokine involved in Th17 cell generation, was noted in CMF from CD (CD-CMFs) when compared to N-CMFs. We hypothesized that decreased PD-L1 expression and

upregulation of IL-6 by CD-CMFs contributes to uncontrolled Th1/Th17 responses during CD immunopathogenesis. Methods: CFSE-proliferation, FACS analysis, and cytokine arrays were used to evaluate regulation of Th cell responses by CMFs. Results: In contrast to N-CMFs that decrease the proliferation of the CD2/CD3/CD28-activated Th0 cells, CD-CMFs enhanced proliferation of the Th cells expressing the Th17 transcriptional factor RORγτ via mechanism involving IL-6. IL-6 neutralization decreased RORγτ+ Th cell proliferation. In contrast to the N-CMFs that suppress Tbet and IFN-γ expression in prepolarized Th1 cells via PD-L1, the PD-L1lowCD-CMFs failed to decrease expression of Th1 transcriptional factor Tbet and IFN-γ. Conclusion: These data suggest that a switch in the CMF phenotype from immunosupressive (IL-6lowPD-L1+) toward inflammatory (IL-6high PD-L1low) contribute to the dysregulation of the Th1/Th17 balance during immunopathogenesis of CD.

OR.12. Notch-hes1 Pathway and TNF-α Synergistically Up-regulate OLFM4 Expression in Human Intestinal Epithelial Cells

Ryuichi Okamoto, Kiichiro Tsuchiya, Tetsuya Nakamura, Mamoru Watanabe. Tokyo Medical and Dental University, Tokyo, Japan

Recent studies have shown that Olfm4 is a robust marker for intestinal epithelial stem cells. However, the molecular mechanism regulating its expression in intestinal epithelial cells (IECs) remains largely unknown. In normal human intestinal tissues, Olfm4 expression was restricted to the stem-progenitor region of the crypt, where Notch-Hes1 pathway is activated. However, in inflamed human intestinal tissues, Olfm4-positive IECs significantly increased in number, and expanded their distribution to the upper part of the crypt. In LS174T cells, stimulation by TNF-α significantly up-regulated the mRNA expression of Olfm4. Also, forced expression of activated Notch1 (NICD1) or Hes1 significantly up-regulated mRNA and protein expression of Olfm4. Surprisingly, NICD1 or Hes1 over-expression had synergistic effect with TNF-α upon expression of Olfm-4 mRNA, reaching up to 2500 fold increase in LS174T cells. Promoter assays using 5' flanking region of the human Olfm4 gene revealed that such a synergistic effect is mediated through transcription via the proximal NF-κB binding site. Consistently, NF-κB-dependent transcription was significantly enhanced by over-expression of NICD1 or Hes1 in LS174T cells. These results suggest that Notch-Hes1 pathway may interact with TNF-α signaling via NF-κB dependent transcription, and thereby synergistically regulate expression of their target gene, Olfm4, in the inflamed mucosal environment.

Mucosal Infections I (1402)
Wednesday, July 6, 14:30-16:00

OR.13. DC-LAMP+ Dendritic Cells are Recruited to Gastric Lymphoid Follicles in Helicobacter Pylori-infected Individuals

Malin Hansson, Malin Sundquist, Susanne Hering, Samuel Lundin, Michael Hermansson, Marianne Quiding-Järbrink. University of Gothenburg, Göteborg, Sweden

Infection with Helicobacter pylori is associated with development of ulcer disease and gastrointestinal adenocarcinoma. The infection is usually chronic, and leads gastric inflammation, but also to a substantial regulatory T cell response. The maturation stage of dendritic cells (DC) is crucial for determining the type of T cell response induced to a particular antigen. Therefore, we investigated the localization and maturation of human DC in H. pylori infected gastric mucosa. Using immunohistochemistry and flow cytometry, we could identify two DC subsets with distinct localization in the human gastric antrum mucosa. DC-SIGN+ DC were scattered throughout the lamina propria and were more numerous in H. pylori-infected individuals compared to in uninfected volunteers. In contrast, DC expressing the maturation marker DC-LAMP (CD208) were only present in H. pylori-infected stomach tissue, and were specifically localized within or close to lymphoid follicles. The DC-LAMP+ DC expressed CD11c and high levels of HLA-DR, but little CD80 and CD86, and were in close association with CD4+ T cells. In conclusion, DC-LAMP+ DC with low co-stimulatory capacity accumulate in the lymphoid follicles in human H. pylori infected gastric tissue, and our results suggest that these DC may contribute to the chronic infection by causing tolerance to H. pylori antigens.

OR.14. The Peripheral Blood Regulatory T Cell Response to Helicobacter Pylori Infection as a Marker for Peptic Ulcer Disease

Anzel Greenaway, J. Winter, Khiyam Hussain, Darren Letley, Emily Staples, John Atherton, Karen Robinson. University of Nottingham, Nottingham, United Kingdom

H. pylori (Hp), the leading cause of peptic ulcer disease (PUD) and gastric adenocarcinoma induces a strong regulatory T cell (Treg) response in the gastric mucosa. Hypothesising that systemic Treg levels would also be elevated by Hp infection, we aimed to characterise peripheral blood Tregs in patients, and their association with Hp-induced disease. Peripheral blood mononuclear cells (PBMCs) isolated from blood donated by 49 infected and 58 uninfected patients, were stained for the Treg markers CD4, CD25, FOXP3, CTLA-4 and IL-10, and analysed by flow cytometry. IL10 mRNA was quantified by real-time RT-PCR. As in the gastric mucosa, compared to uninfected patients, the Hp-positive group had markedly increased levels of systemic CD4+CD25hiIL-10+ cells (p=0.007), with 4.3-fold higher levels of IL10 mRNA (p=0.001). Greater proportions of CTLA-4+ Tregs were present, but systemic CD4+CD25hiFOXP3+ cell frequencies were not altered. Among Hp-infected patients, PUD was present in those with significantly fewer CTLA-4+(p=0.02) and IL-10+ (p=0.002) CD4+CD25hi cells. Hp infection is associated with increased levels of systemic IL-10+ and CTLA-4+ Tregs. Peptic ulceration is less likely to occur in the presence of a sufficient Treg response. This could be used to develop non-invasive diagnostic tests to identify the individuals most at risk of Hp-associated diseases.

OR.15. Dectin-1 and TLR-independent Discrimination of Commensal and Pathogenic C. Albicans by Oral Epithelial Cells
David Moyes, Celia Murciano, Stephen Challacombe, Julian Naglik. King's College, London, London, United Kingdom

The polymorphic fungal pathogen Candida albicans can act as a commensal or pathogen providing an invaluable model for studying host mechanisms discriminating between beneficial and disease-causing microbes. These mechanisms are critical in mucosal immune defense and homeostasis. Previously, we have demonstrated a MAPK mechanism involving c-Fos that enables epithelial cells (ECs) to discriminate between pathogenic and commensal C. albicans by responding specifically to hyphae. Here, we show this mechanism is dependent on fungal burden and that c-Fos is critical in inducing inflammatory mediators. The c-Fos response is independent of cell wall mannan and β-glucans although recognition of these moieties does occur. However, a hyphal specific protein induces both MKP1 and c-Fos activity. Blockade of TLR2, 4 and dectin-1 reveals that, unlike myeloid cells, none of these receptors are involved in EC responses to Candida infection. We therefore propose a mechanism enabling ECs to distinguish between commensal and pathogenic organisms through selective activation MKP-1 and c-Fos. Yeast cells and low numbers of hyphae avoid the MKP-1/c-Fos response resulting in no inflammatory mediators, permitting colonisation of mucosal surfaces without host challenge, whilst high numbers of hyphae are recognised by a novel receptor and activate MKP1/c-Fos, driving a pro-inflammatory response that clears infection.

OR.16. **The Role of β1**-integrins for Yop Translocation in Yersinia Enterocolitica
Birgit Keller[1], Eva Deuschle[1], Alexandra Siegfried[1], Rainer Haas[2], Silvano Retta[3], Reinhard Fässler[4], Ingo Autenrieth[1], Erwin Bohn[1]. [1]University Hospital of Tübingen, Tübingen, Germany; [2]Ludwig-Maximilians-University Munich, Munich, Germany; [3]Molecular Biotechnology Centre, Torino, Italy; [4]Max Planck Institute of Biochemistry, Martinsried, Germany

The enteropathogenic bacterium Yersinia enterocolitica injects effector proteins (Yops) directly into host cells with a Type Three Secretion System. Yops affect several cell functions what finally leads to immune evasion.Former studies using cultured cells as well as a mouse infection model revealed that mainly granulocytes and B cells are targeted by Yops. Furthermore it was shown that an interaction of Yersinia via the adhesion factors YadA and Invasin with β1-integrins on the host cell site is a prerequisite for Yop injection. In this study we wanted to know how YadA, Invasin and β1-integrins contribute to Yop injection *in vitro* and *in vivo*. For this purpose a β-lactamase reporter system was used to monitor Yop injection in cell culture and mouse infection experiments. We will present evidence that (i) YadA and Invasin show striking differences how they contribute to Yop injection, (ii) YadA mediated Yop injection occurs depending on cell type in a β1-integrin dependent and also in a β1-integrin independent manner, (iii) an interaction of YadA with β1-integrins requires beside integrins additional factors for effective Yop injection, and (iv) Yop injection in cells during mouse infection is predominantly YadA mediated.

OR.17. The Protection of Neonates Against Cryptosporidium Parvum Infection Induced by One Administration of PolyIC Needs the Help of Gut Flora
Sonia Lamandé[1], Françoise Drouet[1], Roselyne Mancassola[1], Louis Lantier[1], Catherine Werts[2], Fabrice Laurent[1]. [1]National Institute for Agronomical Research, Tours, France; [2]Pasteur Institute, Paris, France

Neonates are highly susceptible to digestive and respiratory infections. This higher susceptibility to intracellular infections is due to their proneness to develop Th2-type immune responses associated with the presence of a limited number of resident immune cells in their mucosae. A TLR-ligand administration is known to allow both cell recruitment and activation. In this study, we therefore investigated for the first time the role of polyIC in the stimulation of immune responses in the intestine of neonates. We observed that a single intraperitoneal administration of polyIC increased the mRNA expression of a large panel of chemokines and of interferons in the intestine of neonates. This transient intestinal inflammation induced by polyIC was sufficient to control the development of the parasite Cryptosporidium parvum in the intestinal epithelium. This protection depended on TLR3, dendritic cell activation and subsequent IL-12 production. In absence of gut flora, the protection against C. parvum was abolished and the IL-12 production is reduced. These data suggested that a cooperation between TLR3 and other bacterial PRR is required for an efficient control of the infection.

OR.18. **Generation of Memory TCRγδ T Cells Following Oral L. Monocytogenes Infection**
Brian Sheridan, Quynh-Mai Pham, Leo Lefrancois. University of Connecticut Health Center, Farmington, CT

Listeria monocytogenes (LM) has been used as a model organism for studying T cell responses. However, the vast majority of LM immunological studies utilize inoculation routes other than oral. These studies utilize a recombinant LM that expresses an internalin A protein with high affinity for mouse E-cadherin thus mimicking the physiologic route of infection in humans. Our findings reveal a remarkable mucosal TCRγδ T cell response to oral LM infection, whose kinetics mimicked an adaptive T cell response. The responding TCRγδ subset was polyfunctional and was comprised of both IL-17 and IFNγ producers and notably, IL-17/IFNγ double producers. They appeared distinct from “innate” TCRγδ IL-17 producers which are present early after infection and in peripheral lymph nodes of naïve mice. Moreover, this distinct subset of mucosal TCRγδ T cells were retained long-term and underwent a recall response upon oral challenge similar to that of a TCRαβ T cell recall response. However, memory TCRγδ T cells failed to expand following iv rechallenge, suggesting tissue-specific mechanisms regulate their ability to mount a secondary response. Very little is known regarding the anatomy of TCRγδ T cell responses and our results identify a bona-fide mucosal memory TCRγδ T cell population following oral

LM infection.

Food Allergy (1403)
Wednesday, July 6, 14:30-16:00

OR.19. Allergic Sensitization to House Dust Mite Allergens is Dependent on PAR-2 Activation

Courtney Davidson[1], Narcy Arizmendi[1], John Gordon[2], Morley Hollenberg[3], Harissios Vliagoftis[1]. [1]University of Alberta, Edmonton, AB, Canada; [2]University of Saskatchewan, Saskatoon, SK, Canada; [3]University of Calgary, Calgary, AB, Canada

A number of common aeroallergens have serine proteinase activity, which is important for allergic sensitization. Serine proteinases among house dust mite (HDM), and other allergens activate Protease-Activated Receptor-2 (PAR-2). We have shown that PAR-2 activation in the airways leads to allergic sensitization to concomitantly inhaled antigens, implicating PAR-2 in the pathogenesis of asthma. We hypothesized that PAR-2 activation in the airways by HDM allergens is important for the development of allergic sensitization. HDM extract was administered to mice intranasally to induce allergic sensitization. Mice were then challenged with HDM extract intranasally for 4 consecutive days. A group of mice received a blocking anti-PAR-2 antibody before each HDM administration during the sensitization phase. Mucosal exposure to HDM extract induced AHR and airway eosinophilic inflammation. Administration of the anti-PAR-2 blocking antibody during the sensitization phase completely inhibited the development of AHR and airway inflammation. The PAR-2 blocking antibody also decreased the amount of IL-4 and OX-40L mRNA in the airways, suggesting decreased allergic airway sensitization. HDM extract induces PAR-2-dependent allergic airway sensitization in a murine model of asthma. These results will allow us to define the mechanisms of allergic sensitization to allergens with serine proteinase activity.

OR.20. Sublingual Administration of Antigen Effectively Induces Systemic Tolerance Mediated by Regulatory T Cells

Jens Brimnes, Carola Rask, Kaare Lund. ALK-Abello, Hoersholm, Denmark

To investigate sublingual tissue as a site for induction of systemic tolerance, naïve BALB/c mice were treated sublingually with Phleum pratense (Phl p) or ovalbumin (OVA) for two weeks followed by an intraperitoneal challenge with alum-adsorbed Phl p or OVA. Our results show that sublingual treatment of naïve mice leads to the induction of systemic tolerance, as measured by the ability to downregulate a subsequent systemic challenge. In sublingually treated mice both T cell proliferation and secretion of IFN-gamma, IL-4 and IL-5 were significantly reduced compared to buffer-treated mice, in a dose and duration dependent manner. We show that sublingual administration is more efficient in generating systemic T cell tolerance compared to peroral and intranasal administration. Transfer of CFSE-labeled OVA specific DO11.10 cells into wild type mice, demonstrated *in vivo* proliferation of antigen-specific T cells in the cervical lymph nodes of sublingually treated mice. Furthermore, sublingual treatment with Phl p was able to induce tolerance towards OVA, indicating induction of bystander tolerance mediated by regulatory T cells. Similarly, spleen cells from sublingually treated mice could transfer tolerance to naïve mice. These findings show that sublingual treatment of naïve mice is an efficient way of inducing systemic tolerance, most likely mediated by regulatory T cells.

OR.21. Functional Basophils Express the Novel Phenotype SIRP-α$^-$FcεRI$^+$ MHC Class II$^-$ in Humans and Mice

Keiko Wakahara, VuQuang Van, Nobuyasu Baba, Manuel Rubio, Guy Delespesse, Marika Sarfati. Université de Montréal, Montréal, QC, Canada

The nature and the role of basophils in the induction of Th2 responses remain controversial in mice while the human studies are scarce and scattered. Are basophils MHC class II$^+$ antigen-presenting cells? We investigated the phenoptype and function of basophils at steady state and under airway inflammatory conditions in mice. First, IgE$^+$DX5$^+$ basophils were identified as SIRP-α$^-$ and MHC class II$^-$ cells in spleen, bone marrow and lung tissues of naïve and OVA-immunized and aerosol challenged mice. Second, lung SIRP-α$^-$IgE$^+$DX5$^+$CD11c$^-$ basophils, in contrast to SIRP-α$^+$IgE$^-$DX5$^-$CD11c^{high} dendritic cells (DCs), did not present OVA peptide to naïve DO11.10 Tg T cells. We next reevaluated the expression of MHC class II in relation to SIRP-α expression on human basophils in adult and umbilical cord blood. We discriminated SIRP-α$^-$FcεRI$^+$ cells as MHC class II$^-$ CD1c$^-$IL-3R$^+$ basophils from SIRP-α$^+$FcεRI$^+$ cells, identified as MHC class II^{high} CD1c$^+$ DCs. SIRP-α^{low} MHC class II^{high}IL-3R$^+$ cells included plasmacytoid DCs. Human basophils did not upregulate MHC classII in response to activation. In conclusion, basophils are SIRP-α$^-$FcεRI$^+$ MHC class II$^-$ cells, which do not display antigen-presenting function. Accurate identification and isolation of basophils provides the basis for future studies on the function of these cells in human disease. This work was supported by the Canadian Institute for Health and Research (CIHR Grant, MOP-53152).

OR.22. TLR9-dependence of Innate IgE Production and Allergic Sensitization

Cecilia Berin, Wei Wang. Mount Sinai School of Medicine, New York, NY

Although TLR9 ligands have been used to successfully treat human and experimental allergic disease, we found that TLR9 knockout mice paradoxically are resistant to allergic sensitization to peanut. We observed that the main defects of TLR9-/- mice were (1) an inability to mount an IgE response despite normal Th2 cytokine production and IgG responses, and (2) a defect in Peyer's patch (PP) development. We therefore examined the link between TLR9-induced PP defects and IgE production by sensitizing mice via non-intestinal routes. TLR9+/+ and -/- co-housed mice were repeatedly exposed to peanut by oral, intraperitoneal, or cutaneous routes. IgE production and peanut-induced anaphylaxis upon re-

challenge was abrogated in TLR9-/- mice independent of exposure route. We next examined the site and phenotype of IgE-producing cells in wild-type mice orally sensitized to peanut. Cells were fixed and permeabilized to assess intracellular IgE and analyzed by flow cytometry. A distinct population of CD19+ B220+ IgE+ cells were identified in the peritoneal cavity (0.6% of B cells), bone marrow (1.0% of B cells), and spleen (0.14% of B cells), but were surprisingly absent from the mesenteric lymph node or PP. The site-specificity of IgE-expressing cells argues against passive acquisition of IgE via CD23. Peritoneal IgE+ B cells did not express the B-1 markers CD5 or CD11b. Syndecan-1+ plasma cells were negative for IgE at all sites, and the majority of IgE+ B cells retained expression of surface IgM. Defects in IgE+ B cells (decreased numbers and/or level of IgE expression) were observed at both baseline and after sensitization in TLR9-/- mice, suggesting microbial regulation of both innate IgE production and allergic sensitization at sites distal from the gastrointestinal tract.

OR.23. Breastfeeding-induced Immune Tolerance in Neonates: Mechanisms and Possible Clinical Implications

Patricia Macchiaverni, Akila Rekima, Eric Mosconi, Barbara Seitz-Polski, Valerie Julia, Nicolas Glaichenhaus, Valerie Verhasselt. INSERM U924, Valbonne, France

Diseases due to defect in tolerance induction such as allergy, celiac disease or Type 1 Diabetes develop mostly in childhood indicating the necessity of early intervention for primary prevention. Epidemiological studies report that breastfeeding could protect from these diseases. However, data are controversial and the mechanisms unclear. We hypothesized that breastfeeding induced protection might rely on immune tolerance induction and that protection might therefore be induced only in circumstances where both the antigen and tolerogenic factors are found in breast milk. In a mouse model, we demonstrated that optimal tolerance is induced when antigens found in breast milk are associated to maternal specific IgG. In that case, the neonatal FcR expressed on intestinal epithelial cells allows a protected transfer of the antigens bound to maternal IgG across the pup's gut barrier. Moreover, milk borne antigen-IgG immune complexes were found to be potent inducers of FOXP3 Tregs both *in vitro* and *in vivo* . The expression of the inhibitory FcgRIIb in the progeny was not necessary for tolerance induction. Ongoing studies are assessing what are the biochemical characteristics necessary for the tolerogenic properties of milk borne immune complexes and what are the cells responsible for oral tolerance induction in neonates.

OR.24. Lineage-specific **IKKβ Deficiency Reveals a Role for Intestinal Epithelial Cells in Lung Immune Response to Ingested Antigens**

Astrid Bonnegarde-Bernard, Junbae Jee, Haley Steiner, Michael Fial, Ian Davis, Prosper Boyaka. Ohio State University, Columbus, OH

The sites where immune responses are initiated are believed to play a key role at shaping subsequent responses at distant mucosal sites. However, little is known about the relative contribution of intestinal epithelial cells (IEC) to lung reactivity to foreign antigens and the development of allergic and non-allergic asthma. To address the potential regulatory effect of IEC for lung reactivity, control C57BL/6 mice and mice deficient in IKKβ in IEC (IKKβΔIEC) or in macrophage (IKKβΔMac) were orally sensitized by administration of ovalbumin and cholera toxin as adjuvant. All groups developed similar levels of antigen-specific IgE responses, but IgG2a responses were enhanced in IKKβΔIEC and, even more in IKKβΔMac. Subsequent nasal challenges of C57BL/6 mice with ovalbumin induced lung inflammation and increased lung resistance. Interestingly, both lung inflammation and lung resistance were lower in IKKβΔIEC mice and further reduced in IKKβΔMac mice. Taken together, these results show that localized alteration of NF-κB signaling in IECs have broader effects on immune responses to ingested antigen, which were similar, although of lower magnitude, than those seen when NF-κB signaling is altered on macrophages. They also reveal that IECs can shape immune responses at distant mucosal sites of the airways.

Epithelial Cell and Intestinal Barrier I (1500)
Wednesday, July 6, 16:15-17:45

OR.25. AIEC Interaction with CEACAMs Induces Claudin-2 Expression and Barrier Defect in CEABAC10 Mice and Crohn's Disease Patients

Jérémy Denizot[1], Adeline Sivignon[1], Frédérick Barreau[2], Claude Darcha[3], Carlos Chan[4], Clifford Stanners[4], Paul Hofman[5], Arlette Darfeuille-Michaud[1], Nicolas Barnich[1]. [1]Université d'Auvergne, Clermont-Ferrand, France; [2]Université Paris VII, Paris, France; [3]CHU, Clermont-Ferrand, France; [4]McGill University, Montreal, QC, Canada; [5]Université de Nice Sophia Antipolis, Nice, France

Background: Abnormal expression of CEACAM6 observed at the apical surface of the ileal epithelium in Crohn's disease (CD) patients allows Adherent-Invasive Escherichia coli (AIEC) to colonize gut mucosa, leading to inflammation. Moreover, intestinal permeability is significantly increased in CD patients. Aim: Our aim was to investigate whether AIEC infection alters intestinal permeability by modulating tight junction protein expression. Results: Immunohistochemistry on human tissue micro-arrays showed that, together with CEACAM6, pore-forming tight junction protein claudin-2 is strongly expressed in the ileum from CD patients in both quiescent and active phase. AIEC bacteria via type 1 pili significantly increased intestinal permeability and disrupted mucosal integrity *in vivo* in CEABAC10 transgenic mice expressing human CEACAMs. This correlated with abnormal expression of claudin-2 at the plasma membrane of intestinal epithelial cells observed in AIEC-infected CEABAC10 mice. AIEC bacteria were able to translocate *ex vivo* through CEABAC10 intestinal mucosa in a type 1 pili dependent manner. Conclusion: Type 1 pili-mediated AIEC interaction with CEACAM6 abnormally expressed in quiescent phase of CD could disrupt mucosal integrity by inducing claudin-2 expression before the onset of inflammation. Thus, targeting claudin-2 could represent a new strategy for clinicians to preserve intestinal barrier function in CD patients.

OR.26. NOD2 Signaling Enhances TLR2-mediated Responses of Intestinal Epithelial Cells to Bacterial Lipopeptides

Ida Hiemstra, Gerd Bouma, Georg Kraal, Joke den Haan. VU University Medical Center, Amsterdam, Netherlands.

Intestinal epithelial cells (IECs) form a physical barrier between the internal milieu and the intestinal microflora. This barrier function is mainly formed by tight junctions between the IECs. The presence of bacteria in the intestines has been demonstrated to be important for IEC-homeostasis and toll-like receptor 2 (TLR2) mediated recognition of bacterial lipopeptides has been shown to preserve tight junctions and promote cell survival in IECs. We observed, using the IEC cell line CMT93/69, that muramyl dipeptide (MDP) stimulation strongly enhances TLR2 signaling. MDP is a breakdown product of peptidoglycan and released upon bacterial replication in the intestines. MDP pretreatment improved barrier function and enhanced chemokine production induced by TLR2-ligand Pam3CSK4. This enhancement of TLR signaling via MDP pretreatment was seen for both TLR2 and TLR4. Experiments using lentiviral vectors with shRNA constructs for NOD2 and Nalp3, indicated that the MDP receptor involved in these priming effects is NOD2 and not Nalp3. Altogether, these results suggest that MDP primes IECs for upcoming bacterial interaction. Since loss-of-function mutation of NOD2 is associated with increased risk of developing Crohn's disease, lack of this MDP-mediated enhancement of IEC function could be involved in the IEC barrier function defects observed in Crohn's disease patients.

OR.27. Horizontal Cell-cell Communication to Potentiate Epithelial Antimicrobial Host Defense

Tamas Dolowschiak, Cécilia Chassin, Mathias Hornef. Hannover Medical School, Hannover, Germany

Recognition of pathogenic microbes by intestinal epithelial cells results in transcriptional activation and secretion of soluble mediators that attract professional immune cells to the site of infection. This defence mechanism works very efficiently despite the often low number of pathogens and the limited amount of mediators secreted per epithelial cell. We have recently identified epithelial cell-cell communication as critical component of an efficient epithelial innate host defence (PLoS Pathog 2010 Nov 18;6(11):e1001194). Chemokine production after L. monocytogenes infection was primarily observed in neighboring, non-infected cells despite the invasion-dependent nature of Listeria-induced epithelial activation. Horizontal communication was independent of gap junction formation, cytokine secretion, ion fluxes, or nitric oxide synthesis, but required NADPH oxidase (Nox) 4-dependent oxygen radical formation. Here, we present additional results to investigate the impact of epithelial cell-to-cell communication in an *in vivo* setting. Using reporter gene technology, intracellular chemokine staining and flow cytometric analysis, epithelial communication in response to innate immune activation at the very early stage of microbial infection was examined. In conclusion, our results provide a novel concept of a coordinated epithelial host response upon microbial challenge to maintain mucosal homeostasis and provide an efficient protection from infection with enteropathogenic microorganisms.

OR.28. Resistin-like Molecule-β Contributes to Host Defense During Citrobacter Rodentium Infection

Kirk Bergstrom[1], Caixia Ma[1], Meera Nair[2], Ho-Pan Sham[1], Jennifer Lau[1], Colby Zaph[3], David Artis[2], Bruce Vallance[1]. [1]Child and Family Research Institute, Vancouver, BC, Canada; [2]University of Pennsylvania, Philadelphia, PA; [3]University of British Columbia, Vancouver, BC, Canada

Resistin-Like Molecule-β (RELMβ) is a secreted peptide produced by goblet cells in the intestinal tract following colonization of the intestine by commensal bacteria. However, its function in this regard is not clear. We hypothesized that RELMβ promotes host defense against enteric bacterial pathogens. To test this, we used the Citrobacter rodentium model of infectious colitis. Our results show C. rodentium infection dramatically increased RELMβ protein in rectal goblet cells and stool within the first week. Infection of RELMβ-/- mice with C. rodentium led to greater morbidity between 6 and 10 days post-infection (DPI), compared with C57BL/6 (WT) mice, as determined by loss of body mass. Histological analysis of RELMβ-/- tissues revealed more severe inflammatory damage in the cecum and higher frequency of ulceration in the colon compared to WT mice. This was associated with markedly elevated bacterial burdens (10-100 fold) in the cecal tissues of RELMβ-/- mice. Immunolocalization revealed bacteria deep in the colonic crypts of RELMβ-/- but not WT mice. Interestingly, these pathologies were adjacent to regions of overt goblet cell hyperplasia, suggesting a role for RELMβ in modulating epithelial function to promote host defense. These results point to a novel protective role for RELMβ during enteric bacterial infection.

OR.29. The Toll-interacting Protein Regulates Susceptibility to Acute and Chronic Colitis

Michel Maillard[1], Eric Bernasconi[1], Holm Uhlig[2], Nicolas Barnich[3], Mathias Chamaillard[4], Pierre Michetti[1], Dominique Velin[1]. [1]CHUV, Lausanne, Switzerland; [2]University of Oxford, Oxford, United Kingdom; [3]INSERM, Clermont-Ferrand, France; [4]INSERM, Lille, France

Toll-like receptor (TLR) signals are key to maintaining host-microbial interactions. The Toll-interacting-Protein (Tollip) is an ubiquitously-expressed inhibitor of Iinterleukin-1 receptor, TLR-2 and TLR-4. We hypothesized that Tollip might control gut immune homeostasis. Histological analysis of colons and small intestines from unchallenged Tollip-deficient mice did not show any inflammatory changes. However, induction of acute colitis with oral dextran-sulfate sodium (DSS) 1.5% showed that Tollip-deficient mice had more severe weight loss and rectal bleeding than WT mice. This was corroborated by increased histological scores of colitis, and upregulation of IL-1beta and IL-6 in diseased colons. Using bone-marrow chimeras, we observed that Tollip deficiency in non-hematopoietic cells was sufficient to increase mice susceptibility to acute colitis. In the epithelium, Tollip deficiency was associated with early tight junction dissolution and increased apoptosis upon DSS exposure. Chronic colitis in IL-10 deficient mice was also aggravated by Tollip deficiency and resulted in elevated IL-17 and IFN-gamma expression. Finally, lack of Tollip had a dramatic impact on

intestinal microbial content leading to overrepresentations of the segmented filamentous bacteria in the small intestine. Overall, our data suggest that control of excessive microbial-derived signals is essential to maintain epithelial integrity, control gut flora and prevent excessive inflammatory responses.

OR.30. The Notch-1 Signaling Pathway is Required for Functional Colonic Barrier in WT Mice

Guilia Roda, Aventika Chitre, Cecilia Berin, Lloyd Mayer, Stephanie Dahan. Mount Sinai Medical School, New York, NY

Introduction: The presence of LPLs promotes mucosal barrier function in the RAG1-/- transfer model of colitis putatively due to the Notch-1 signaling pathway. Aim: To determine whether the Notch pathway is mandatory for barrier function *in vivo*. Methods: WT mice received intra-rectal injections of a Notch-1 or scrambled siRNA sequence. Colonic tissues were either mounted into Ussing chambers to assess permeability or subjected to Real-Time PCR for Notch-1, Hes-1, CDX-2, Villin, Occludin, Claudin-5, β-catenin, and E-cadherin. Results: The Notch-1 mRNA expression was decreased in the Notch-1 siRNA treated WT mice (p=0.001). Hes-1, CDX-2, β-catenin and E-cadherin mRNA expression were decreased in the Notch-1 siRNA treated WT mice (p=0.01, p=0.009, p<0.0001 and p=0.0004). These findings correlated with a decrease in villin, occludin, and claudin-5 mRNA expression (p=0.0002, p=0.0002 and p=0.002). While no differences in resistance were seen between the groups, there was a significant decrease in flux in mice that received the Notch-1 siRNA (10925 ± 3389 ng of Dextran-FITC/ml/min/cm2 vs. 2912 ± 1054 ng, p=0.01). Conclusion: Local knock down of Notch-1 impaired expression of the genes related to Notch-1 and to barrier function affecting epithelial barrier integrity.

IBD and Mouse Models I: T Cell Subsets (1501)
Wednesday, July 6, 16:15-17:45

OR.31. $CD4^+CD25^+$ Regulatory T Cells Suppress the Developmental Pathway from Th17 to Alternative Th1 Cells via Th17/Th1 Cells

Takanori Kanai, Tomohisa Sujino, Yuichi Ono, Atsushi Hayashi, Tomomitsu Doi, Shinta Mizuno, Katsuyoshi Matsuoka, Tadakazu Hisamatsu, Haruhiko Ogata, Toshifumi Hibi. University of Tokyo, Tokyo, Japan

Th17 cells are increased in IBD patient and both Th17 and Th1 cells are positively pathogenic. Recently there are two types of Th17 cells, non-pathogenic Th17 cells under TGF-b, or pathogenic Th17 cells under IL-23 condition *in vitro*. We aim to clarify not only the developmental pathway of Th17, Th17/Th1, and Th1 cells, but also the position of $CD4^+CD25^+Foxp3^+$ regulatory T (T_R) cells in their developmental pathway using *in vivo* adoptive transfer experiments. We use naïve $CD4^+CD45RB^{high}$ T cells and/or $CD4^+CD25^+$ T_R cells obtained from $ROR\gamma t^{gfp/+}$ reporter mice or Ly5.1- and Ly5.2-derived mice. We found there are three types of colitogenic $CD4^+$ Th1 cells (IL-$17A^-$IFN-γ^+): 1) $ROR\gamma t^-T\text{-}bet^+$ classical Th1 cells directly differentiated from naïve T cells, 2) $ROR\gamma t^+T\text{-}bet^+$ Th1-like cells, and 3) $ROR\gamma t^-T\text{-}bet^+$ alternative Th1 cells terminally differentiated via the pathway of $ROR\gamma t^+T\text{-}bet^-$ Th17 (IL-$17A^+$IFN-γ^-)→$ROR\gamma t^+T\text{-}bet^+$ Th17/Th1 (IL-$17A^+$IFN-γ^+)→$ROR\gamma t^+T\text{-}bet^+$ Th1-like (IL-$17A^-$IFN-γ^+) cells. $CD4^+CD25^+$ T_R cells suppressed the development of alternative Th1 cells at the step Th17/Th1 alternative Th1 cells, resulting in the accumulation of Th17 and Th17/Th1 cells in mice in which the development of colitis is suppressed. The present study demonstrates that Th17 and Th17/Th1 cells become colitogenic $ROR\gamma t^-T\text{-}bet^+$ alternative Th1 cells via $ROR\gamma t^+T\text{-}bet^+$ Th1-like cells.

OR.32. *In vivo* Expanded and Activated Treg do not Require TCR Stimulation to Prevent Colitis

Katharina Forster[1], Trupti Panchal[1], Sheela Manek[1], Catherine Streutker[1], Kenneth Croitoru[2]. [1]University of Toronto, Toronto, ON, Canada; [2]Mount Sinai Hospital, Toronto, ON, Canada

Regulatory T cells (Treg) are important in controlling inflammatory immune responses. Here we investigated whether T cell receptor (TCR) stimulation is required for Treg function in the T cell transfer model of colitis. OVA-specific DO11.10/$Rag2^{-/-}$ $CD4^+CD45RB^{low}$ Treg were unable to home, expand or prevent colitis in absence specific antigen. Feeding OVA SCID mice restored the ability of DO11.10/$Rag2^{-/-}$ $CD4^+CD45RB^{low}$ cells to protect mice from colitis driven by wildtype naïve T cells. Spleen cells from OVA fed SCID recipients of DO11.10/$Rag2^{-/-}$ $CD4^+CD45RB^{low}$ cells had significantly more transgenic T cells within the $CD4^+$ T cell population than unfed controls. Notably, DO11.10/$Rag2^{-/-}$ $CD4^+CD45RB^{low}$ Treg suppressed colitis weeks after OVA feeding ceased. To differentiate the requirement of antigen for homing and expansion from the requirement of antigen for continued suppression of colitis, we transferred DO11.10/$Rag2^{-/-}$ $CD45RB^{low}$ Treg into SCID recipients and fed OVA, before reconstituting the recipients with colitogenic BALB/c T cells. OVA fed recipients of DO11.10/$Rag2^{-/-}$ $CD45RB^{low}$ Treg were once more protected, while disease developed in untreated controls. TCR signaling is necessary for initial activation, homing and expansion of Treg but dispensable for continued *in vivo* function. In IBD, the antigen trigger driving development of colitis remains unknown. Efforts towards expanding or activating regulatory T cells are dependent on antigen non-specific approaches. Funded by the CCFC.

OR.33. T Cell Specific Overexpression of Smurf2 Contributes to Minor Intestinal Inflammation Preventing Colon Cancer Development by Downregulation of IL6 in Mice

Heike Dornhoff, Christoph Becker, Jürgen Siebler, Markus Neurath. Institute of Molecular Inflammation and Cancer Research, Erlangen, Germany

Alterations of the TGFb signaling pathway are involved in colorectal cancer. Overexpression of a new identified ubiquitin ligase Smurf2, which is a

regulator of the TGFb signaling pathway leads to an upregualtion of the TGFb receptor II and the Smad signaling in T-Lymphocytes, previously described targets for degradation. The mechanism behind that elevated expression of the TGFb receptor II and phosphorylation of Smad2 and -3 is not a proteasomal but rather an endolysosomal degradation of the WTSmurf2 form by its splicevariant, which is additionally Smad7 dependent. Generated Smurf2 transgenic mice showed a higher sensitivity towards TGFb in T cells and were therefore unable to proliferate in the presence of TGFb compared to the controls. Furthermore, they additionally produced lower amounts of proinflammatory cytokines *in vivo* like IL6 that has been shown to promote colon carcinogenesis, resulting in a lower colon tumor number and -size in transgenic mice in an AOM-DSS colitis model. Immunohistochemical stainings for CD4, granulocytes and CD11c revealed a significant reduction of inflammation in the gut of transgenic mice. And also stainings for IL6, IL6Ra and pStat3 are markedly downregulated in transgenics denoting the critical TGFbeta/Smurf2 dependent IL6 signal transduction in T cells and the relevance for Smurf2 in tumor development.

OR.34. **RORγt**-dependent Th17 Cells are Pathogenetic for Colitis-associated Tumor Growth

Maria Martin, Rebecca Kesselring, Stefan Fichtner-Feigl. University of Regensburg, Regensburg, Germany

IL17A and RORγt play a fundamental role in the pathogenesis of IBD. In this study we tried to elucidate the role of Th17 cells in colitis-associated tumorigenesis. We initially investigated human tissue of colorectal cancer from patients with ulcerative colitis and found that those tumors are infiltrated by $CD4^+IL17A^+$ T cells. Further studies conducted in the axozymethane/dextran-sodium-sulfate model in mice revealed the importance of T cells for colon tumorigenesis, as $Rag1^{-/-}$ mice developed neoplastic changes (aberrant crypt foci), but no macroscopic tumor nodules. In addition, we could demonstrate that the immunologic milieu in the colon of WT mice was characterized by Th17 cells mediating chronic colitis and that those cells infiltrated β-$catenin^+$ colon tumors. In contrast, in $ROR\gamma t^{-/-}$ mice, Th17 cells were absent and therefore $CD4^+IFN\text{-}\gamma^+$ Th1 cells induced intestinal inflammation. However, this Th1-mediated colitis in $ROR\gamma t^{-/-}$ mice did not support exophytic tumor growth from present aberrant crypt foci. WT and $ROR\gamma t^{-/-}$ mice revealed transformed β-$catenin^+$ intestinal epithelial cells, but the proliferation rate was reduced in the absence of Th17 cells. These results indicate the importance of the immunological milieu for colitis-associated cancer development. The RORγt-dependent induction of a Th17 proinflammatory response is the basis for tumorigenesis in the intestine.

OR.35. IL-21 Producing T Helper Cells that Co-express CCR9 Target Accessory Organs of the Digestive System for Autoimmunity

Helen McGuire[1], Cindy Ma[1], William Hughes[1], David Fulcher[2], Marika Falcone[3], Cecile King[1]. [1]Garvan Institute of Medical Research, Darlinghurst, NSW, Australia; [2]Westmead Hospital, Westmead, NSW, Australia; [3]San Raffaele Scientific Institute, Milan, Italy

This study describes a CD4+ T helper (Th) subset marked by co-expression of the cytokine interleukin (IL)-21 and the gut-homing chemokine receptor CCR9 (Tccr9 cells). Although Tccr9 cells were observed in healthy mice and humans, they were enriched in the inflamed pancreas and salivary glands of NOD mice and in the circulation of most Sjögren's syndrome patients. Tccr9 cells expressed large amounts of IL-21, ICOS, the transcription factors Bcl6 and cmaf, and supported antibody production from B cells, thereby resembling T follicular helper (TFH) cells. However, Tccr9 cells in the pancreas displayed limited expression of CXCR5 and SAP and provided “help” to CD8+ T cells for the development of diabetes. By contrast, CCR9 expressing CXCR5+ TFH cells were observed in the peyers patches and mesenteric lymph nodes and adoptive transfer experiments indicated that these cells could be the precursors of pancreatic Tccr9 cells. The presence of ectopic GC observed in the pancreas and salivary glands of NOD mice may provide a suitable environment for the maintenance and/or generation of a TFH-like transcriptome in CCR9+ Th cells and contribute to the development of autoimmunity.

OR.36. Expression of the Transcriptional Repressor B-lymphocyte-induced Maturation Protein -1 (Blimp-1) is Differentially Regulated During T Helper Differentiation and Represses the Production of the Inflammatory Cytokine IL-17

Luciana Benevides[1], Soofia Salehi[1], Naomi Keech[1], David Arribas-Layton[1], Jessica Willen[1], Deepti Dhall[2], Eric Meffre[3], Stephan Targan[1], Gislaine Martins[1]. [1]University of California-Los Angeles, Los Angeles, CA; [2]CSMC, Los Angeles, CA; [3]Yale University, New Haven, CT

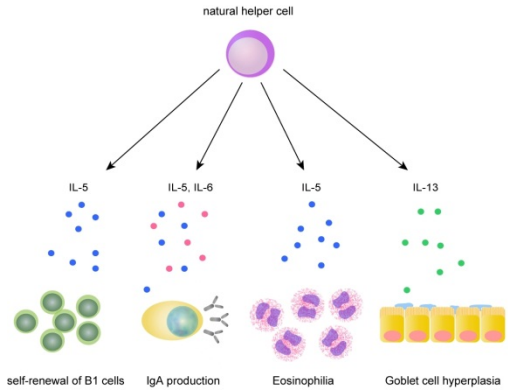
We have previously shown that T cell-specific deletion of Blimp-1 (Blimp-1CKOmice) results in spontaneous development of colitis associated with accumulation of CD4+T cells in the colonic mucosa, suggesting that Blimp-1 is required to control T cell responsiveness in the intestinal mucosa. The mechanisms underlying Blimp-1 functions in T cells are not fully understood. Here we show that TCRbeta+CD4+T cells from Blimp-1CKOmice produce more IL-17 (A and F) than control mice *in vivo*. Moreover, sorted naïve (CD44LowCD4+T cells) from Blimp-1CKOmice produce significantly more IL-17 (protein and mRNA) than naïve cells from control mice upon *in vitro* stimulation, indicating an intrinsic role for Blimp-1 in controlling IL-17 production. Using cells from Blimp-1 reporter mice, we also find that Blimp-1 expression is upregulated in Th1 and Th2cells, but suppressed during Th17 differentiation *in vitro*. Blimp-1CKO CD4+T cells produced more IL-17 (and caused more severe disease) than controlCD4+T cells in a model of colitis induced by adoptive transfer of naïveCD4+T cells into RAG1KOmice. Genomic sequence inspection indicates that Blimp-1 could directly repress IL-17A and IL17F genes. Experiments to confirm this hypothesis are underway. Together the results described above indicate that regulation of IL-17 production is one of the mechanisms by which Blimp-1 controls T cell function and promote intestinal mucosa homeostasis.

Lungs and Upper Respiratory Tract (1502)
Wednesday, July 6, 16:15-17:45

OR.37. Th2-type Innate Immune Responses and the Natural Helper Cell

Kazuyo Moro, Shigeo Koyasu. Keio University School of Medicine, Tokyo, Japan

Natural helper (NH) cells are newly identified lymphocyte which respond to a combination of IL-2 and IL-25 or IL-33 and produce large amounts of Th2 cytokines. We identified NH cells in tiny lymphoid cluster of adipose tissues which we termed fat-associated lymphoid cluster (FALC). IL-5 and IL-13, produced by NH cells induce eosinophilia and goblet cell hyperplasia, and play a role in helminth infection and allergic diseases such as asthma. NH cells can also produce Th2 cytokines constitutively without any stimulation, and support the self-renewaling of B1 cells and IgA production by B cells. After we reported NH cells, other groups also reported novel Th2 cytokine producer cells, such as MPPtype2 cells, nuocytes, and innate helper type 2 (Ih2) cells. There are similarities and differences between these newly identified cell populations and NH cells. MPPtype2 cells can differentiate to other myeloid cells, making this cell type distinct from the others. MPPtype2 cells, nuocytes, and Ih2 cells were reported to respond to IL-25 alone, but NH cells do not respond to IL-25 without IL-2 but respond strongly to IL-33. Localization of NH cells in FALC is also one of intriguing differences from other cells which are found in lymph node and/or spleen.



OR.38. The Mucosal Environment Modifies the Development of Specific CD8 Tmem Pools and their Dependence on IL-15 for Survival

Katherine Verbist, Mary Field, Kim Klonowski. University of Georgia, Athens, GA

Memory CD8 T cells (Tmem) are critical for rapid and efficient control of secondary encounters with intracellular pathogens. Although the majority of intracellular pathogens enter via mucosal surfaces, much of our understanding of Tmem biology has been amassed predominantly from experimental models of acute, systemic infections. It is becoming clearer, however, that the route of infection influences the phenotype and function of resultant Tmem pools. Our studies demonstrate that CD8 Tmem generated in response to a mucosal influenza infection are numerically and functionally preserved in the absence of IL-15, a common gamma chain cytokine implicated in maintaining Tmem long-term. Importantly, by alternating between systemic and intranasal deliveries of vesicular stomatitis virus, we can modify the phenotype of the resultant Tmem pool from a documented IL-15 dependent to an IL-15-independent Tmem phenotype akin to those Tmem generated in response to influenza infection. Together these data demonstrate that the mucosal environment directly affects the development and programming of particular subsets of Tmem ultimately impacting their requirement for IL-15 and subsequent long-term survival, necessitating a need for re-evaluation of the current model of CD8 Tmem maintenance.

OR.39 MiRNAs Regulate Bacterial Infection in Lungs

Hock Tay, Gerard Kaiko, Maximilian Plank, Philip Hansbro, Joerg Mattes, Paul Foster. University of Newcastle, Callaghan, Australia

Rationale: MicroRNAs (miRNAs) are small non-coding RNA that can bind to multiple target messengerRNA (mRNA) to repress protein production. While the expressions of miRNAs are shown to be dysregulated in human diseases, the roles of miRNAs in the airways during bacterial infection remain unknown. Methods: BALB/c mice were challenged with low infectious dose of non-typeable Haemophilus Influenzae (NTHi). miRNAs that were differentially expressed in the airways were identified by microarray and quantified by qPCR. Expression of specific miRNAs were inhibited using miRNA inhibitors (antagomirs) versus scrambled control to determine the effect of miRNA on cellular infiltrates, bacterial clearance and gene expression during lung infection. Results: Interestingly knockdown of a specific miRNA followed by infection in the lungs resulted in increasing bacterial load and cellular infiltrates in the airways compare to the control groups. Conclusions: Our study suggests that miRNA may play important roles in regulating the innate immune response to bacterial infection in lungs.

OR.40. Innate Lymphoid Cells Mediate Influenza-induced Airway Hyperreactivity Independent of Adaptive Immunity

Ya-Jen Chang[1], Hye Young Kim[1], Lee Albacker[1], Nicole Baumgarth[2], Andrew McKenzie[3], Dirk Smith[4], Rosemarie DeKruyff[1], Dale Umetsu[1]. [1]Children's Hospital Boston, Boston, MA; [2]University of California, Davis, CA; [3]Medical Research Council, Cambridge, United Kingdom; [4]Amgen, Inc., Seattle, WA

Asthma is a major public health problem affecting 300 million people worldwide. Although allergic reactions mediated by Th2 cells comprise a significant trigger of asthma symptoms, respiratory infection, for example with influenza virus, more often results in severe symptoms. However, the pathogenic mechanisms by which influenza infection causes asthma are not fully known. We now show in a mouse model that influenza infection induces airway hyperreactivity (AHR), a cardinal feature of asthma, through a novel pathway that is independent of Th2 cells and adaptive immunity, but requires an IL-13-IL-33 axis. Infection with influenza A virus, which activates the NLRP3 inflammasome, greatly increased alveolar macrophage

production of IL-33, which in turn activated a non-T, non-B innate lymphoid cell type called natural helper cells/nuocytes that produced IL-13 and was required for the development of AHR. These results are the first to show that natural helper cells/nuocytes, described previously in the intestines in the context of helminth infection, are present in the lungs, and that natural helper cells/nuocytes are required for the development of AHR induced with influenza. In addition, these studies may lead to improved therapies for acute severe exacerbations of asthma associated with viral infection.

OR.41. Role of Invariant Natural Killer T Cells During Experimental Influenza A Virus Infection

François Trottein. Pasteur Institute, Lille, France

Invariant Natural Killer T (iNKT) cells represent a population of non-conventional "innate-like" ab T lymphocytes that recognize lipid antigens presented by the CD1d molecule. During viral infection, iNKT cells provide innate surveillance against virally infected cells through the swift production of IFN-g. We assessed the *in vivo* physiological contribution of iNKT lymphocytes on the host (C57BL/6 mice) response and viral pathogenesis during influenza A virus infection (H3N2). Upon infection, iNKT cells became activated in the lungs to rapidly produce IFN-g, and surprisingly enough, IL-22, a cytokine known to play a key role in mucosal defence. Relative to wild-type animals, C57BL/6 mice deficient in iNKT cells developed a more severe broncho-pneumonia and had an accelerated fatal outcome. During this presentation, we will present the mechanisms by which iNKT cells produce IL-22 and discuss the potential role of this cytokine during the early stage of acute influenza A virus infection.

OR.42. The Development of Inducible Bronchus-associated Lymphoid Tissue (iBALT) in Weanling Mice is Suppressed by Regulatory T Cells

Shen Yun Foo[1], Vivian Zhang[2], Amit Lalwani[2], Chuan En Lam[1], Paul Foster[1], Simon Phipps[2]. [1]University of Newcastle, Callaghan, NSW, Australia; [2]University of Queensland, St. Lucia, QLD, Australia

Unlike other lymphoid structures, inducible bronchus-associated lymphoid tissue (iBALT) organogenesis is not pre-programmed but occurs in response to chronic inflammation or infection. However, the molecular immune processes that underlie iBALT formation remain poorly understood. Here we show that a single exposure to LPS was sufficient to promote the formation of multiple iBALTs in a dose- and MyD88-dependent manner. iBALTs were supplied by high endothelial venules, provided a niche for lymphocyte proliferation, and contained clusters of B220+ B cells and CD3+ T cells. iBALT formation was associated with increased cytokine (IL-6, TNF, IL-21) and chemokine (CCL2, CXCL9, CXCL13) gene expression and was dependent on CD40-CD40L interactions. Consistent with the finding that iBALT occur more frequently in children than adults, only 25% of weanling (28 day old) mice as compared to 80% of neonatal (7 day old) mice developed iBALT. Remarkably, antibody-mediated depletion of regulatory T cells altered cytokine/chemokine lung expression and increased the formation of iBALT in LPS-exposed weanling mice. Thus, our data suggest that activation of the toll-like receptor 4/MyD88 signalling pathway in the neonatal period can trigger a series of coordinated events that result in iBALT formation, and that this process is in part controlled by regulatory T cells.

Regulatory T Cells (1503)
Wednesday, July 6 16:15-17:45

OR.43. High Abundance of Helios+ Foxp3- T Cells in Peyer's Patches Reveals Plasticity of Natural Regulatory T Cells in the Gut

Ulrich Steinhoff, Viktoria Sprenger, Denise Winkler, Charlotte Wach, Anne Hellhund, Alexander Visekruna. University of Marburg, Marburg, Germany

CD4+ Foxp3+ regulatory T cells (Tregs) are important for immune homeostasis. The transcription factor Helios has been suggested as specific marker for natural Tregs. Here, we demonstrate that in Peyer's Patches (PPs), a substantial percentage (30 %) of CD4+ T cells belongs to a Helios+ Foxp3- population while these cells are nearly absent in other tissues examined. Interestingly, in germ-free (GF) animals, almost 50% of all CD4+ T cells in the PP are Helios+ Foxp3- cells. Interestingly, c-Rel deficient mice have a diminished frequency of these lymphocytes. Since c-Rel controls the development of thymic natural Tregs and GF animals display higher levels of Helios+ Foxp3+ Tregs in the gut than SPF mice, we suggest that Helios+ Foxp3- T cells represent former natural Tregs which originated from thymic Helios+ Foxp3+ Tregs. The conversion and preferential generation of T follicular helper cells from Foxp3+ Tregs in PPs has recently been described. Treatment of SPF mice with IL-2/JES6-1 complex resulted in disappearance of Helios+ Foxp3- cells and expansion of Helios+ Foxp3+ Tregs in the PPs. Taken together, these observations suggest a high plasticity of Tregs in the PPs probably due to their close proximity to the gut flora.

OR.44. Mucosal Tolerance Dependent upon Natural Killer (NK) Cells to Resolve Myelin Oligodendrocyte Glycoprotein (MOG)-induced Experimental Autoimmune Encephalomyelitis (EAE) by Recruiting Regulatory T (Treg) Cells into the CNS

David Pascual, Massimo Maddaloni, Carol Riccardi, Eduardo Huarte. Montana State University, Bozeman, MT

Recently, the tolerizing agent, MOG fused to reovirus protein σ1 (MOG-pσ1), was shown to resolve EAE at the peak of disease within 24 hrs of mucosal treatment. We hypothesized that innate cells must be involved in the resolution of EAE. NK cells were suspected to be involved, and subsequently the NK cell subset, interferon-producing killer dendritic cells (IKDCs), was shown to be essential in EAE resolution following single dose treatment. To understand its mechanism of action, studies revealed that activated IKDCs were recruited subsequent MOG-pσ1 treatment, and disease resolution was abated upon NK1.1 cell depletion. These IKDCs were found to kill activated CD4+ T cells and mature dendritic cells in a

perforin-dependent manner, thus, contributing to EAE remission. Additionally, IKDCs were responsible for MOG-pσ1-mediated IL-10+ Treg cell recruitment to the CNS since *in vivo* depletion of IKDCs resulted in the loss of Treg cell recruitment. The capacity of IKDCs to rapidly migrate to the CNS and inhibit the proliferation of encephalitogenic CD4+ T cells provides for one mechanism of MOG-pσ1's therapeutic effect. These data further confirm the feasibility of using pσ1 as a mucosal delivery platform for tolerance induction applied specifically to treat autoimmunity. Supported by AI-078938.

OR.45. A Critical Role of Gut-homing T Cells in Oral Immunological Tolerance

Barbara Cassani[1], Eduardo Villablanca[1], Francisco Quintana[2], Paul Love[3], Adam Lacy-Hulbert[1], William Blaner[4], Tim Sparwasser[5], Howard Weiner[2], J. Rodrigo Mora[1]. [1]Massachusetts General Hospital, Boston, MA; [2]Brigham and Women's Hospital, Boston, MA; [3]National Institute of Child Health and Human Development, Bethesda, MD; [4]Columbia University, New York, NY; [5]Institute of Infection Immunology, Hannover, Germany

Oral immunological tolerance (OT) induces systemic non-responsiveness to orally administered antigens (food and commensal microbiota), and it has been investigated as a potential treatment for autoimmune conditions. However, the mechanisms underlying OT remain poorly understood. Lymphocyte migration to the gut requires the integrin α4β7 and chemokine receptor CCR9, whose induction critically depends on the vitamin A metabolite all-trans retinoic acid (RA). RA additionally potentiates the generation of Foxp3+ TREG cells, which are required for OT. We show that OT was abolished in vitamin A-depleted (RA-depleted) mice. Moreover, using two experimental models we found that OT was abrogated in CCR9-/- mice or when the α4β7-ligand MAdCAM-1 was blocked in wild type (wt) mice, indicating that gut-homing receptors are critically required for oral tolerization. Importantly, OT was rescued in CCR9-/- mice by adoptively transferring wt T cells, but not CCR9-/- or integrin β7-/- T cells, demonstrating that bona-fide gut-homing T cells are necessary and sufficient for OT induction. Noteworthy, TREG depletion prevented OT in CCR9-/- mice adoptively transferred with wt CD4 T cells, indicating that homing and/or differentiation of TREG in the gut is required for OT. Our results highlight a heretofore unrecognized link between RA-dependent gut-homing T cells and immunological tolerance.

OR.46. Gut-draining Lymph Nodes Display an Intrinsic Capacity for Efficient Induction of Foxp3+ Regulatory T Cells

Sascha Cording[1], Manuela Buettner[2], Benjamin Wahl[2], Markus Heimesaat[3], Stefan Bereswill[3], Oliver Pabst[2], Ulrike Bode[2], Alf Hamann[2], Jochen Huehn[1]. [1]Helmholtz Centre for Infection Research, Braunschweig, Germany; [2]Hannover Medical School, Hannover, Germany; [3]Charité University Medicine, Berlin, Germany

Foxp3+ regulatory T cells (Tregs) play an important role in the maintenance of mucosal tolerance. They have been shown to develop both in the thymus as well as in the periphery upon antigen recognition under tolerogenic conditions. We have recently shown an extraordinarily efficient Foxp3+ Treg induction in gut-draining mesenteric lymph nodes (mLN) upon oral antigen-administration. To investigate the impact of the commensal microflora on this process, we performed de novo Treg induction in antibiotics-treated mice. Interestingly, we observed no difference in Treg induction when antibiotics-treated mice were compared with untreated control mice. To find out, whether the high Treg inducing capacity is due to an intrinsic property of mLN or to their anatomical localisation, we performed LN transplantation experiments by removing the 'non-tolerogenic' popliteal LN and replacing it by mLN. Strikingly, using a systemic antigen-administration protocol we here observed a higher Treg induction in transplanted mLN when compared to transplanted control LN. Taken together, our data suggest that effective de novo Treg induction within mLN is neither dependent on the presence of a commensal microflora nor on the anatomical localisation, but is an intrinsic property of the gut-draining LN itself.

OR.47. CD8+ T Suppressor Cells in the Normal Intestine Lose their Suppressor Activity in Response to TGF-β

Keren Rabinowitz[1], Yuanyuan Wang[2], Cecilia Berin[1], Avi Ma'ayan[1], Damien Chaussabel[2], Lloyd Mayer[1]. [1]Mount Sinai School of Medicin, New York, NY; [2]Baylor Institute for Immunology Research, Dallas, TX

In the normal state, the interaction between IEC and LP lymphocytes gives rise to a population of CD8+ T cells with suppressor function (Ts) that can be identified phenotypically and functionally in normal LPL. Ts isolated from the LP of CD patients show a defect in their suppressive activity. Increased levels of TGF-β are reported in CD patients. Aim: Characterize the requirement for expansion of Ts. Define what factors leads to the loss of suppressor activity mediated by Ts in CD patients. We developed a method for generating Ts lines from freshly isolated LPLs from normal controls, UC and CD patients. Cells were stimulated with anti-CD3 mAb, IL7 and IL15. After 14 days CD8+ T cells were purified by magnetic bead sorting, and cultured with IL7 and IL15. Ts lines were analyzed for suppressor activity, surface molecule expression and cytokine secretion profiles. RNA from the 3 groups was used for microarray. Ts lines did not express CD28, ICOS, CTLA4, perforin and granzyme B, but expressed integrin α4β7, CD8β, CD101, CD56 and CD122. Ts lines generated from the LP of normal and UC patients were able to suppress CD4+ T cell proliferation in a contact dependent fashion. In contrast, Ts lines generated from CD patients had a markedly decreased capacity to mediate suppression. Microarray analysis showed differential genes expression in multiple genes regulated by TGF-β. Interestingly when introducing TGF-β or supernatants from CD tissue cultures into the suppressor assay it reduced suppressor activity in a dose dependent fashion. We developed a method for generating Ts lines from the LP and showed that Ts lines from controls and UC but not CD patients exhibit contact dependent regulatory function. Introducing TGF-β reduced the suppressor activity of Ts, which could explain the defect in Ts in Crohn's disease.

OR.48. Intestinal Homeostasis: Role of Regulatory CD8+Foxp3+ T Cells

Diana Fleissner[1], Jost Langhorst[2], Jan Buer[1], Astrid Westendorf[1]. [1]University Hospital Essen/Institute of Medical Microbiology, Essen, Germany; [2]University Duisburg-Essen, Essen, Germany

Immunomodulatory CD8+Foxp3+ T cells are less well defined in terms of their generation and function in the intestinal mucosa. In this study we demonstrate that intestinal antigen exposure leads to the peripheral induction of antigen-specific CD8+Foxp3+ T cells in transgenic mice expressing the model antigen hemagglutinin exclusively in intestinal enterocytes and concomitantly bearing hemagglutinin-specific CD8+ T cells. Antigen-experienced CD8+ T cells that express Foxp3 and exhibit strong immunosuppressive activity might be necessary in controlling intestinal homeostasis in this transgenic mouse model. Furthermore, frequency of CD8+Foxp3+ T cells is reduced in the peripheral blood of patients with ulcerative colitis. As these cells might play yet an underestimated role in the maintenance of intestinal homeostasis, we have investigated human and murine CD8+Foxp3+ T cells generated by stimulating naïve CD8+ T cells in the presence of TGF-beta and retinoic acid, mediators that are abundant produced in the intestinal mucosa. These CD8+Foxp3+ fully competent regulatory T cells show strong expression of regulatory molecules CD25, Gpr83 and CTLA-4, exhibit cell-cell contact-dependent immunosuppressive activity *in vitro* and interfere with inflammation *in vivo*. Thus, our study illustrates a previously unappreciated critical role of CD8+Foxp3+ T cells in controlling intestinal homeostasis.

Oral Presentations: Thursday, July 7

Epithelial Cell and Intestinal Barrier II (2400)
Thursday, July 7, 14:30-16:00

OR.49. Epithelial Cell-intrinsic Notch Signaling Plays an Essential Role in the Maintenance of Gut Immune Homeostasis

Yuuki Obata[1], Daisuke Takahashi[2], Masashi Ebisawa[2], Kisa Kakiguchi[3], Shigenobu Yonemura[3], Koji Hase[3], Hiroshi Ohno[3]. [1]Chiba University, Chiba, Japan; [2]Yokohama City University, Yokohama, Japan; [3]RIKEN, Kobe, Japan

Intestinal epithelial cells (IEC) possess important functions in the first line defense against diverse microorganisms on the luminal surface. Epithelial stem cells constantly give rise to transient amplifying cells, which subsequently differentiate into absorptive and secretory cell lineages. Notch signaling plays a critical role in the cell fate decision of these two types of IEC. We here show that mice with IEC-specific deletion of Rbpj (RBP-J$^{\Delta IEC}$), a transcription factor responsible for Notch signaling, spontaneously develop Th17-dominant chronic colitis. Intestinal microbiota was responsible for the colitis development, because depletion of microbiota abolished the development of colitis in RBP-J$^{\Delta IEC}$ mice. Furthermore, bacterial translocation was enhanced in the colonic mucosa of RBP-J$^{\Delta IEC}$ mice before the colitis onset, suggesting attenuated epithelial barrier functions in these mice. While the formation of tight junction and the expression of antimicrobial molecules remained intact, RBP-J$^{\Delta IEC}$ mice displayed significant reduction of epithelial turnover coupled with goblet cell hyperplasia. These data suggest that a high frequency of epithelial turnover maintained by epithelial-intrinsic Notch signaling is essential for the establishment of barrier function. Thus, Notch signaling plays a vital role in the maintenance of gut immune homeostasis by ensuring epithelial integrity.

OR.50. Formyl Peptide Receptor-1 Expressed on Epithelial Cells Regulates Cell Motility, Commensal-epithelial Cross-talk and Mucosal Barrier Function

Giovanna Leoni[1], Mohammad Alam[1], Phillip Swanson[1], Mauro Perretti[2], Charles Parkos[1], Andrew Neish[1], Asma Nusrat[1]. [1]Emory University, Atlanta, GA; [2]William Harvey Research Institute, London, United Kingdom

Barrier properties of the intestinal epithelium play a pivotal role in mucosal permeability and thus regulate exposure of the mucosal immune system to commensal and pathogenic microbes. Commensal microbes and their products contribute to these processes in the intestinal mucosa by regulating integrity of the intestinal epithelium. We have recently reported that N-formyl peptide receptor-1 (FPR-1) is expressed in the intestinal epithelium and mediates G-protein coupled signaling to regulate intestinal epithelial cell motility. However, the mechanism by which FPR-1 transduces signals to control epithelial functions is not understood. In the current study, we observed that exposure of epithelial cells to FPR-1 agonists N-formyl methionyl peptide (fMLF), Annexin 1 N-terminal peptide Ac2-26 as well as intact viable commensal bacteria induce rapid generation of reactive oxygen species (ROS). Analysis of the signaling pathways identified a role of PI3K, Vav2 a guanine nucleotide exchange factor and Rac1 GTPase in mediating NADPH oxidase-dependent ROS generation, phosphorylation/activation of focal adhesion kinase (FAK), and induction of epithelial motility and barrier properties. Taken together, these results support an important role for FPR-1 stimulated signaling pathways in promoting critical homeostatic properties of intestinal mucosa.

OR.51. The Functions of IL-22 in Mucosal Immunity

Wenjun Ouyang, Naruhisa Ota, Kit Wang. Genentech, South San Francisco, CA

IL-22 is an IL-10 family member of cytokines. It signals through IL-22R and IL-10R2 chains. IL-22R is specifically expressed on various epithelial cells. IL-22, on the other hand, is primarily produced by leukocytes. IL-22, thus, mediates the crosstalk between the immune system and tissue epithelial cells. During C. rodentium infection in the colon, IL-22 is upregulated. Multiple pathways play essential role in regulation of IL-22. We found

both IL-23 and lymph toxin (LT) pathway are required for the production of IL-22 in the colon during C. rodentium infections. Multiple cell types including LTi, NK, and myeloid cells are able to response to IL-23 and secrete IL-22. IL-22 is required for host defense against C. rodentium infection. In the absence of IL-22 pathway, all mice are succumbed during the second week infection. The colon from IL-22 deficient mice demonstrated much severe epithelial damage upon the infection. IL-22 is able to induce various antimicrobial peptides from colon epithelial cells. The induction of Reg family c-type lectins is dependent on IL-22 during the infection. Recombinant RegIII is able to partially rescue the phenotypes observed in IL-22 deficient mice. Finally, IL-22 pathway in this model is linked with lymph toxin pathway. IL-22 is able to rescue the lethal phenotype when LT pathway is blocked. In addition, IL-22 is also participated in the formation of lymph follicles in the colon. Thus, IL-22, though its function on epithelial cells, also plays a role in neolymphogenesis in inflamed mucosal tissues.

OR.52. Intestinal Epithelial Cell-specific MyD88 Signaling Promotes Homeostasis by Regulating Expression of the Polymeric Immunoglobulin Receptor

Aubrey Frantz, Eric Rogier, Maria Bruno, Charlotte Kaetzel. University of Kentucky, Lexington, KY

Commensal bacteria enhance innate immune functions of intestinal epithelial cells (IECs) through MyD88-dependent Toll-like receptor signaling. We demonstrated that expression of the polymeric immunoglobulin receptor (pIgR), the epithelial IgA transporter, was significantly reduced in IECs of MyD88-deficient mice, and in the human IEC-line HT-29 following shRNA-mediated knockdown of MyD88. To assess the role of IEC-specific MyD88 signaling *in vivo*, we generated mice with an IEC-specific deletion of MyD88 by crossing MyD88 "floxed" mice with mice expressing Cre recombinase under the control of the IEC-specific Vil1 promoter. Analysis of isolated IEC from wild-type, MyD88-IEC-hemizygous and MyD88-IEC-null mice revealed that expression of MyD88 and pIgR were highly correlated ($r = 0.942$, $p < 0.0001$). In contrast, expression of the pro-inflammatory factors TNF and MIP2 was not correlated with IEC-specific MyD88 expression. MyD88-IEC-null mice were found to have defective epithelial barrier function, resulting in increased bacterial translocation to mesenteric lymph nodes. Severity of acute DSS-induced or chronic T cell-mediated colitis in wild-type mice was correlated with reduced expression of pIgR in IEC. These findings suggest that maintenance of pIgR expression by MyD88-dependent signaling promotes intestinal homeostasis and protects against inflammation. Supported by the NIH and the Crohn's & Colitis Foundation of America.

OR.53. Secretory Antibodies Regulate Commensal Microbiota and Protect Against DSS-induced Colitis

Finn-Eirik Johansen[1], Dag Henrik Reikvam[1], Alexander Erofeev[1], Rejoanol Islam[1], Vedrana Grcic[1], Peter Gaustad[1], Muriel Derrien[2], Anders Sandvik[1], Frode Jahnsen[1], Hauke Smidt[2]. [1]University of Oslo and Oslo University Hospital, Oslo, Norway; [2]Wageningen University, Wageningen, Netherlands

Perturbations in the homeostatic relationship between gut microbiota and host is clearly involved in inflammatory bowel disease. We have investigated gut homeostasis when an important mediator for host protection against commensal microbes is missing. Polymeric Ig Receptor knock out (pIgR KO) mice fail to transport dimeric IgA and pentameric IgM to the gut lumen and are therefore deficient in secretory antibody formation. Specific pathogen-free pIgR KO mice showed enhanced susceptibility to DSS-induced colitis. Both pIgR KO and wild type mice were resistant to colitis when they were gavaged twice daily with a concoction of antibiotics show to deplete almost all intestinal bacteria. Interestingly, we found alterations in the phylogenetic composition of intestinal bacteria in pIgR KO mice versus wild type mice, both in the normal situation and when colitis was induced with DSS. Furthermore, colonic epithelial cells of untreated pIgR KO mice expressed elevated levels of antimicrobial and pro-inflammatory genes compared with untreated wild type mice, and these differences depended on the presence of intestinal bacteria. These findings show that although the absence of secretory antibodies can partly be compensated by enhanced innate antimicrobial responses, mucosal homeostasis is disturbed in pIgR KO mice making them more prone to intestinal inflammation.

OR.54. M Cells as an Effcient Way for Crohn's Disease-associated Adherent-invasive Escherichia Coli Expressing Long Polar Fimbriae to Cross the Epithelial Barrier

Benoit Chassaing, Arlette Darfeuille-Michaud. Clermont Université, Université Auvergne, Clermont-Ferrand, France

Background: Ileal lesions of patients with Crohn's disease (CD) are colonized by adherent-invasive Escherichia coli (AIEC). We recently identified that long polar fimbriae (LPF) are involved in the targeting of Peyer's patches, the site of the earliest CD lesions. We aimed to identify the role played by M-cells in the interaction between AIEC bacteria and PP. Methods: Wild-type LF82 bacteria and the LPF negative mutant were tested for their abilities to interact *in vitro* with M-cells using the co-culture model (Caco2 - RajiB) and with murine M-cells *ex vivo* using ileal loop assays. Results: AIEC LF82 bacteria translocate at a very high level through M-cell monolayers, but mainly after bacterial growth in the presence of bile salts. β-galactosidase assays confirmed that bile is a key gastrointestinal factor that activates LPF promoter. The lpfA mutant was highly impaired in its ability to translocate through M-cells, and confocal analysis confirmed that AIEC bacteria target M-cells dependently on LPF expression. Inhibition experiments with anti-glycoprotein2 antibodies revealed that GP2, which is involved in the binding of piliated bacteria through FimH, is not involved in the interaction of LPF positive bacteria with M-cells. Conclusions: LPF are a key factor for AIEC to target M-cells.

IBD Pathogenesis (2401)
Thursday, July 7, 14:30-16:00

OR.55. Mitochondrial Stress Mechanisms Fuel Chronic Intestinal Inflammation via PKR in Human IBD and Murine Models of Colitis

Eva Rath[1], Emanuel Berger[1], Tiago Nunes[2], Bo Liu[3], Nick Hoogenraad[4], Miquel Sans[2], Balfour Sartor[3], Dirk Haller[1]. [1]Technische Universität München, Freising-Weihenstephan, Germany; [2]Hospital Clínic i Provincial, Barcelona, Spain; [3]University of North Carolina, Chapel Hill, NC; [4]La Trobe University, Melbourne, VIC, Australia

Endoplasmic reticulum (ER) unfolded protein responses (UPR) in intestinal epithelial cells (IEC) contribute to the development of chronic intestinal inflammation. In this study we characterized the interrelated role of ER- and mitochondrial (mt) UPR in the epithelium of patients with inflammatory bowel diseases (IBD) and murine models of colitis. Proteome- as well as immunohistochemical and Western blot analysis of primary IEC from IBD patients and the murine models revealed strongly activated ER- and mtUPR as reflected by increased expression of the ER chaperone glucose-regulated protein (GRP)78 and mitochondrial chaperonin (CPN)60. This was associated with an induction of the dsRNA-activated protein kinase (PKR). Mitochondrial specific stress-induction in a murine IEC line triggered the phosphorylation of eukaryotic translation initiation factor (eIF2)α through the recruitment of PKR. Using pharmacological inhibitors and siRNA, we identified the mtUPR-induced eIF2α phosphorylation and transcription factor activation (CHOP, cJun) to be dependent on the mitochondrial protease ClpP, the cytoplasmic kinase PKR as well as MEK and JNK. Moreover, Pkr-/- mice showed almost complete resistance to DSS-induced colitis and lack DSS-induced epithelial CPN60 induction. These results demonstrate a novel mechanism for mitochondrial stress-integration into the disease-relevant ER signaling cascade and suggest PKR to link metabolic-, inflammatory- and immune responses.

OR.56. NKG2D Activation Drives Th17 Response in Crohn's Disease

Benjamin Pariente, Iulia Mocan, Matthieu Camus, Nicolas Dulphy, Antoine Toubert, Matthieu Allez. Hôpital Saint-Louis, Paris, France

CD4+ T cells expressing NKG2D exhibit cytotoxic and pro-inflammatory properties in Crohn's disease (CD) Aims: To assess the impact of NKG2D pathway on Th17 responses in CD. Methods: Lamina propria lymphocytes (LPL) and peripheral blood lymphocytes (PBL) from 44 CD patients were isolated. Analysis was performed by flow cytometry and Reverse-Transcription Polymerase Chain Reaction (Q-RT-PCR). Results: CD4+NKG2D+ T cells were highly positive for IL17 intracellular staining (34.5±25.3%) and expressed significantly higher levels of IL17 than their CD4+NKG2D- counterparts ($p=0.001$). Messenger RNAs of IL-17 and RORC were expressed preferentially in CD4+NKG2D+ T cells compared to CD4+NKG2D- subset ($p=0.02$). CCR6 and IL23R were also significantly more expressed on CD4+NKG2D+ T cells ($p<0.05$). Higher proportion of IL17 producing T cells co-expressed both NKG2D and CD161, as compared to IL17 negative cells (52.5±23.7% vs. 4.5±3.2%). Targeting NKG2D by its ligands expressed on P815 cell lines in co-stimulation with TCR significantly increased IL17 production by CD4+ T cells compared to stimulation through TCR alone ($p<0.05$). CD4+NKG2D+ population secreted significantly higher IL17 in presence of IL23 as compared to IL15 condition ($p<.05$). Conclusion: CD4+NKG2D+ T cells have typical features of Th17 cells and iinteractions between NKG2D and its ligands strongly influence IL17 production in CD.

OR.57. Cytotoxic Natural Killer T Cells Bearing IL-13Rα2 Populate the Mucosa in Ulcerative Colitis (UC)

Ivan Fuss[1], Bharat Joshi[2], Manijeh Phillips[3], Zhiqiong Yang[1], Chuli Yi[1], Peter Mannon[1], Claudio Fiocchi[3], Raj Puri[2], Warren Strober[1]. [1]National Institutes of Health, Bethesda, MD; [2]Center for Biologics Evaluation FDA, Bethesda, MD; [3]Cleveland Clinic Foundation, Cleveland, OH

In prior studies, we showed that oxazolone colitis, a murine model of UC, is driven by invariant Natural Killer T (NKT) cells which secrete increased amounts of IL-13. In related human studies, we showed that UC LPMC also produce significant amounts of IL-13, arising in this case, from non-invariant NKT cells. Moreover, these NKT cells were directly cytotoxic for HT-29 epithelial cells, thus establishing a possible mechanism of injury in UC. Recently it has been shown that IL-13Rα2 can function as a signaling receptor for IL-13. In the present studies we determined the relationship of cells bearing this receptor to the occurrence of inflammation in human ulcerative and oxa-colitis. In flow cytometric studies we found that peripheral blood T cell populations of UC patients contain a higher percentage of cells that bear both CD161 (NKT) and IL-13Rα2 markers as compared to Crohn's dx cell population. This correlated with the presence of large numbers of CD161+ LP cells expressing IL-13Rα2 in UC patients as compared to CD patients. In further studies we demonstrated that depletion of IL-13Rα2 cells from UC patient LPMC with a cytotoxic agent, mutated IL-13 coupled to pseudomonas exotoxin (IL-13PE), that binds with high affinity to IL-13Rα2-bearing cells led to 1) loss of CD4+ cells producing IL-13 and 2) reduced cytotoxicity for HT-29 targets. Finally, to examine the pathogenicity of the IL-13Rα2 bearing cells we treated oxa-colitis mice with the IL-13PE and such treatment led to both amelioration of inflammation and decreased IL-13 secretion. These studies show that UC is uniquely characterized by large numbers of IL-13Rα2-bearing, CD4+ NKT cells that produce IL-13 and manifest cytotoxicity for epithelial cells.As such cells mediate oxa-colitis, these studies suggest the IL-13Rα2 cells may direct the pathogenesis of UC.

OR.58. Role of IL-10 Receptor in Early-onset Inflammatory Bowel Disease

Christopher Moran[1], Subra Kugathasan[2], Christoph Klein[3], Anne Griffiths[5], Mark Silverberg[4], Aleixo Muise[5], Scott Snapper[1]. [1]Massachesetts General Hospital for Children, Boston, MA; [2]Emory-Children's Center, Atlanta, GA; [3]Hannover Medical School, Hannover, Germany; [4]Mount Sinai Hospital, Toronto, ON, Canada; [5]Hospital for Sick Children, Toronto, ON, Canada

Introduction: Mutations in interleukin-10 receptor (IL-10R) genes cause a form of infantile inflammatory bowel disease (IBD). However, IBD genome-wide association studies (GWAS) have not reported associations with IL-10R gene polymorphisms. Associations in the IL10R genes may have gone undetected because these GWAS were neither powered to examine rare polymorphisms (<5%) nor young patients. We hypothesized that IL-10R polymorphisms play a role in very early-onset IBD (≤10yo; VEO-IBD). Methods: IL-10R genes were sequenced in 188 VEO-IBD patients and 185 controls. Odds ratios (OR) were calculated under a dominant genetic model using Fisher's exact test. Results: We identified a VEO-IBD patient with a novel homozygous mutation in the conserved GT splice donor site of intron 5 of the IL10RA gene that resulted in a novel stop codon. IL10 did not activate STAT3 phosphorylation at tyrosine 705 in peripheral blood mononuclear cells isolated from our patient as compared to a healthy control. Thus, the putative truncated protein could not induce IL10-mediated signaling. Further sequencing of 188 patients identified five IL-10RA polymorphisms that associated with ulcerative colitis and two IL-10RB polymorphisms in the 3' untranslated region that associated with Crohn's disease. Conclusions: These results suggest that IL-10R polymorphisms are associated with VEO-IBD.

Ulcerative Colitis SNPs	OR (Unadjusted p value)
rs10892202	0.30 (p=0.009)
rs4252249	0.30 (p=0.014)
rs4252270	0.30 (p=0.014)
rs2228054	3.06 (p=0.008)
rs2228055	2.52 (p=0.029)
Crohn's Disease SNP	**OR (Unadjusted p value)**
rs8178561	0.10 (p<0.001)
rs1058867	2.12 (p=0.004)

OR.59. Intestinal CXCR4+IgG+ Immature Plasma Cells Contribute to the Pathogenesis of Ulcerative Colitis through IgG-Immune Complex-FcγR Signaling

Tadakazu Hisamatsu, Michihide Uo, Jun Miyoshi, Kazuaki Yoneno, Nagamu Inoue, Haruhiko Ogata, Takanori Kanai, Toshifumi Hibi. Keio University, Tokyo, Japan

In health the vast majority of intestinal plasma cells (PCs) are IgA-producing PCs, on the other hand, there is a massive influx of IgG-producing PCs into the inflamed mucosa in UC. However, the precise mechanism of infiltration and their involvement in pathogenesis of UC remain unclear. To clarify these unresolved questions, we analyzed the characteristic features of intestinal PCs in UC. Methods: 1) Microarray gene expression analysis of isolated intestinal PCs was performed and the expression of surface antigens was analyzed by flow cytometry. 2) The cytokine production by LPMCs stimulated by plate IgG-immune complex (IC) was measured. Target cells expressing FcγR were identified by flow cytometry. Results: 1) PCs in inflamed mucosa of UC were CD38highCD19+CD20-CD27low immature plasmablast-like phenotype. IgG+ PCs in UC specifically expressed chemokine receptor CXCR4 and lacked the expression of CCR10. 2) IgG-IC stimulation induced the production of TNF-α from LPMCs. CD14+ intestinal macrophages increased in IBD mucosa expressed FcγRs, and produced a large amount of TNF-α by IgG-IC stimulation. Blocking of FcγR signaling selectively inhibited IgG-IC-induced TNF-α production. Conclusion: In UC, intestinal IgG+ PCs may infiltrate into the inflamed mucosa via CXCL12-CXCR4 axis and involve the pathogenesis by exacerbating mucosal inflammation through IgG-IC-FcγR signaling.

OR.60. Appendicitis, Protection Against Colitis and the Role of Colonic Regulatory T Cells

Annie Luo, Watson Ng, Rajkumar Cheluvappa, Michael Grimm. University of New South Wales, Sydney, NSW, Australia

Appendicectomy for intra-abdominal inflammatory conditions protects against later colitis. We have recapitulated this observation in a mouse model, showing that appendicitis and appendicectomy offered protection against trinitrobenzene sulfonic acid (TNBS) induced colitis. The protective effect of appendicitis was associated with an expansion of regulatory T cells (T_{regs}) which preferentially migrated to the colonic lamina propria (cLP). Flow cytometric analysis of the whole colon from appendicitis mice showed a dramatic increase in cLP T_{regs} with an approximately 2.5 fold increase in $CD4^+Foxp3^+$ T_{regs} and a 6 fold increase in $CD8^+Foxp3^+$ T_{regs} compared to control mice. Protection was associated with the production of IL-10 by these cLP T_{regs} and neutralisation of IL-10 function completely abrogated the protective effect. Immunohistochemical data showed $Foxp3^+$ T cells were presented as isolated cells in the cLP and there was no difference in cell distribution between the treatment groups. We found colonic lymphoid follicles consistently contained a large number of $FoxP3^+$ T cells; therefore the difference is likely to be related to T_{reg} numbers in lymphoid follicles. These data suggest that appendicitis mediated protection against colitis through the creation of an immune-regulatory environment by favouring the differentiation of immuno-suppressive T_{regs}.

IgA, Polymeric Receptor and Peyer's Patch B Cell Lymphopoiesis (2402)

Thursday, July 7, 14:30-16:00

OR.61. Mice Lacking IgA Exhibit Defective IgG Antibody Responses to Polysaccharide Immunizations

Jennifer Wilson-Welder[1], Girish Kirimanjeswara[1], Rachael Racine[1], Joseph Petrosino[2], Dennis Metzger[1]. [1]Albany Medical College, Albany, NY; [2]Baylor College of Medicine, Houston, TX

In order to understand the role of IgA in mucosal protection against respiratory pathogens studies have been performed using mice with a targeted gene deletion that causes lack of IgA but normal expression of other Ig isotypes. Surprisingly, IgA-/- mice exhibit specific defects in both IgA and IgG serum antibody responses to the polysaccharide (PS) component of pneumococcal conjugate vaccine. IgG antibody responses to the protein component of the conjugate vaccine were not affected. Spleenic and peritoneal cavity B cell subsets in IgA-/- mice were present in similar numbers as compared to wild-type mice. Studies using bone marrow chimeras showed that reconstitution of IgA-/- mice with IgA+/+ B cells failed to restore serum antibody responsiveness to the PS component of the conjugate vaccine. Preliminary sequencing of the intestinal microbiota showed that wild-type and IgA-/- mice are markedly different. Reconstitution of IgA-/- mice with the intestinal microbiota from wild-type mice increased the serum antibody response to PS vaccine antigens. Taken together, these results indicate that the environment (systemic priming by the intestinal flora) that is present during B cell activation is critical for the response to polysaccharide vaccine antigen. Supported by NIH grants RO1 41715 and R21 83878.

OR.62. Impaired Cellular Immunity in the Murine Neural Crest-specific Conditional Knockout of Endothelin Receptor-B Model of Hirschsprung's Disease

Ankush Gosain, Aaron Heneghan, Joseph Pierre, Kenneth Kudsk. University of Wisconsin, Madison, WI

Hirschsprung's disease (HD) is characterized by aganglionosis resulting from failure of neural crest cell (NCC) migration to the distal hindgut. Without treatment, HD patients develop bowel obstruction, Hirschsprung's associated enterocolitis (HAEC), and death. HD patients suffer recurrent HAEC even after colostomy or surgical resection of the aganglionic segment. Recent reports indicate that signaling pathways involved in NCC migration may be involved in the development of secondary lymphoid organs. We hypothesize that there exist gastrointestinal mucosal immune defects in HD that result in susceptibility to HAEC. Conditional mutagenesis was employed to knock out Ednrb, which is required for NCC migration, in the neural crest. Wnt1-Cre(+/-)R26R(YFP/+)Ednrb(flex3/flex3) (Ednrb-null) and Wnt1-Cre(+/-)R26R(YFP/+)Ednrb(flex3/+) (Ednrb-het) animals were sacrificed prior to the development of clinical enterocolitis and the small intestine and spleen harvested. Ednrb-null animals demonstrated fewer Peyer's Patches (PP) and smaller spleens with decreased cellularity, primarily B-lymphocytes, versus Ednrb-het. Ednrb-null spleens demonstrated increased IgM(+)IgD(hi) mature B-lymphocytes and decreased IgM(+)IgD(lo) transitional B-lymphocytes versus Ednrb-het. In contrast, Ednrb-null PP demonstrated decreased mature B-lymphocytes versus Ednrb-het. Additionally, Ednrb-null animals had decreased levels of secretory IgA. These findings may explain susceptibility to HAEC and suggest a role for the neural crest in the development of gastrointestinal mucosal immunity.

OR.63. A Novel Human IgA Monoclonal Antibody Protects Against Tuberculosis

Jennifer Woof[1], Sucharitha Balu[2], Rajko Reljic[3], Richard Pleass[4], Juraj Ivanyi[2]. [1]University of Dundee, Dundee, United Kingdom; [2]King's College London, London, United Kingdom; [3]St. George's, University of London, London, United Kingdom; [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Antibodies have been shown to be protective in passive immunotherapy of tuberculous infection using mouse experimental models. Here we report on the properties of a novel human IgA1, constructed using a scFv clone (2E9), selected from an antibody phage library. The purified antibody monomer revealed high binding affinities for the mycobacterial α-crystallin (Acr) antigen and for the human FcαRI (CD89) IgA receptor. Intranasal inoculations of 2E9IgA1 significantly inhibited pulmonary H37Rv infection in mice transgenic for human CD89, but not in CD89-negative littermate controls, suggesting that binding to CD89 was necessary for the passive protection imparted by IgA. 2E9IgA1 added to human whole blood or monocyte cultures, inhibited luciferase-tagged H37Rv infection, though not for all tested blood donors. The demonstration of the essential role of FcαRI (CD89) for human IgA-mediated protection is important for understanding of the mechanisms involved and also for translation of this approach toward development of passive immunotherapy of tuberculosis.

OR.64. Maintenance of Intestinal Homeostasis Requires Expression of the Polymeric Immunoglobulin Receptor by Both Mother and Offspring

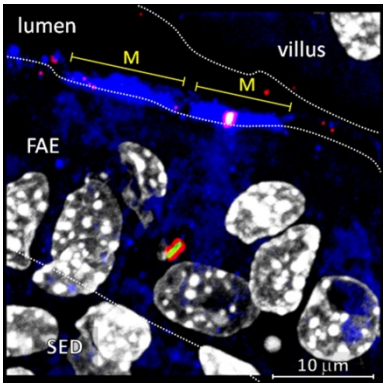
Eric Rogier, Maria Bruno, Aubrey Frantz, Charlotte Kaetzel. University of Kentucky, Lexington, KY

The polymeric immunoglobulin receptor (pIgR) transports locally synthesized polymeric IgA across mucosal and glandular epithelia to form secretory IgA (SIgA), which shapes the composition of the commensal microbiota and protects against mucosal pathogens. In newborn mammals, intestinal SIgA is supplied from both maternal milk and endogenous transport across intestinal epithelial cells. To test the hypothesis that both sources of SIgA contribute to intestinal homeostasis, we employed a unique breeding scheme in which female Pigr-hemizygous mice were crossed with male Pigr-null mice, and vice versa, to generate pIgR-sufficient and -deficient offspring that had or had not received SIgA in maternal milk. At 8-10 weeks of age, acute colitis was induced by administration of 2% DSS in drinking water. Symptoms of colitis appeared earlier in pIgR-deficient than pIgR-sufficient mice, regardless of maternal genotype. Expression of pIgR and other protective factors in colonic epithelial cells was reduced following DSS treatment, to a greater extent in offspring of pIgR-deficient mothers. These findings suggest that SIgA in maternal milk may enhance early development of the intestinal epithelial barrier, and that locally transported SIgA contributes to intestinal homeostasis and protects against inflammation. Supported by the NIH and the Crohn's & Colitis Foundation of America.

OR.65. Secretory Immunoglobulin A: A Forgotten Actor in the Relationship Between the Microbiota and the Intestinal Immune System

Nicolas Rol[1], Laurent Favre[2], Jalil Benyacoub[2], Blaise Corthésy[1]. [1]University State Hospital, Lausanne, Switzerland; [2]Nestec Research Center, Lausanne, Switzerland

Secretory IgA (SIgA) is able to deliver minute quantities of sizeable cargos in the form of SIgA-based immune complexes into intestinal Peyer's patches (PP). Such a novel feature led us to speculate that SIgA interacts with commensal bacteria and contributes in bacterial/host homeostasis. Lactobacillus rhamnosus (LPR), free or as a SIgA-based complex, was thus administered into a mouse ligated intestinal loop containing a PP. Both forms of the bacterium were observed in the subepithelial dome (SED) region of PP, yet with different kinetics. We found out that bacteria delivered alone were actually rapidly coated with endogenous (natural) SIgA present in intestinal secretions. Transport was strictly mediated by M cells, leading to the targeting of intact complex to dendritic cells (DC) located in the SED region. Impact of LPR alone or as SIgA-based complex on DC isolated from PP, mesenteric lymph nodes and spleen was investigated *ex vivo*; this unraveled marked differences in surface display of activation and homing markers between DC of mucosal or systemic origin. The sum of the data brings new evidence on the involvement of SIgA in the mechanisms by which the mucosal immune system permanently senses the content of the intestine. Lactobacillus rhamnosus found complexed with secretory immunoglobulin A (respectively in green and red) after administration in a mouse ileal ligated loop. These bacteria, in the form of SIgA-based complexes, are shown transiting through an M cell (in blue) in a Peyer's patch. 3D reconstruction image following acquisition by laser scanning confocal microscopy. FAE: Follicular associated epithelium; M: M cell; SED: Subepithelial dome. Cells are stained white DAPI (in white).



OR.66. Complexes Between NF-**κB p65 and STAT3 are Key Actors in I**nducing AID Expression and IgA Production in CD40L Plus IL-10-treated Human Blood B Cells

Sandrine Lafarge[2], Hind Hamzeh-Cognasse[2], Yolande Richard[3], Bruno Pozzetto[2], Michel Cogné[4], Fabrice Cognasse[1], Olivier Garraud[1]. [1]Etablissement Français du Sang, Saint Etienne, France; [2]Université de Lyon, Saint Etiennet, Saint Etienne, France; [3]Institut Cochin, Paris, France; [4]CNRS, Limoges, France

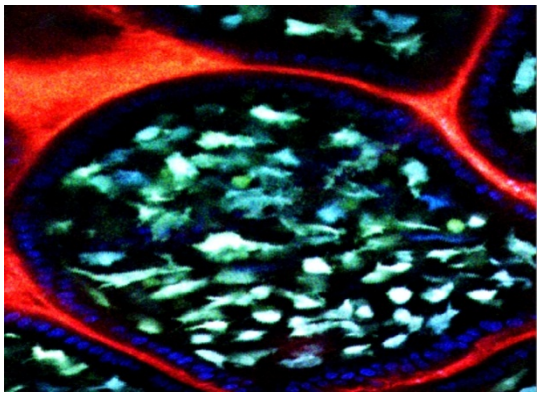
The STAT3 transcription factor pathway plays an important role in many biological phenomena. Here, we analyzed the respective and mutual roles of STAT3 in IL-10 induced terminal B-cell differentiation and in IgA production. We identified optimal conditions for inducing *in vitro* IgA production by purified blood naïve B-cells using IL-10 and soluble CD40L. Soluble CD40L consistently induced the phosphorylation of NF-kB p65 but not of STAT3, while IL-10 induced the phosphorylation of STAT3 but not of NF-kB p65. IL-10 and sCD40L thus appear synergistic in driving the terminal maturation of B-cells into IgA-producing plasma cells, which was confirmed by attempts to block either NF-κB p65 or STAT3 leading to a profound alteration of the production of mRNA for AID. Finally, the STAT3 pathway was directly activated by IL-10 but not by IL-6. This novel role for STAT3 in B-cell development reveals potential targets for eliciting post-immunization IgA at mucosal interfaces.

Dendritic Cells (2403)
Thursday, July 7, 14:30-16:00

OR.67. Small Intestine Trans-epithelial Conduits Assist Lamina Propria Dendritic Cells in Sampling Luminal Antigen

Jeremiah McDole, Leroy Wheeler, Rodney Newberry, Mark Miller. Washington University School of Medicine, St. Louis, MO

The intestinal immune system is charged with the difficult task of protecting a large environmentally exposed surface from potential pathogens, while simultaneously preventing inflammatory responses to innocuous foreign antigen from food and commensal microbiota. The presentation of foreign antigens by dendritic cells (DCs) to lymphocytes is the pivotal event guiding immune responses. Recent studies determined that the lamina propria (LP) DC population is primarily comprised of dichotomous (CD103+ tolerogenic or CX3CR1+ inflammatory) DCs. These remarkable discoveries suggest that the balance between tolerance and immunity rests upon which LP DC subtype participates in the immune responses. However a key and missing component in this model balancing tolerance and immunity is *in vivo* knowledge of how and which LP DC subtype(s) acquire luminal antigen. Using two photon microscopy on the living intestine we identify a novel mechanism delivering luminal antigens to intestinal DCs, which we termed small intestine trans-epithelial conduits (siTECs). siTECs were not present in the stomach or colon, and preferentially delivered soluble antigens. *in vivo* imaging revealed that siTECs selectively delivered luminal antigen to CD103+ DC. These findings describe a novel antigen delivery mechanism restricted to the small intestine with characteristics poised to promote intestinal

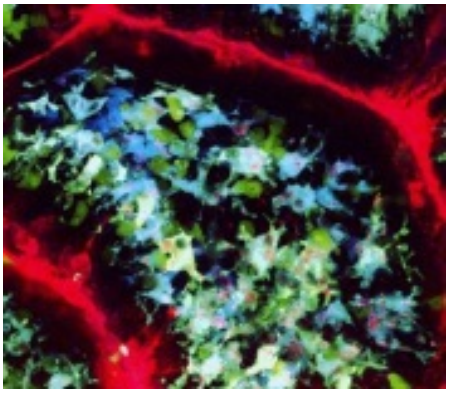


tolerance. Lamina propria dendritic cells in villi of the small intestine

OR.68. Intravital Imaging Captures Sampling of Intestinal Antigens by CD103+ DCs

Julia Farache, Steffen Jung, Guy Shakhar. Weizmann Institute of Science, Rehovot, Israel

Adaptive mucosal immunity relies on sampling of antigen from the intestinal lumen and its delivery to mesenteric lymph nodes (MLNs). Two dendritic cell (DC) populations, CX3CR1+ and CD103+ DCs participate in this process. While CX3CR1+ DCs are believed to sample intestinal content, including the flora, by extending transepithelial dendrites (TEDs) into the lumen, CD103+ DCs are the cells that migrate and initiate the adaptive immune response in the MLNs. It remains unclear how CD103+ DCs sample antigen. We used two-photon microscopy in live mice and FACS analysis to study how DCs in the ileum migrate and sample antigens. CD103+ DCs were observed to be more motile than their CX3CR1+ counterparts, and vigorously patrolled the intetine in search of antigens. Bacteria and microspheres were efficiently taken up by CD103+ DCs which phagocytosed them by either extending thick finger-like projections or migrating fully into the lumen. This sampling behavior was stimulated by LPS. Our findings focus attention on CD103+ DCs in the ileum and shows where and how they can sample antigens from the lumen before migrating to MLNs to initiate immune responses. CD11c+ dendritic cells (green and blue) in the villus capturing antigen (red) from the intestinal lumen.



OR.69. Dendritic Cells Mediate the Selective Transmission of R5 Human Immunodeficiency Virus (HIV)-1 through the Intestinal Mucosa

Mariangela Cavarelli[1], Chiara Foglieni[1], Maria Rescigno[2], Gabriella Scarlatti[1]. [1]San Raffaele Scientific Institute, Milan, Italy; [2]IFOM, Milan, Italy

HIV-1 variants using CCR5 as coreceptor (R5) are preferentially transmitted in sexual or mother-to-child infection route. Several mechanism were implied to play a role at mucosal level. We tested the hypothesis that HIV-1 can gain access into the intestinal mucosa by inducing dendritic cells (DCs) to migrate between epithelial cells and sample virus. An *in vitro* dual-chamber co-culture system of human colonic cell line Caco-2 and monocyte derived-DCs was developed to mimick the mucosa. R5 and CXCR4-using (X4) viruses were incubated on the apical side of the epithelial cell monolayer. Both R5 and X4 viruses were transcytosed across epithelial cells, captured by underlying DCs and efficiently transferred to lymphocytes to replicate. However, only R5 viruses induced DC's dendrites elongation through the Caco-2 monolayer as revealed with confocal and transmission electron microscopy. The process occurred within 30 minutes. The epithelial barrier was preserved and maintained a regular expression of tight junctions. CCL5 pre-treatment of DCs abolished the migration suggesting that it is viral envelope mediated. Our results clarify the earliest events in the establishment HIV-1 infection at mucosal level describing a mechanism of preferential transmission of R5 viruses. The central role of DCs will need further consideration in adjuvant and vaccine development.

OR.70. Cellular Retinol Binding Protein II (CRBPII), Dietary Vitamin A and Dendritic Cell-epithelia Associations Promote All Trans Retinoic Acid (ATRA) Release by Lamina Propria Dendritic Cells

Keely McDonald, Matthew Leach, Kaitlin Brooke, Leroy Wheeler, Ellen Li, Rodney Newberry. Washington University School of Medicine, St. Louis, MO

Dendritic cells (DCs) release all trans retinoic acid (ATRA) to imprint a mucosal phenotype on lymphocytes. Retinaldehyde dehydrogenase (RALDH), the enzyme catalyzing the final and irreversible step in ATRA production, was preferentially expressed by small intestine CD103+ LP DCs when compared with DCs from Peyer's patches, or other lymphoid tissues. An *in vitro* reporter assay confirmed that CD103+ LP DCs released ATRA, and this was enhanced by exposure to retinol or ATRA, and inhibited by an RALDH inhibitor. Supplemental enteral vitamin A increased RALDH expression by LP CD103+ DCs. Mice deficient in CRBPII, an enterocyte specific protein enhancing vitamin A absorption, had diminished RALDH expression by CD103+ LP DCs despite systemic vitamin A sufficiency. Bone marrow chimeras confirmed that CRBPII expression by non-bone marrow derived cells rescued RALDH expression. Furthermore CRBPII-/- mice had decreased fecal IgA levels and their CD103+ LP DCs had a decreased capacity to promote IgA class switch. CD103+ LP DCs preferentially associated with the small intestine epithelium and LP DCs from mice with impaired DC-epithelia associations had reduced RALDH expression. These findings demonstrate a role for dietary vitamin A, epithelial CRBPII expression, and epithelia-DC associations to confer ATRA releasing capacity on LP DCs.

OR.71. Defining Molecular Contributions of Colonic CX3CR1+ Macrophages in Steady State and Inflammation

Ehud Zigmond, Tegest Aychek, Diana Rashkovan, Ki-wook Kim, Simon Yona, Steffen Jung. Weizmann Institute of Science, Rehovot, Israel

The intestinal immune system is pivotal for the discrimination of harmful and innocuous challenges, responding robustly to pathogens, yet remaining tolerant to the commensal microflora. Maintenance of this critical balance has been attributed to mucosal mononuclear phagocytes residing both in organized lymphoid and throughout the subepithelial connective tissue. Studies in mice recently identified two main distinct lamina propria-resident

cell populations defined by their origin and differential functions: (1) CD103+ dendritic cells (DCs) that are derived from dedicated DC precursors and are - like classical DCs - poised to migrate to draining lymph nodes and (2) monocyte-derived CX3CR1+ macrophages that are non-migratory and thus likely fulfill local functions in lamina propria [Varol, Zigmond & Jung, Nat Immunol 2010]. Here we will report on our progress in the study of specific contributions of CX3CR1+ macrophages to the maintenance of gut homeostasis and its breakdown following irritant and pathogen challenge. Specifically, we employ a recently established combination of cell ablation and monocyte transfer to seed the intestinal tract of intact animals with manipulated CX3CR1+ macrophages (Varol et al. Immunity 2009). Moreover, we will present results obtained from newly generated mice that express a conditionally or constitutive active Cre recombinase under the CX3CR1 promoter.

OR.72. MyD88-dependent Signals Synergize with Retinoic Acid to Educate Gut-associated Dendritic Cells

Eduardo Villablanca[1], Sen Wang[1], Jaime De Calisto[1], Joseph Napoli[2], William Blaner[3], Ulrich von Andrian[4], J. Rodrigo Mora[1]. [1]Massachusetts General Hospital, Boston, MA; [2]University of California, Berkeley, CA; [3]Columbia University, New York, NY; [4]Harvard Medical School, Boston, MA

Gut-associated dendritic cells (GALT-DC) can metabolize dietary vitamin-A into all-trans retinoic acid (RA), which is required for inducing gut-tropic lymphocytes and Foxp3 Treg induction. How GALT-DCs are programmed to synthesize and secrete RA is a key unresolved question. We hypothesized that RA “educates” DC to induce its own synthesis and imprint gut-homing lymphocytes. Here we show that GALT-DC isolated from vitamin A-depleted mice exhibited an impaired RA synthesizing capacity *in vivo*. Moreover, spleen-DC pre-treated with RA (RA-DC), but not untreated spleen-DC, expressed high levels of retinal dehydrogenases (raldh 1 and 2) and induced high levels of CCR9 and α4β7 on T cells. RA-mediated DC education was dependent of the RAR and RXR nuclear receptor and also required ERK1/2. Since ERK1/2 is implicated in TLR signaling, we hypothesized that the TLR-adaptor MyD88 is also necessary for RA-mediated DC education. Indeed, we demonstrated that MyD88 plays a critical role in RA-mediated DC education *in vitro* and *in vivo*. Moreover, RA- and TLR-mediated signals synergized and were sufficient to educate extra-intestinal murine and human DC with gut-homing imprinting capacity. Thus, our data highlight a hitherto unanticipated crosstalk between RA- and MyD88-dependent signals, which synergize to confer DC with gut-homing imprinting capacity.

Innate Immunity II (2500)
Thursday, July 7, 16:15-17:45

OR.73. Epithelial MicroRNAs Regulate Gut Mucosal Immunity via Epithelium-T Cell Crosstalk

Moshe Biton. Hebrew University, Jerusalem, Israel

Colonic host-flora homeostasis requires epithelium-lymphocyte cooperation, yet many players of this process are mostly unknown. Here we report that epithelial microRNAs mediate a mucosal-immune crosstalk necessary for mounting protective Th2 responses. Abolishing microRNA induction by Dicer1 deletion in the gut epithelium predisposes the organism to parasite infection and Th1-biased inflammation with hallmarks of inflammatory bowel disease. We found that one specific microRNA, miR-375, is induced by the Th2 factor IL-13 via activation of the PI3K signaling pathway. miR-375 regulates the expression of the epithelial cytokine TSLP that facilitates Th2 responses. This microRNA-regulated process constitutes a positive feedback loop, amplifying the Th2 anti-parasite response. Finally, miR-375-deficient mice have reduced intestinal levels of goblet-derived RELMβ, a Th2-regulated key anti-parasite cytokine. These findings implicate epithelial microRNAs as key regulators of gut homeostasis and mucosal immunity.

OR.74. Microbiota Regulates Host Innate Response via Inhibition of MiR-10a which Targets CD40 and IL-12/IL-23p40

Xiaochang Xue, Ting Feng, Suxia Yao, Yingzi Cong. University of Texas Medical Branch, Galveston, TX

Commensal flora plays important roles in the regulation of intestinal mucosal immune system, and in the pathogenesis of inflammatory bowel diseases (IBD). MicroRNAs appear to play important roles in both innate and adaptive immunity. However, how microbiota regulates microRNA expression, thus contributes to pathogenesis of IBD is still largely unknown. We found that microbiota negatively regulated intestinal miR-10a expression, as intestine of SPF mice expressed much lower level of miR-10a compared to that in germ-free mice. Commensal bacteria downregulated DC miR-10a expression via TLR-TLR ligand interactions through a Myd88-dependent, but NFκB-independent pathway. We identified CD40 and IL-12/IL-23p40, key molecules for innate immune responses to commensal bacteria and in the pathogenesis of IBD, as targets of miR-10a. Ectopic expression of miR-10a precursor inhibited, whereas miR-10a inhibitor promoted expression of CD40 and IL-12/IL-23p40. In patients with Crohn’s disease and in colitic IL-10 deficient mice which have high level of gut bacterial translocation and express high level of IL-12/IL-23p40 and CD40, intestinal miR-10a expression is much lower than that of control normal mice. Collectively, our data demonstrated that microbiota regulates host innate response and colitis development through inhibiting miR-10a expression as miR-10a negatively regulates expression of IL-12/IL-23p40 and CD40 as its target genes.

OR.75. Intestinal Gr-1highCD11b+CD11c+ Cells Prevent T Cell-dependent Colitis

Hisako Kayama, Yoshiyasu Ueda, Kiyoshi Takeda. Osaka University, Suita, Japan

Adequate activation of CD4+ T lymphocytes is essential for host defense against invading pathogens; however, exaggerated activity of effector CD4+ T cells induces tissue damage leading to inflammatory disorders such as inflammatory bowel diseases. Recently, several unique subsets of

intestinal innate immune cells have been identified. However, the direct involvement of innate immune cell subsets in the suppression of T cell-dependent intestinal inflammation is poorly understood. Here we report the identification of a unique intestinal Gr-1high CD11b+ CD11c+ cell subset, which we have named regulatory myeloid (Mreg) cells, that is responsible for prevention of intestinal inflammation through inhibition of T cell responses. These cells inhibit CD4+ T cell proliferation in a cell contact-dependent manner, and prevent T cell-dependent colitis. The suppressive activity of Mreg cells is abrogated in the absence of the IL-10/Stat3 pathway. Mreg cells inhibit T cell proliferation by two steps. Initially, Mreg cells preferentially interact with T cells through highly expressed adhesion molecules, then, they fail to activate T cells because of defective expression of co-stimulatory molecules including CD80 and CD86. Thus, Mreg cells, which show preferential interaction with T cells and IL-10/Stat3-dependent downregulation of co-stimulatory signals, are a novel innate immune cell subset maintaining intestinal homeostasis.

OR.76. Development and Activation of Human Intestinal Innate Lymphoid Cells

Patricia Aparicio, Ferry Cornelissen, Natalie Papazian, Tom Cupedo. Erasamus Medical Center Rotterdam, Rotterdam, Netherlands

Human RORC-expressing innate lymphoid cells (RORC+ ILC) are found in mucosal tissues and are essential for mucosal homeostasis and early immunity. An important ILC-derived effector cytokine is IL-22, which activates intestinal epithelial cells and induces production of antimicrobial proteins. We analyzed the requirements for development and activation of human RORC+ ILC in human intestines. In fetal human small intestines that have not yet been colonized by microbiota, RORC+ ILC appear during the end of the first trimester, indicating that intestinal ILC are developmentally programmed. However, while these cells are phenotypically indistinguishable from adult ileum-derived RORC+ ILC, they lack IL22 transcripts. This suggests that intestinal colonization might be required for ILC activation. To address this issue, ILC were co-cultured with Caco-2 intestinal epithelial cells that were prestimulated with TLR ligands to mimic bacterial sensing. After TLR-stimulation, epithelial cells were able to activate RORC+ ILC and induce production of IL-22, both through soluble mediators and direct cell contact. In conclusion, human intestinal RORC+ ILC are developmentally programmed and locate to the fetal intestinal mucosa as resting cells. Upon bacterial colonization, epithelial cells can activate ILC to produce cytokines such as IL-22 in order to maintain mucosal homeostasis under microbial pressure.

OR.77. NKT Cells Can Sense Oxidative Stress: Implication in COPD Pathogenesis

Muriel Pichavant[1], Sandrine Bekaert[2], Gaelle Remy[1], Dale Umetsu[3], François Trottein[1], Isabelle Tillie-Leblond[1], Didier Cataldo[2], Philippe Gosset[1]. [1]INSERM, Lille, France; [2]Université de Liège, Liège, Belgium; [3]Children's Hospital Boston, Boston, MA

COPD, or chronic obstructive pulmonary disease, is a major cause of disability induced by cigarette smoke (CS), and characterized by low airflow. We hypothetised that CS-induced changes in lung functions via natural killer T (NKT) cell activation, like in our previously described ozone-model. *in vivo*, NKT cell recruitment, activation and role were evaluated in wild-type (WT) mice chronically exposed to CS, compared to NKT cell-deficient (Jalpha18(-/-)) mice. *In vitro*, mouse and human NKT cell activation was evaluated in response to airway epithelial cells (AEC) and dendritic cells (DC) exposed to CS extracts. First, we validated the COPD model, by demonstrating inflammation in the lungs of CS-exposed WT mice, as well as an increase of lung resistance and lung remodeling. This phenotype was associated with an increased number in activated NKT cells, producing more IL-17. Jalpha18(-/-) mice showed no changes in lung function and reduced inflammation. *In vitro*, CS-treated AEC and DC induce NKT cell activation, in a CD1d-dependant manner. Activation of NKT cells by CS-treated AEC and DC involves oxidative stress since pre-treatment with anti-oxidant (N-acetylcysteine) abolishes NKT cell activation. Our data demonstrate that NKT cells can sense oxidative stress and could represent a unique target for effective COPD therapy.

OR.78. TSLP and IL-33 Promote the Differentiation of Multi-potent Progenitor Type 2 Cells into a Nuocyte-like Cell Population

Steven Saenz, Mark Siracusa, David Artis. University of Pennsylvania, Philadelphia, PA

$CD4^+$ T helper (Th) 2 cytokine responses are required for immunity to helminth infections and promote chronic inflammation associated with allergic diseases. Recent studies demonstrated that the epithelial-derived cytokines IL-25 (IL-17E), IL-33 and/or TSLP promote Th2 cell-dependent immunity and inflammation at barrier surfaces through the induction of MPP^{type2} cells and other innate lymphoid cells including natural helper cells (NHCs), nuocytes, or innate helper 2 (Ih2) cells. While these four cell populations were described as being lineage negative (Lin^-) and c-kit^+, only MPP^{type2} cells exhibited multi-potent potential suggesting that they may have the potential to differentiate into innate lymphoid cell lineages. Here we show that IL-25-elicited MPP^{type2} cells (c-kit^+, $T1/ST2^{neg}$) express the receptor for TSLP (TSLPR) and that stimulation with TSLP can induce the expression of the IL-33R (T1/ST2). Further, activation of IL-25-elicited MPP^{type2} cells with TSLP and IL-33 yielded a nuocyte-like cell population. The ability of MPP^{type2} cells to respond to TSLP and IL-33 and differentiate into a nuocyte-like cell population indicates that MPP^{type2} cells may represent a progenitor cell for nuocytes and other innate lymphoid cells. These findings indicate that coordinate interactions between epithelial cell-derived cytokines can promote Th2 cytokine responses at barrier surfaces through eliciting extramedullary hematopoiesis of innate lymphoid cell lineages.

IBD and Mouse Models II: T Cell Subsets (2501)
Thursday, July 7, 16:15-17:45

OR.79. The S1P Receptor Agonist FTY720 Prevents Onset of Colitis but Fails to Control Established Disease in a Murine T Cell Transfer Model

Matthias Hesse, Donald Hodges, Anna Lindquist, James Ooi, Raymond Winquist, Matthew Harding. Vertex Pharmaceuticals Inc., Cambridge, MA

Unregulated activation of T lymphocytes by commensal gut antigens leads to development of inflammatory bowel disease (IBD). Preventing migration or activation / proliferation of colitogenic T cells are potential therapeutic strategies for IBD. Sphingosine 1-phosphate (S1P) receptors are essential for lymphocyte trafficking through secondary lymphoid organs. The S1P receptor agonist FTY720 sequesters lymphocytes by preventing migration from lymphoid tissues and reduces inflammation in several animal disease models. We evaluated FTY720 in a CD4+CD25- T cell transfer colitis model. Prophylactic administration (1 and 10 mg/kg FTY720 starting Day-2 after transfer) significantly diminished T cell engraftment. Animals did not develop disease symptoms and there was no gross colon pathology or T cell proliferation in the intestinal mucosa at Day-28. In contrast, therapeutic administration (1 and 10 mg/kg starting on Day-17) had no effect. Treated mice experienced clinical disease, colon pathology, and significant activation and proliferation of T cells in the intestinal mucosa. However, FTY720 significantly reduced the proportion of T cells in the peripheral compartment. These results suggest that blocking initial trafficking of colitogenic cells to the gut can prevent mucosal inflammatory disease. However, once colitogenic T cells are present in the mucosa, a therapeutic strategy targeting activation and proliferation is necessary for optimal disease modification.

OR.80. Natural Killer Cells Suppress a Murine Model of Colitis by Targeting the Early Stage of T Cell Development

Osamu Yamaji[1], Teruji Totsuka[1], Takashi Nagaishi[1], Michio Onizawa[1], Masahiro Suzuki[1], Naoto Tsuge[1], Takanori Kanai[2], Mamoru Watanabe[1]. [1]Tokyo Medical and Dental University, Tokyo, Japan; [2]Keio University, Tokyo, Japan

Background: We previously reported that IL-7$^{-/-}$RAG$^{-/-}$ mice receiving naïve T cells failed to induce colitis. Such abrogation of colitis may be associated not only with the lack of IL-7, but also with the induction of T cell apoptosis at an early stage of colitis development. Natural killer (NK) cells may be associated with the suppression of pathogenic T cells, and may induce apoptosis of CD4$^+$ T cells. Methods and Results: To further investigate these roles of NK cells, RAG$^{-/-}$ and IL-7$^{-/-}$RAG$^{-/-}$ mice that had received naïve T cells were depleted of NK cells using anti-asialo GM1 antibody. NK cell depletion at an early stage during colitogenic effecter/memory T cell (T_{EM}) development resulted in exacerbated colitis in recipient mice even in the absence of IL-7. Increased CD44$^+$CD62L$^-$ T_{EM} and unique CD44$^-$CD62L$^-$ T cell subsets were observed in the T cell-reconstituted RAG$^{-/-}$ recipients when NK cells were depleted, although Fas, DR5 and IL-7R expressions in the CD44$^-$CD62L$^-$ subset differed from that in T_{EM} subset. Conclusions: These results suggest that NK cells suppress colitis severity in the T cell-reconstituted recipient mice through targeting of colitogenic CD4$^+$CD44$^+$CD62L$^-$ T_{EM} cells and, possibly, of the CD4$^+$CD44$^-$CD62L$^-$ subset present at the early stage of T cell development.

OR.81. CD69 Mediates Type I Interferon-induced Tolerogenic Signals to Mucosal CD4 T Cells that Attenuates their Colitogenic Potential

Katarina Radulovic, Calin Manta, Valerio Rossini, Jan Niess. Ulm University, Ulm, Germany

Background: Here, we report that the early activation antigen CD69 is critical for the development of oral tolerance and regulation of intestinal inflammation. Methods: The expression of CD69 by CD4 T cells isolated from the small intestinal (siLP) and colonic lamina propria (cLP) was determined in specific pathogen free (SPF), germ-free (GF) B6 mice and T cell receptor (TCR) transgenic animals. Results: In GF mice the absence of the intestinal microflora is associated with reduced CD69 expression. The oral challenge of OT-II/RAG$^{-/-}$ animals with OVA induced CD69 expression by 47% of the CD4 T cells. CD69-positive but not CD69-negative CD4 T cells in oral antigen-challenged OT-II/RAG$^{-/-}$ expressed LAP/TGF-β but not IFNγR, CD122, TGFβRII, IL-21R1 or T-bet, GATA3, RORγt and Foxp3 indicating a regulatory phenotype. CD69 activation induced TGF-β expression by CD4 T cells. Microarray analyzes of CD4 T cells showed 323 differentially expressed genes in CD69$^{-/-}$ animals. CD4 T cells from CD69$^{-/-}$ animals are characterized by increased IFNγ and TNFα production. Oral tolerance is impaired in CD69$^{-/-}$ and IFN-I receptor 1-deficient (IFNAR$^{-/-}$) B6 mice as compared to B6 and OT-II/RAG$^{-/-}$ animals. Transfer of CD69$^{-/-}$ CD45RB(high) CD4 T cells in RAG$^{-/-}$ hosts induced an accelerated colitis as compared to hosts transplanted with B6 CD45RB(high) CD4 T cells. The treatment of B6 and OT-II/RAG$^{-/-}$ animals with the strong type I IFN inducer poly(I:C) induced CD69 expression but not in IFNAR$^{-/-}$ animals. The treatment of RAG$^{-/-}$ hosts transplanted with B6 CD4 T cells with poly(I:C) attenuated transfer colitis but not in hosts transplanted with CD69$^{-/-}$ or IFNAR$^{-/-}$ CD4 T cells. Conclusion: The activation antigen CD69 may help control the potential harmful impact of the intestinal microflora to the host.

OR.82. Small Molecule Tyrosine Kinase Inhibitors for the Treatment of Intestinal Inflammation

Maninder Sidhu[1], Carmen Alonso[1], Bayasi Guleng[1], Sun-Young Chang[1], MyDoanh Chau[2], Alfred Ajami[2], Hans-Christian Reinecker[1]. [1]Massachusetts General Hospital and Harvard Medical School, Boston, MA; [2]Antisoma, Inc., Cambridge, MA

Background: We developed a series of dendritic cell autoimmune modifiers (DCAMs) based on small molecule Flt3 receptor tyrosine kinase inhibitors for the inhibition of intestinal inflammation and oral delivery. Methods: Two or three oral doses of DCAMs were administered simultaneously or after induction of DSS colitis in 4-6 wks old female C57BL/6 mice. The severity was assessed histologically. Post treatment

cytokine gene expression analysis was performed by qPCR from the MLNs. Bone marrow derived macrophages (BMM) were utilized to define the mechanisms by which DCAMs can modify responses to microbial signals. Results: Treatment with two new compounds resulted in reduced mucosal inflammation and significantly shortened recovery from severe DSS colitis. Reduction of mucosal inflammation by the effective DCAMs was associated by significantly higher IL-10 expression and reduced TNF-α expression. Pro-inflammatory cytokine expression of IL-1β, IL-6 and TNF-α by BMM in response to TLR4 and TLR9 activation was significantly reduced in the presence of DCAMs. Surprisingly, DCAMs were able to directly induce IL-10 expression in macrophages and significantly enhanced IL-10 expression induced by LPS. Conclusions: We have identified promising candidates for further development into potent orally available drugs for the prevention of colitis and promotion of mucosal recovery in IBD.

OR.83. TL1A Drives Innate and Adaptive Immune Responses During the Development of Chronic Colitis

Kathrin Michelsen[1], Michelle Wong[1], Brian Ko[1], Lisa Thomas[1], Deepti Dhall[1], Timothy Zheng[2], Linda Burkly[2], Stephan Targan[1]. [1]Cedars-Sinai Medical Center, Los Angeles, CA; [2]Biogen Idec, Cambridge, MA

TL1A, a TNF superfamily member, mediates a strong co-stimulation of TH1 and TH17 responses. Expression of TL1A and its receptor DR3 is increased in inflamed mucosa of Crohn's Disease patients and in murine models of ileitis. We have shown that neutralizing TL1A antibodies attenuate clinical signs of chronic DSS-colitis by attenuating TH1 and TH17 responses. However, it remains to be elucidated if TL1A is essential for the development of chronic colitis. TL1A-/- mice developed less severe acute (induced by innate immune cells) and chronic (T cell mediated) DSS-colitis suggesting that TL1A plays an important role during innate and adaptive immune responses. We observed reduced inflammation and reduced IL-17 and IFN-γ production in lamina propria mononuclear cells (LPMC) in TL1A-/- mice. To confirm our data we used a second, T cell driven model of chronic colitis. Transfer of WT CD4+CD45RBhigh T cells into Rag1-/- mice resulted in severe colitis within 5-7 weeks. Cohorts receiving TL1A-/- CD4+CD45RBhigh T cells did not develop colitis. Restimulation of LPMC or MLN from mice receiving TL1A-/- CD4+CD45RBhigh T cells produced significantly less IL-17 and IFN-γ. These data demonstrate that TL1A plays a crucial role in the development of chronic colitis by affecting TH1 and TH17 responses.

OR.84. Sphingosine-1-phosphate Receptors Mediate T. Gondii-induced Ileitis and Parasite Trafficking to the CNS

Daniel Mielcarz[1], David Foureau[1], Javier Ochoa-Repáraz[1], Dominique Buzoni-Gatel[2], Lloyd Kasper[1]. [1]Dartmouth Medical School, Lebanon, NH; [2]Institut National de la Recherche Agronomique, Nouzilly, France

We investigated lymphocyte and dendritic cell trafficking to the intestine and CNS in an experimental model of pathogen-driven inflammatory bowel disease (IBD). Inbred B6 mice develop an acute necrotizing ileitis 7-9 days following oral infection with Toxoplasma gondii associated with massive inflammatory infiltrates and morphologic tissue changes. Treatment with an analogue inhibitor of sphingosine-1-phosphate (S1P) reduced clinical and inflammatory changes in the small intestine, as well as reducing weight loss following infection. Untreated mice succumbed to infection by day 10, while all treated mice survived the acute phase of infection. Reduced Th1 cytokine expression as well as an increased parasite burden was observed in the small intestine following S1P blockade. Enhanced trafficking of CD103+ DCs and accumulation of FoxP3+ Treg in the MLN was observed, with reduced numbers of CD4+ lymphocytes in both the small intestinal lamina propria and the CNS. The brain parasite burden and inflammation of treated mice was reduced as well. Taken together, these results indicate that S1P is required for both lymphocyte trafficking to the intestine and parasite trafficking to the CNS following oral infection by T. gondii.

Vaccines I (2502)
Thursday, July 7, 16:15-17:45

OR.85. Intranasal Rotavirus Peptide Vaccine Requires CD40 for an Efficient Induction of Protective Th1 Cells

Fernando Esquivel[1], Lourdes Gutierrex-Xicotencatl[2], Rogelio Hernandez[3], Eduardo Garcia[4], Ernesto Esquivel[1]. [1]UAEM, Cuernavaca, Mexico; [2]CISEI, INSP, SSA, Cuernavaca, Mexico; [3]INNSZ, SSA, Mexico, DF, Mexico; [4]IIB, UNAM, Mexico, DF, Mexico

Previous work has shown that the synthetic peptide corresponding to the sequence 289-302 of the rotavirus protein VP6, which is an IE^d-restricted Th cell epitope, can induce a Th cell-dependent protection against a rotavirus infection when intranasally (i.n.) administered. However, the precise mechanism of protection still remains unclear. $CD40^{-/-}$, $IL\text{-}4^{-/-}$ and $CCR9^{-/-}$ mice were immunized i.n. 3 times with the peptide and cholera toxin and orally challenged with a murine rotavirus. As controls, heterozygots mice were used. It was found that only $CD40^{-/-}$ mice showed a protection reduction of about 70 % compared to the control mice. The fact that the protection partially depended on CD40 and was independent on IL-4, suggested that the protection was mediated by Th1 cells. In this way, when mesenteric lymph node cells from normal immunized mice were stimulated *in vitro* with the peptide, the Th cells showed a Th1 phenotype. Furthermore, when the peptide was inoculated in the food pad a strong DTH reaction was induced. These results indicate that after i.n. immunization with the peptide, unlike IL-4 and CCR9, CD40 is essential for an efficient induction of protective intestinal Th cells that at least partially may present a Th1 phenotype.

OR.86. Unraveling Molecular Signatures of Immunostimulatory Adjuvants in the Female Genital Tract

Madelene Lindqvist[1], Intawat Nookaew[2], Jens Nielsen[2], Ali Harandi[1]. [1]University of Gothenburg, Gothenburg, Sweden; [2]Chalmers University of Technology, Gothenburg, Sweden

Sexually transmitted infections (STIs) unequivocally present a major public health concern in both industrialized and developing countries. Previous efforts in development of vaccines designed for systemic immunization to counter a large number of STIs in humans have been unsuccessful. There is currently a drive to develop mucosal vaccines and adjuvants for delivery through the genital tract to confer protective immunity to STIs. Identification of molecular signatures that can be used as biomarkers of adjuvant potency and toxicity can inform rational development of potent and safe mucosal adjuvants. We have previously shown that CpG ODN (Tengvall S et al, Journal of virology 2006) and the invariant natural killer T cell agonist alpha-galactosylceramide (Lindqvist M et al, Journal of Immunology, 2009) could function as equally potent vaginal adjuvants. Here, we studied global gene expression and signature molecules/pathways in the mouse vagina in response to these 2 classes of experimental adjuvants through systems biology combined with immunological read outs. Our integrated analysis of genome-wide transcriptome data unraveled signature pathways, processes and networks shared by or otherwise exclusive of the experimental vaginal adjuvants in the mouse vagina. These results could inform rational development of effective mucosal adjuvants for vaccination against STIs.

OR.87. Co-delivery of Mucosal Chemokine Plasmids in a Systemically Administered DNA Vaccine Elicits Systemic and Mucosal Immune Responses in Rhesus Macaques

David Weiner[1], Michele Kutzler[2], Kimberly Kraynyak[1], Albert Sylvester[2], Arielle GInsberg[1], Diane Carnathan[2], Noshin Kathuria[2], Amir Khan[3], Bapi Pahar[4], Zina Moldoveanu[5], Jiri Mestecky[5], MIchael Betts[1], Preston Marx[4], David Weiner[1]. [1]University of Pennsylvania, Philadelphia, PA; [2]Drexel University College of Medicine, Philadelphia, PA; [3]Inovio Biomedical, Woodlands, TX; [4]Tulane National Primate Research Center, Covington, LA; [5]University of Alabama at Birmingham, Birmingham, AL

Using a rhesus macaque model, we created optimized rhesus CCL27, CCL25, and CCL28 plasmid adjuvants, and co-immunized with optimized/consensus macaque pol and sooty mangabey consensus gag/env antigens. Macaques (n=5) were immunized intramuscularly with antigenic plasmids plus/minus each chemokine. In the periphery, we observed significant IFN-gamma in all groups (~6,000 SFU each), while intracellular cytokine staining showed a trend toward more functional CD8+T cells in mucosa of the CCL27 co-immunized macaques. Statistically significant antigen-specific IgA in sera, genital and duodenal washes of chemokine-vaccinated macaques was also observed. A repeated low dose intravaginal challenge with SmmE660 was carried out, and vaccine-specific immune responses were characterized to determine the functionality and phenotype of vaccine-induced T and B cell immunity at the mucosa and in systemic compartments. The results of this study will be critical to the development of an effective vaccine against HIV. This is the first example of the use of mucosal chemokines to influence a DNA vaccine strategy, suggesting a novel approach for manipulation of vaccine-induced immune responses. This work is supported by funding through the NIH/NIAIDS.

OR.88. Evaluation of Beta-glucan Particles as Mucosal Delivery System in the Peyer's Patch Regions of the Murine Small Intestine

Rebecca De Smet, Tine Demoor, Stephanie Verschuere, Marijke Dierendonck, Bruno De Geest, Claude Cuvelier. Ghent University, Ghent, Belgium

Oral vaccination is essential to generate protective local immunity against intestinal pathogens. However, antigen delivery to the inductive sites for mucosal immunity (the small intestine Peyer's patches, PP) has proven to be particularly challenging. We evaluated the potential of β-glucan microparticles to deliver antigen transmucosally in the PP regions of the murine small intestine. Beta-glucan microparticles (3-5 µm) were prepared from Saccharomyces cerevisiae and loaded with Alexa Fluor 488-conjugated bovine serum albumin. Particles were administered to male C57BL/6 mice (8-10 weeks old) via intestinal loops at a particle concentration of 100×10^6/ml. After one hour of incubation, transmucosal particle transport and uptake in the PP was evaluated by flow cytometry, confocal microscopy and transmission electron microscopy. Using flow cytometry, we could not observe any particle uptake in the main antigen presenting cell population, the dendritic cells. Interestingly, flow-cytometric analysis indicated a modest but clear uptake of particles in the B-cell population. Confocal microscopic images showed yeast particles localized in the Follicle Associated Epithelium. Moreover, transmission electron microscopy demonstrated transcellular transport of yeast particles in M-cells. Our data suggest that M-cells, but not subepithelial dendritic cells, are crucial for the transmucosal transport of β-glucan particles from the intestinal lumen to the PP.

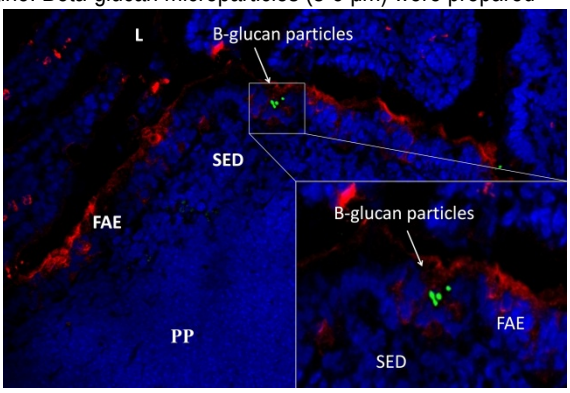


Figure 1. Confocal image of β-glucan particle uptake in the Follicle Associated Epithelium. PP = Peyer's patch, SED = Subepithelial Dome, FAE = Follicle Associated Epithelium, L = Lumen

OR.89. Potent IgA Elicited Against the Highly Conserved gp41 Epitope, QARVLAVERY, Protects Against Direct HIV-1 Infection of TZM-bl Cells and Prevents HIV-1 Transcytosis *in vitro*

Sumiti Jain, Kenneth Rosenthal. McMaster University, Hamilton, ON, Canada

Since mucosal surfaces are the predominant site of HIV-1 entry and transmission, the induction of site-specific protective immunity will be critical for the success of future prophylactic therapies. We have developed a mucosa-targeting vaccination model that use heterologous vectors expressing chimeric Gag VLPs. Notably, a highly conserved gp41 epitope QARVLAVERY, which was inserted within the VLP construct, elicited potent epitope-specific IgA in the serum, vaginal washes (VW) and fecal pellets (FP) of immunized mice. This epitope exhibited unique properties in our model which have important implications for mucosal vaccination: 1) It induced significantly greater epitope-specific IgA compared to ELDKWA in comparable immunization models, in both C57Bl/6 and Balb/c mice, and in different immunization schemes; 2) It elicited an unusual ~1:1 ratio of epitope-specific IgG:IgA in the serum; 3) QARVLAVERY-specific IgA was detected within days, even prior to IgG in the serum; 4) Preliminary evidence implicates a role of innate B-cells in early IgA induction in response to QARVLAVERY; 5) QARVLAVERY-specific Abs were protective against lab strains and acute infectious molecular clones in direct neutralization assays, as well as transcytosis assays. Collectively, our results highlight this highly conserved epitope, which makes an attractive epitope to be included in mucosal vaccine models.

OR.90. Airway Epithelial Cells Regulate TLR5-mediated Mucosal Adaptive Responses

Laurye Van Maele[1], Laure Janot[2], Delphine Fougeron[1], Arnaud Didierlaurent[4], Bertrand Dubois[5], Martin Rumbo[6], Simon Jeffs[7], Delphine Cayet[1], Staelle Chamaillard[1], Yves Lemoine[1], Selma Boulenouar[1], Tracy Hussell[3], François Erard[2], David Hot[1], Bernard Ryffel[2], Arndt Benecke[8], Jean-Claude Sirard[1]. [1]CIIL, Lille, France; [2]Institut de Transgenose, Orléans, France; [3]National Heart and Lung Institute, London, United Kingdom; [4]Glaxosmithkline, Rixensart, Belgium; [5]Université Lyon, Lyon, France; [6]Facultad de Ciencias Exactas, La Plata, Argentina; [7]Imperial College of London, London, United Kingdom; [8]Institut des Hautes Etudes Scientifiques, Bures-sur-Yvette, France

Adaptive immunity depends on activation of antigen-presenting cells (APC) like dendritic cells. Toll-like receptors (TLR) that detect microbial signals can be instrumental in the direct APC stimulation and their agonists are then considered as adjuvants. Here we addressed whether TLR stimulation of non-hematopoietic cells is required to initiate mucosal adaptive immunity. Upon nasal administration of flagellin, lung radioresistant cells were sufficient for promoting the TLR5-mediated T cell- and antibody-mediated immunity. The lung signature in response to flagellin was limited to the broncho-alveolar epithelial compartment and was associated to the production of a restricted set of mediators including the chemokine CCL20, known to promote APC recruitment in mucosal tissues. Our results suggest that TLR-mediated signaling in epithelium is sufficient to transmit activating signals to APC and initiate adaptive immunity. In conclusion, the adaptive immune-enhancing effect of microbial signals on epithelial cells can be harnessed for improving vaccines.

Celiac Disease (2503)
Thursday, July 7, 16:15-17:45

OR.91. IL-21 Promotes IL-15 Mediated Activation of Intraepithelial Lymphocyte in Celiac Disease (CD) and Type II Refractory CD

Raja El Machhour, Nicolas Montcuquet, Georgia Malamut, Christophe Cellier, Nadine Cerf-Bensussan, Bertrand Meresse. Université Paris Descartes, Paris, France

Recent studies point to a possible participation of Interleukin (IL)-21 produced by gluten-reactive T cells in Celiac disease (CD). Furthermore, genome-wide association studies for CD have identified risk variants in the region harboring IL21. Yet, the role of IL-21 in the pathogenesis of CD remains to determine. Several studies have shown that IL-21 can act in synergy with IL-15, to regulate NK and CD8+T cell expansion and function. Interestingly, IL15 is overproduced by enterocytes and macrophages in the intestine of celiac patients and in patients with type II refractory CD (RCDII), the malignant complication associated with CD. IL-15 inhibits apoptosis of intraepithelial T lymphocytes (IEL) in both CD and RCDII and promotes their cytotoxicity against the intestinal epithelium. Here, we show that IL-21 combined with IL-15 promotes survival and proliferation of transformed IEL lines from RCDII. Furthermore, we demonstrated that IL-21 acts on the human monocytic cell line THP-1 and the intestinal epithelial cell line T84 to promote IL-15 transpresentation that induces survival and activation of IEL in both CD and RCDII. Altogether, our data indicate that IL-21 potentiates the effect of IL-15 and thereby could provide the missing genetic link with IL-15 in CD.

OR.92. Rod-shaped Bacteria from the Small Intestinal Microbiota of Children with Celiac Disease Affect T-lymphocyte and Epithelial Cell Function

Marie-Louise Hammarström, Maria Hedberg, Veronika Sjöberg, Grzegorz Pietz, Olof Sandström, Olle Hernell, Sten Hammarström. Umea University, Umea, Sweden

Between 1985 and 1996 the incidence of childhood CD in Sweden was 4 times higher than either before or after. The jejunal microbiota of CD-children born during the “epidemics” had a different composition from that of CD-children born after the "epidemics" and controls with enrichment of rod-shaped Clostridiales, Prevotella, and Actinomyces bacteria. Do these bacteria contribute to the etiology or pathogenesis of CD? Isolated bacteria were characterized. Genome wide microarray and real-time qRT-PCR analysis of jejunal T- and epithelial cells of CD patients was performed. Seven

rod-shaped bacterial species isolated from CD biopsies were studied. Two were unknown. One, tentatively named Anaerobacterium umeaense, represented a new species in a new genus, induced TNF-alpha in tight monolayer cells. Five Prevotella species caused increased permeability in tight epithelial monolayers. In active CD there was a marked production of interleukin-17A (IL-17A) by intraepithelial lymphocytes while IL-17A was barely detected in CD patients on a gluten-free diet. *Ex vivo* challenge with a mixture of the CD associated bacteria plus gluten peptides caused strong IL-17A upregulation in biopsies from treated CD patients. The CD associated bacteria affect the function of the intestinal T cells and epithelial cells possibly constituting a risk factor for CD.

OR.93. Tolerance to Ingested Deamidated Gliadin in Mice is Maintained by Splenic IL-10-producing T Cells

Anne Kozijn[1], M. du Pré[1], Mariette ter Borg[1], Lisette van Berkel[1], Dicky Lindenbergh-Kortleve[1], Lise Torp Jensen[2], Yvonne Kooy-Winkelaar[3], Frits Koning[3], Louis Boon[4], Edward Nieuwenhuis[5], Ludvig Sollid[6], Lars Fugger[7], Janneke Samsom[1]. [1]Erasmus Medical Center, Rotterdam, Netherlands; [2]Aarhus University Hospital, Aarhus, Denmark; [3]Leiden University Medical Center, Leiden, Netherlands; [4]Bioceros B.V., Utrecht, Netherlands; [5]Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, Netherlands; [6]University of Oslo and Oslo University Hospital, Rikshospitalet, Oslo, Norway; [7]Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford, Oxford, United Kingdom

Permanent intolerance to gluten or celiac disease occurs in approximately 1:100 individuals. This high frequency raises the question whether oral tolerance to gluten differs from that to other food proteins. Using mice transgenically expressing HLA-DQ2 and a gliadin-specific humanized T cell receptor, we compared gluten-specific T cell responses with the tolerogenic mucosal T cell responses to the model food protein OVA. Consistent with earlier data, the OVA-specific response occurred in the mesenteric lymph nodes (MLN) and induced Foxp3+ Treg cells. In contrast, deamidated gliadin feed induced dominant T cell division in the spleen and very little in MLN. The gliadin-reactive T cells had an effector-like phenotype and secreted large amounts of interferon-γ (IFN-γ) but also interleukin-10 (IL-10). Despite their effector-like phenotype, gliadin-reactive T cells exhibited regulatory capacity as transfer of the cells suppressed a gliadin-induced delayed type hypersensitivity response. This effect was IL-10-mediated as IL-10 neutralization enhanced IFN-γ production *in vitro*. These data infer that the default T cell response to deamidated gliadin feed is tolerance, which is not conditioned by the mucosal immune system but instead relies on differentiation of splenic IL-10-producing T cells. Current experiments are directed at identifying triggers that abrogate splenic IL-10-producing T cell differentiation and induce loss of tolerance to gliadin.

OR.94. IL-15 Impairs Regulatory Responses to Dietary Proteins

Emma Ramiro-Puig, Julie Schulthess, Julien Ettersperger, Natalia Korneychuck, Nicolas Montcuquet, Bertrand Meresse, Nadine Cerf-Bensussan. INSERM, Paris, France

To define the impact of IL-15 on the response to a dietary antigen, mice with transgenic intestinal overexpression of IL-15 (IL-15Tge) were crossed with OTII mice with ovalbumin-specific (OVA) CD4+ T cells. OTIIxB6 and OTIIxIL-15Tge mice were exposed to control or OVA-containing diet until three months. OVA-fed OTIIxB6 mice had normal growth and intestinal appearance while OVA-fed OTIIxIL-15Tge mice showed growth retardation and atonic and dilatated duodenum (not observed in OTIIxIL-15Tge fed a control diet). No decrease in villous/crypt ratio was observed upon OVA feeding of OTIIxB6 mice. In contrast this ratio, already decreased in OTIIx IL-15Tge mice compared to OTIIxB6 mice fed a control diet, further decreased upon OVA feeding. OVA feeding of OTIIxB6 mice decreased the numbers of OVA-specific CD4+T cells and induced OVA-specific FoxP3+ Treg in lamina propria (LP). In IL-15+/OTII+ mice fed with OVA, LP OVA-specific CD4+T cells did not decrease but contained an increased proportion of FoxP3Treg. FoxP3 Treg induced in IL-15Tge mice were functional but preliminary results suggest that effector CD4+ and CD8+ T cells become resistant to Treg. Altogether, these results suggest that IL-15 interferes with the regulatory mechanisms which avoid undesired immune responses to dietary proteins.

OR.95. T Cell Receptor Negative Intraepithelial Lymphocytes in Refractory Celiac Disease: Epithelial Cell Lysis via DNAM-1/αEβ7 and Dendritic Cell Induced Expansion

Frederike Schmitz[1], Jennifer Tjon[1], Yuching Lai[1], Wilma Kroes[1], Richard Lemmers[1], Marco Schreurs[2], Anton Langerak[3], Yvonne Kooy-Winkelaar[1], C. Mulder[2], Jeroen van Bergen[1], Frits Koning[1]. [1]Leiden University Medical Center, Leiden, Netherlands; [2]VUMC, Amsterdam, Netherlands; [3]Erasmus MC, Rotterdam, Netherlands

Approximately 2-5% of adult celiac disease (CD) patients develop refractory celiac disease (RCD). RCD type II is characterized by a marked expansion of aberrant intra-epithelial lymphocytes (IEL) that lack surface TCR/CD3 expression and can develop into gastrointestinal lymphoma. We performed extensive characterization of aberrant IEL lines from RCDII patients. Phenotypic analyses included karyotyping, TCR rearrangement analysis, microarray, Q-PCR and flowcytometry. The experiments revealed that these aberrant IEL lines had incomplete or out-of-frame TCR rearrangements, expressed many NK cell markers, including KIR2DL4, and displayed striking similarities to pre-T/NK cells. In functional experiments, epithelial cell lines and enterocytes were specifically lysed via the NK cell receptor DNAM-1 and the adhesion receptor αEβ7 indicating that the aberrant cells can contribute directly to tissue damage. Moreover, dendritic cells induced strong proliferation of these cell lines which may be linked to their expansion *in vivo*. In duodenal biopsies, cells matching the unique phenotype of aberrant IEL (defined as CD3-CD45+CD7+CD103+CD19-CD14-CD56-CD34-CD127-) constituted 1-5 % of the lymphocyte population of healthy individuals and CD patients, and a larger fraction in RCD. These findings indicate that aberrant IEL expanded in RCDII patients do not derive from mature TCR/CD3+ IEL, but instead may derive from a novel precursor lymphocyte.

OR.96. Developing a Murine Enteropathy Model: Intraluminal Administration of p31-43, a Gliadin Peptide, Induces Histological Changes in the Intestinal Mucosa

Romina Araya, Nestor Rulli, Fernando Chirdo. Universidad Nacional de La Plata, La Plata, Argentina

There exists no animal model for Celiac Disease, a chronic enteropathy triggered by peptides derived of wheat proteins. Among them, p31-43 gliadin drives several innate pathogenic mechanisms. In mice, the damage of intestinal mucosal by intraperitoneal poly (I:C) (PIC) has been described. We evaluated the induction of histological changes in intestinal mucosa by PIC and p31-43 as a experimental model of enteropathy. Injection of PIC into intestinal ligated loops of C57BL/6J mice, but not i.p., resulted in histological changes (villus high/crypt depth ratio) after 12 and 72 hs post treatment. The structure of the intestinal villi was clearly disrupted, while crypt depth was unaffected. Strikingly, p31-43 induced similar changes and exacerbated the damage when administered together with PIC. No such changes were observed in animals treated with an unrelated peptide. Injection of PIC, p31-43 or the combination of both into ligated loops resulted in increased epithelial cell regeneration. Injection of PIC into ligated loops, but not i.p. induced IFNb and IL-15 in small intestine at 2 and 12hs post induction, respectively. This study presents further evidence that stimulation of innate immunity in the small intestine produces a severe enteropathy and showed that p31-43 induces mucosal damage in genetically unmanipulated animals.

Oral Presentations: Friday, July 8

Microbiota (3400)
Friday, July 8, 14:30-16:00

OR.97. Deciphering Microbiota-driven Cell Signaling Modulation in the Human Gut using a Functional Metagenomic Approach

Omar Lakhdari, Antonietta Cultrone, Tomas de Wouters, Malgorzata Nepelska, Julien Tap, Karine Gloux, Fabien Dumetz, Dusko Ehrlich, Nicolas Lapaque, Joël Doré, Hervé Blottière. INRA, Jouy en Josas, France

The intestinal microbiota is a complex community which exerts functions often associated with beneficial effects for its host, including contribution to mucosal homeostasis and maturation of the immune system. The microbiota complexity associated to the inability to culture the vast majority of these microbes lead to the development of new and powerful approaches namely metagenomics. To study the interactions between intestinal epithelial cells (IECs) and commensal bacteria, a high throughput cell-based functional metagenomic approach was established targeting NF-κB pathway. A human IEC clone, HT-29-kB-SEAP-25 stably transfected with the pNiFty2-SEAP plasmid was used to screen metagenomic libraries bearing large DNA fragments (~40 kb) derived from the human intestinal bacterial fractions. A high throughput screening platform was established to screen metagenomic libraries for NF-κB modulation. Screening of metagenomic libraries led to the identification of several bioactive metagenomic clones modulating the NF-κB pathway. Sequencing, annotation and transposon mutagenesis allowed the identification of putative genes implicated. For one stimulatory clones derived from a Bacteroides-related strain, we identified 2 loci involved in the NF-κB stimulatory effect. The mechanism is currently under investigation. Thus, we have established a high throughput functional metagenomic approach to identify genes involved in microbiota-driven NF-κB modulation in gut epithelium.

OR.98. Overexpression of PepT1 Increases the Susceptibility of Mice to Colitis in a Process Involving NOD2 Activity

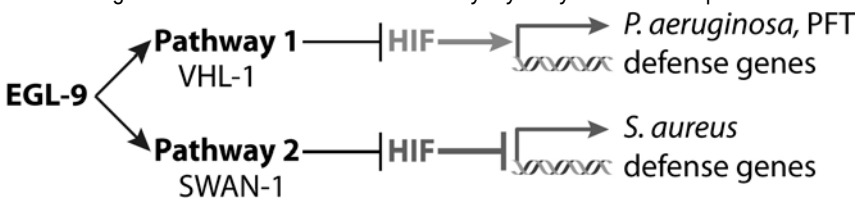
Guillaume Dalmasso, Hang Nguyen, Hamed Laroui, Yutao Yan, Saravanan Ayyadurai, Moiz Charania, Shanthi Sitaraman, Didier Merlin. Emory University, Atlanta, GA

Expression of the human di/tripeptide transporter hPepT1 is induced in inflamed colonocytes from patients with inflammatory bowel disease (IBD). DSS induced more severe inflammation in both beta-actin-hPepT1 and villin-hPepT1 Tg mice than in wild-type (WT) littermates. beta actin-hPepT1 mice, but not villin-hPepT1 animals, exhibited increased susceptibility to TNBS-induced colitis (a model of Th1 inflammation that shares features with Crohn's disease) compared to WT mice. WT animals receiving hPepT1-expressing immune cells were more susceptible to TNBS-induced colitis than were mice receiving WT mouse-derived immune cells. These results demonstrate that hPepT1 expression in both IECs and immune cells contributes to the inflammatory activity of the transporter during colitis. Treatment with broad-spectrum antibiotics attenuated the susceptibility to colitis of Tg mice to levels similar to those of WT animals, suggesting a requirement for commensal bacteria if hPepT1 overexpression is to aggravate intestinal inflammation. Finally, the exacerbation of intestinal inflammation upon hPepT1 overexpression was NOD2-dependent because Nod2−/− and hPepT1 Tg/Nod2−/− littermates were similar in susceptibility to DSS-induced colitis. Expression of hPepT1 in colonic epithelia and immune cells during inflammation increases the severity of colitis. This may be mediated by bacterial peptide transport by hPepT1, which triggers intracellular pro-inflammatory responses via the NOD signaling pathway.

OR.99. How Animals Detect Intestinal Infection Without TLRs, NLRs or Inflammasomes - Role of Hypoxia Signaling

Lyly Luhachack, Javier Irazoqui. Massachusetts General Hospital, Harvard Medical School, Boston, MA

Background: The goals of our research are to investigate evolutionarily conserved host defense pathways, and to use the acquired knowledge to understand their function in mammalian IECs during healthy homeostasis with the microbiota, infection by pathogens, or chronic inflammation. For this purpose we use the genetically tractable model host C. elegans, which exhibits pathogen-specific responses that are independent of TLR, NLR, and NF-κB, and thus is ideal for elucidating novel, yet evolutionarily conserved, host defense pathways in an unbiased and efficient manner *in vivo*. Results: We first established that C. elegans mounts a pathogen-specific intestinal epithelial cell host response to infection. Next, we discovered a novel critical role for hypoxia signaling in controlling this response. Specifically, hypoxia-inducible factor (HIF-1) acted as a repressor and an inducer of distinct host defense responses. Lifting HIF-1-mediated repression required SWAN-1, known to repress HIF-1 by a pathway that is independent of hydroxylation and VHL-1-mediated degradation of HIF-1, and conserved in humans. Conclusions: Our results show that HIF-1 has dual roles in the control of host defense, and suggest that it may also play dual roles during human intestinal inflammation. Prolyl-hydroxylase EGL-9 represses HIF-1 via two pathways. Pathway 1, which involves hydroxylation of HIF-1 followed by its poly-ubiquitylation by VHL-1 and its destruction by the proteasome, is more important for repressing HIF-1 transcriptional activator activity. Pathway 2, independent of hydroxylation but dependent on SWAN-1, represses HIF-1 transcriptional repressor activity by an unknown mechanism.



OR.100. High Fat Diet Favors AIEC Colonization and Inflammation in CEABAC10 Mice by Modulating Microbiota Composition

Margarita Martinez-Medina, Jérémy Denizot, Arlette Darfeuille-Michaud, Nicolas Barnich. Université d'Auvergne, Clermont-Ferrand, France

Background: Host genotype, diet and intestinal microbiota play a role in Crohn's disease (CD). Abnormal expression of CEACAM6 by ileal epithelium in CD patients allows Adherent-Invasive Escherichia coli (AIEC) to colonize gut mucosa, leading to inflammation. Aim: Our aim is to evaluate the effects of lipid rich diet in terms of intestinal permeability, AIEC colonization, gut microbial composition and inflammation using CEABAC10 mice expressing human CEACAM6. Results: High fat diet during a 7 week period significantly increased intestinal permeability in CEABAC10 mice, but not in WT mice. Increased persistence of AIEC LF82 in stools was observed in high fat-treated CEABAC10 mice, but not in CEABAC10 mice receiving classic food or in WT mice irrespectively of type of diet, and AIEC colonization was associated with an increase of TNF-α and IL-12 release. Moreover, altered intestinal microbiota was observed in AIEC-infected CEABAC10 mice fed with high-fat compared to classic food, with increased abundance of E. coli and Bacteroides spp., and eradication of bacteria from the Clostridium leptum group in colonic mucosa. Conclusion: These results strongly support the multifactorial theory of CD aetiology and gives weight to fat intake combined with AIEC infection and abnormal expression of CEACAM6 as risk factors for CD.

OR.101. PSA Produced by the Human Commensal Bacteroides Fragilis Controls Murine Inflammatory CNS Demyelination in a TLR2 Dependent Mechanism Mediated by Tolerogenic CD103$^+$ Dendritic Cells

Javier Ochoa-Repáraz[1], Kiel Telesford[1], Yan Wang[1], Daniel Mielcarz[1], Sakhina Begum-Haque[1], Dennis Kasper[2], Lloyd Kasper[1]. [1]Dartmouth Medical School, Lebanon, NH; [2]Harvard Medical School, Boston, MA

Recent published results in EAE, an animal model of human multiple sclerosis (MS) support the potential role for gut commensal bacteria. We have previously shown that the oral administration of a highly purified antigen of Bacteroides fragilis polysaccharide A (PSA) can protect against EAE, in an IL-10 dependent mechanism. We hypothesize that PSA plays a critical role in maintaining the balance between regulatory and inflammatory cell subsets in humans with MS. We assessed the capacity of PSA to modify the phenotype of FoxP3+Tregs in EAE mice. CD103$^+$ dendritic cells (DCs) from WT mice showed enhanced uptake levels of fluorochrome labeled PSA when compared to TLR2 deficient mice. FoxP3$^+$Treg frequencies were enhanced in CD4$^+$T cells when co-cultured with CD103$^+$DCs and exposed to PSA. This FoxP3$^+$Treg conversion by PSA was enhanced in WT EAE mice when compared to TLR2$^{-/-}$ EAE mice. IL-10 production by PSA-induced FoxP3$^+$Tregs converted from cells of EAE mice was also impaired in diseased TLR2 deficient mice. Our results show that the human commensal antigen PSA generates a tolerogenic dendritic cell phenotype that could restore the impaired function of FoxP3$^+$Tregs of those with MS.

OR.102. Leptin-induced CCR7 Expression on DC can be Downregulated by Probiotic Bacteria

Hafid Al-Hassi[1], Aravinth Murugananthan[1], Elizabeth Mann[1], David Bernardo[1], Nicholas English[1], Cheng Tee[1], Ailsa Hart[1], Alexandra Blakemore[1], Andrew Stagg[2], Stella Knight[1]. [1]Imperial College London, London, United Kingdom; [2]Queen Mary University of London, London, United Kingdom

Background: Dendritic cells (DC) migrate to lymph nodes on expression of CCR7, and control immune activity. CCR7 increases on blood DC on exposure to the proinflammatory adipokine, leptin. Increased mesenteric fat and leptin levels occur early in Crohn's disease, suggesting leptin-mediated change in intestinal CCR7 expression on DC as a pro-inflammatory mechanism. Probiotic bacteria, (PRO) are anti-inflammatory and increase intestinal DC IL-10 production. We have assessed the effects of leptin and PRO on blood and colonic DC migration. Methods: We applied

flow-cytometry analysis to assess expression of CCR7, with and without 4-8 hours leptin treatment, and the effects of PRO on DC from blood and colonic biopsies from healthy human controls. Function was determined by transwell migration towards CCL19. Results: Leptin treatment increased functional CCR7 expression on DC from both tissues in a concentration-dependent fashion (P<0.01). In contrast, PRO decreased the expression of CCR7 on blood and tissue DC (p<0.004 and P<0.04) respectively. Ongoing production of the signaling isoform of the leptin receptor was found in DC expressing CCR7. Conclusions: Leptin treatment increased migration of DC by upregulating CCR7 on blood and colonic DC. Probiotic bacteria may have a beneficial effect by reducing the leptin-induced migration of DC.

Intestinal Inflammation and Innate Immunity (3401)
Friday, July 8, 14:30-16:00

OR.103. T-bet Deficiency Promotes IL-17 Expression in the Innate Immune Compartment in Inflammatory Bowel Disease (IBD)
Nick Powell[1], Emilie Stolarczyk[1], James Canavan[1], Espe Perucha[1], Thomas MacDonald[2], Graham Lord[1]. [1]King's College London, London, United Kingdom; [2]Barts and the London School of Medicine and Dentistry, London, United Kingdom

The IL-23/IL-17 axis is a key effector pathway involved in mediating IBD. Cellular sources of IL-17 have recently been extended to include innate immune cells. The mechanisms controlling innate IL-17 expression are unknown but T cells lacking the transcription factor T-bet favor Th17 development. Recently, a novel colitis model was described in mice lacking T-bet in the innate immune system. Intestinal inflammation in TRUC (T-bet-/-xRAG2-/- ulcerative colitis) mice is initially dominated by TNF over-expression, but over time response to TNF blockade fails indicating that alternative pathways emerge. We have identified innate immune cells that express IL-17A and IL-22 at the expense of interferon-γ in the colonic lamina propria and mesenteric lymph nodes of TRUC mice. These cells were Thy1high, CCR6+, RORγt+, NKp46-, CD11c-, CD11b- and Gr-1-. Thy1+ innate lymphoid cells favoring IL-17A expression in the context of T-bet deficiency were also observed in other models of intestinal inflammation. We also report preliminary data identifying reduced T-bet expression in IL-17 expressing CD3- RORγt+ cells in mucosal lesions of patients with IBD. In the absence of T-bet innate Thy1+ cells preferentially express IL-17A and IL-22. These novel cells are expanded in intestinal inflammation implying that they are pathologically relevant *in vivo*.

OR.104. TLR4-D299G Represents a Key Mediator of Inflammation-associated Cancer Progression via STAT3 in the Intestinal Epithelium
Annette Eyking[1], Birgit Ey[1], Michael Rünzi[2], Andres Roig[3], Guido Gerken[1], Daniel Podolsky[3], Elke Cario[1]. [1]University Hospital of Essen, Essen, Germany; [2]Kliniken Essen-Süd, Essen, Germany; [3]UT Southwestern Medical Center, Dallas, TX

A common missense mutation (D299G) has recently been identified in the human TLR4 gene. The aim of this study was to determine the effects of TLR4-D299G on intestinal epithelial cell (IEC) biology and molecular function. We used IEC lines (Caco-2) stably overexpressing HA-tagged wildtype (WT) TLR4, mutant TLR4-D299G or TLR4-T399I. We performed gene expression profiling using DNA microarray analysis and confirmed findings by realtime qRT-PCR, western/ELISA/confocal immunofluorescence and functional assays. We tested in-vivo tumorigenicity using the CD-1 nu/nu mouse xenograft model and human colonic specimens. We found that TLR4-D299G confers a gain-of-function phenotype in IEC, driving inflammation-associated epithelial-mesenchymal transition and cancer progression. But TLR4-D299G did not induce pro-inflammatory ER stress (Bip/grp78). In contrast, expression of TFF2 and TFF3 was decreased in TLR4-D299G IEC, implying wound healing defects. All of these changes were not evident in clones expressing TLR4-WT, TLR4-T399I or mock. In TLR4-D299G, STAT3 was tyrosine-phosphorylated and cellular invasion was blocked by a STAT3 inhibitor (but not COX-2) *in vitro* and *in vivo*. Primary human colon cancers with TLR4-D299G showed more aggressive behaviour than TLR4-WT which correlated with elevation of STAT3 and inflammation-associated genes (A2M, C5, CHI3L1, TFPI). In Conclusion, TLR4-D299G links aberrant innate immunity and inflammation-associated cancer progression.

OR.105. Endoplasmic Reticulum Stress-induced Enteritis in Xbp1-**Deficient Mice is Dependent on NFκB Signaling**
Lukas Niederreiter[1], Timon Adolph[2], Felix Offner[3], Teresa Fritz[2], Alexander Moschen[2], Barbara Enrich[2], Edina Sarcevic[3], Nicole Kaneider[2], Ann Hwee Lee[4], Laurie Glimcher[4], Herbert Tilg[2], Richard Blumberg[5], Arthur Kaser[1]. [1]University of Cambridge, Cambridge, United Kingdom; [2]Innsbruck Medical University, Innsbruck, Austria; [3]Academic Teaching Hospital Feldkirch, Feldkirch, Austria; [4]Harvard School of Public Health, Boston, MA; [5]Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Background: IRE1/XBP1 signaling is initiated in response to endoplasmic reticulum (ER) stress. Deletion of Xbp1 in intestinal epithelial cells (IECs) results in murine enteritis, and XBP1 variants confer genetic risk for inflammatory bowel disease. Xbp1-/- epithelia lack Paneth cells and exhibit increased JNK activation. We hypothesized that hypomorphic XBP1 might overactivate NFκB via IRE1α. Methods: NFκB signaling was studied in MODE-K IECs after silencing of Xbp1 and Ern1 (IRE1α) expression and stimulation with TNFα. IKK2 was blocked *in vivo* in Xbp1flox/flox.Villin-Cre-ERT2 mice via the specific inhibitor BAY11-7082. Results: TNFα-stimulated XBP1-silenced MODE-Ks exhibited increased phosphorylation of IKKs, IkBα, and nuclear NFκB p65, along with increased NFκB p65 DNA binding activity and IκBα mRNA expression, which upon co-silencing of IRE1α was restored to normal. Treatment of Xbp1flox/flox.Villin-Cre-ERT2 mice with BAY11-7082 significantly reduced the severity of enteritis. Furthermore, BAY11-7082 restored epithelial hyperproliferation and the elevated apoptosis rate in Xbp1-deleted epithelia to normal, and prevented the loss of Paneth cells. Conclusions: Unresolved ER stress in IECs due to XBP1 hypofunction overactivates NFκB signaling, presumably secondary to hyperactivated IRE1α. This pathway may play an important role in enteritis development, which can be prevented by IKK2 blockade.

OR.106. NLRP6 Controls Epithelial Self-renewal and Colorectal Carcinogenesis upon Injury

Sylvain Normand[1], Anne Delanoye-Crespin[1], Aude Bressenot[2], Ludovic Huot[1], Teddy Grandjean[1], Laurent Perin-Biroulet[2], Yves Lemoine[1], David Hot[1], Mathias Chamaillard[1]. [1]Institut Pasteur de Lille, Lille, France; [2] Nancy-Université, Vandoeuvre-lés-Nancy ,France

The colonic epithelium self-renews every 3-5 days, but our understanding of the underlying processes preserving wound healing from carcinogenesis remains incomplete. Here, we demonstrate that NLRP6 suppresses inflammation and carcinogenesis by regulating tissue repair. While NLRP6 expression was lowered in diseased colon, NLRP6-deficient mice were highly susceptible to experimental colitis. Upon injury, NLRP6 deficiency deregulated regeneration of the colonic mucosa and processes of epithelial proliferation and migration. Consistently, absence of NLRP6 accelerated colitis-associated tumor growth in mice. A gene-ontology analysis on a whole-genome expression profiling revealed a link between NLRP6 and self-renewal of the epithelium. Collectively, the integrity of the epithelial barrier is preserved by NLRP6 that may be manipulated to develop drugs capable of preventing adenoma formation in inflammatory bowel diseases.

OR.107. Caspase 8 in Intestinal Epithelial Cells Regulates Immune Homeostasis in the Gut

Claudia Günther, Christoph Becker, Markus Neurath. Uniklinikum Erlangen, Erlangen, Germany

The intestinal epithelium is an important barrier of our body against the external environment characterized by the bacterial flora and food antigens present in the gut lumen. Excessive infiltration of bacteria through this cell layer is believed to result in deregulated intestinal immune response and to the pathogenesis of inflammatory bowel disease. However the regulation of epithelial cell death and its role in intestinal homeostasis remains poorly understood. We demonstrate that deletion of Caspase8 in intestinal epithelial cells results in spontaneous inflammatory lesions in the terminal ileum and that conditional knock-out mice for Caspase8 are highly susceptible to experimental colitis. Caspase8 deficiency results in an impaired expression of antimicrobial peptides. This leads to an attachment of bacteria to the epithelial cell surface which causes a destroyed barrier function and consequently the translocation of bacteria into the lamina propria. Treating Caspase8 Δ IEC mice with antibiotics to decrease the bacterial concentration in the gut partially rescued the severe course of the colitis. Taken together, our data demonstrate for the first time a critical role of Caspase8 in regulating the epithelial integrity and intestinal homeostasis, and have important implications for the understanding the mechanism controlling the pathogenesis of human inflammatory bowel disease.

OR.108. Local Factors Influence Intestinal Epithelial Cell Endoplasmic Reticulum Stress

Michael McGuckin[1], Indrajit Das[1], Thu Tran[1], Rohan Lourie[1], Chin Wen Png[1], Rajaraman Eri[2], Denis Crane[3], Timothy Florin[1]. [1]Mater Medical Research Institute, Brisbane, QLD, Australia; [2]University of Tasmania, Launceston, TAS, Australia; [3]Griffith University, Brisbane, QLD, Australia

Endoplasmic reticulum (ER) stress in intestinal secretory cells has been linked with intestinal inflammation and IBD. To determine whether glucocorticosteroids, TNFα and IL-10 influence ER stress pathways we used human colonic LS174T cells with tunicamycin-induced ER stress, and Winnie mice with ER stress due to misfolding mutations in Muc2. Muc2 precursor accumulated in Winnie goblet cells accompanied by a 40±3-fold increase in intestinal Grp78 (key indicator of ER stress) and elevated expression of unfolded protein response (UPR) genes, which can trigger inflammation. The glucocorticosteroid dexamethasone significantly reduced inflammation, but also reduced expression of ER stress and UPR markers ($P<0.001$) and restored mature Muc2 production ($P<0.001$), suggesting direct and/or indirect effects on ER stress. *In vitro* in the absence of inflammatory cytokines, upregulation of GRP78 (15.1±1.3-fold) and UPR markers in tunicamycin-treated cells was almost completely inhibited by dexamethasone ($P<0.001$), demonstrating a direct protective effect. TNFα alone had no effect on ER stress but exacerbated the increase in GRP78 and UPR markers during tunicamycin-induced misfolding, whereas IL-10 increased GRP78 without affecting the UPR genes. These experiments show that local factors found in the intestine can modulate epithelial cell ER stress providing avenues for therapeutic amelioration of ER stress induced intestinal inflammation.

Mucosal Infections II (3402)
Friday, July 8, 14:30-16:00

OR.109. Development of CD4 Th1 and Th17 Cells After Vaginal Infection with Chlamydia

Stephen McSorley, Lin-Xi Li. University of Minnesota, Minneapolis, MN

CD4 T cells are required for protective immunity to Chlamydia infection but the *in vivo* activation and differentiation during vaginal infection is poorly understood. We have generated Chlamydia-specific MHC class-II tetramers and used a column enrichment strategy to visualize endogenous CD4 responses to vaginal infection. Chlamydia-specific T cells expanded in the draining lymph node and rapidly developed a Th1 (T-bet+) phenotype and secreted IFN-γ *ex vivo*. A small percentage of Chlamydia-specific Th1 cells also expressed the Treg marker Foxp3, while Th2 or Th17 cells were not detected in any secondary lymphoid tissues. Dendritic cells were required for the clonal expansion of Chlamydia-specific T cells in response to infection. Following resolution of infection, Th1 cells redistributed to systemic tissues and persisted for up to 6 months. In contrast to the draining lymph node, Chlamydia-specific CD4 Th17 cells were readily detected within the vaginal mucosa during active disease. These data indicate that Th1 and Th17 populations are required for the resolution of bacterial infection at the vaginal mucosa.

OR.110. Genital Tract Immune Responses to Neisseria Gonorrhoeae are Directed by the Pathogen for its Own Benefit

Michael Russell, Yingru Liu. University at Buffalo, Buffalo, NY

We have previously shown that Neisseria gonorrhoeae elicits Th17-driven innate responses in a mouse model of genital tract infection. We have further proposed that N. gonorrhoeae suppresses adaptive immune responses that might afford protective immunity. As TGF-β is an immunoregulatory cytokine involved in the generation of Th17 cells, we hypothesized that N. gonorrhoeae stimulates TGF-β production and thereby also inhibits the development of Th1/Th2-driven adaptive immune responses. Studies on mouse spleen cells and genital tract tissues *in vitro*, and on mice infected with N. gonorrhoeae *in vivo* showed that TGF-β is generated *in vitro* and becomes elevated in the female genital tract in response to N. gonorrhoeae. This leads to the suppression of Th1 and Th2 immune responses *in vitro* and *in vivo*. Blockade of TGF-β with antibody reverses the suppression and allows Th1 and Th2 responses to emerge against N. gonorrhoeae. Mice treated with anti-TGF-β antibody clear primary gonococcal infection faster, and develop Th1- and Th2-dependent responses and immune memory, such that secondary challenge is resisted and anti-gonococcal antibody responses are developed. The results support the concept that a well-adapted pathogen proactively elicits from its host the pattern of response that is favorable to its own survival.

OR.111. Transmission of Murine Cytomegalovirus in Breast Milk: A Model of Natural Infection in Neonates

Lynn Puddington, Sara Paveglio, Elizabeth Lingenheld, Li Zhu, Leo Lefrancois, Carol Wu. University of Connecticut Health Center, Farmington, CT

Vertical transmission of viruses in breast milk can expose neonates to pathogens at a time when the capacity of their immune system to control infections is limited. We developed a mouse model to study the outcomes of acquiring murine cytomegalovirus (MCMV) while being breastfed by mothers with latent infection. Breast milk leukocytes collected from lactating mice were examined for the presence of MCMV IE-1 mRNA by RT-PCR and Southern analysis. Similar to human mothers with latent cytomegalovirus infection, reactivation of MCMV occurred specifically in the lactating mammary gland of mice with latent infection. Interestingly, breast milk collected from mothers with latent infection transferred infectious virus when injected (i.p.) into neonatal mice. We found that MCMV was transmitted naturally from infected mothers to breastfed neonates, with MCMV IE-1 mRNA or infectious virus present in multiple organs including the brain. In fact, one day of nursing was sufficient to transmit MCMV from latent mothers to breastfed neonatal mice. The relevance of this mouse model to cytomegalovirus transmission in humans will prove useful in future studies designed to elucidate the immunological and pathological ramifications of neonatal infection acquired via this natural route.

OR.112. Th22 Cells Constitute a Highly HIV Susceptible T Cell Subset that is Associated with Epithelial Integrity in the Sigmoid Mucosa and Systemic Immune Activation

Connie Kim[1], Duncan Chege[1], Zenita Alidina[2], Erika Benko[1], Lucy Shin[1], Sanja Huibner[1], Colin Kovacs[1], Gabor Kandel[3], Charu Kaushic[2], Rupert Kaul[1]. [1]University of Toronto, Toronto, ON, Canada; [2]McMaster University, Hamilton, ON, Canada; [3]St. Michaels Hospital, Toronto, ON, Canada

Th22 cells are a unique CD4 T cell subset with tissue repair and regenerative properties. These cells are defined by their propensity to produce IL-22 independent of Th1- or Th17-defining cytokines. Here we investigate the impact of HIV infection and antiretroviral therapy (ART) on Th22 cells in the blood and the sigmoid mucosa to determine their impact on gut epithelial integrity and immune activation. Blood and sigmoid biopsies were collected from: HIV uninfected (HIV-; n=8), HIV infected therapy-naïve (HIV+Rx-; n=9), and HIV infected long-term ART treated (HIV+Rx+; n=16) men. Frequency and absolute number of sigmoid Th22 cells and IL-22 producing CD4 T cells were significantly reduced in untreated HIV infection, but were comparable in uninfected and ART treated participants. Th22 cells in the sigmoid positively correlated with epithelial integrity and negatively correlated with systemic immune activation in the ART treated group. Th22 cells expressed higher frequency and density of HIV co-receptor/binding molecules (CCR5/α4β7) compared to Th1, Th17, and IL-22+ CD4 T cells, strongly suggesting enhanced HIV susceptibility in this subset. Our data suggest that Th22 cells constitute a preferential target for HIV replication and play an important role in maintaining epithelial integrity and HIV disease progression.

OR.113. Muc5ac: A Critical Component Mediating the Rejection of Enteric Nematodes

Sumaira Hasnain[1], Christopher Evans[2], Michelle Roy[2], Amanda Gallagher[3], Kristen Kindrachuk[4], Luke Baron[4], Burton Dickey[2], Mark Wilson[5], Thomas Wynn[4], Richard Grencis[3], David Thornton[3]. [1]Mater Medical Research Institute, Brisbane, QLD, Australia; [2]MD Anderson Cancer Center, Houston, TX; [3]University of Manchester, Manchester, United Kingdom; [4]National Institutes of Health, Bethesda, MD; [5]National Institutes for Medical Research, London, United Kingdom

Trichuriasis, caused by the intestinal nematode Trichuris, is a disease that affects up to a billion people worldwide. Most of our understanding comes from the mouse model of this disease (Trichuris muris), which is used to dissect the immune-mediated effector mechanisms that elicit the expulsion of the nematode. De novo expression of Muc5ac, a mucin not normally expressed in the intestinal tract, is induced in the caecum of mice resistant to T. muris infection. Therefore, in this study we investigated the role of Muc5ac, which is detected shortly before worm expulsion and associated with the development of Th2 T cell mediated interleukin-13 (IL-13)-dependent resistance to this nematode. Muc5ac-deficient mice were incapable of expelling T. muris from the intestine and harboured long-term chronic infections, despite developing strong Th2 responses. Importantly, human MUC5AC had a direct detrimental effect on the nematode's vitality *in vitro*. Moreover, absence of Muc5ac caused a significant delay in the expulsion of two other gut-dwelling nematodes (Trichinella spiralis and Nippostrongylus brasiliensis). Thus, for the first time we identify a single mucin, Muc5ac,

as a direct and critical effector of Th2-driven expulsion of intestinal nematodes.

OR.114. Duodenal Helminth Infection Alters Barrier Function of the Colonic Epithelium via Adaptive Immune Activation

Chien-wen Su, Jess Kaplan, Mei Zhang, Allan Walker, Haining Shi. Massachusetts General Hospital and Harvard Medical School, Charlestown, MA

Intestinal helminth infection is a major public health problem, particularly in the developing world, and can have significant effects on host responses to other enteric pathogens and antigens. The mechanisms underlying these effects are not well understood. In this study, we investigated the impact of infection with the murine small intestinal nematode Heligmosomoides polygyrus on epithelial barrier function in the colon. We found that H. polygyrus infection produced a significant increase in colonic epithelial permeability, as evidenced by detection of elevated serum levels of the tracer horseradish peroxidase following rectal administration. The loss of barrier function was associated with clear ultrastructural changes in the tight junctions of colonic epithelial cells and alterations in the distribution and expression of E-cadherin. These parasite-induced abnormalities were not observed in SCID mice, but did occur in SCID mice that were adoptively transferred with wild-type T cells, indicating a requirement for adaptive immunity. Furthermore, the helminth-induced increase in gut permeability was not seen in STAT6 KO mice. These results demonstrate that one of the mechanisms by which helminths exert their effects involves the lymphocyte- and STAT6-dependent breakdown of the intestinal epithelial barrier, which may facilitate the movement of lumenal contents across the mucosa, helping to explain how helminth infection can alter the immune response to enteric antigens.

Food Allergy and Microbiota (3403)
Friday, July 8, 14:30-16:00

OR.115. Dietary Intervention with Synbiotics Protects Against Allergic Disease via Induction Of Galectin-9 by Intestinal Epithelial Cells

Sander de Kivit[1], Eirikur Saeland[2], Aletta Kraneveld[1], Henk van de Kant[1], Bastiaan Schouten[1], Betty van Esch[1], Jan Knol[3], Aline Sprikkelman[4], Leontien van der Aa[4], Leon Knippels[3], Johan Garssen[1], Yvette van Kooyk[2], Linette Willemsen[1]. [1]Utrecht University, Utrecht, Netherlands; [2]VU University Medical Center, Amsterdam, Netherlands; [3]Danone Research, Wageningen, Netherlands; [4]Emma Children's Hospital, Amsterdam, Netherlands

Prebiotic galacto- and fructo-oligosaccharides (scGOS/lcFOS) resembling oligosaccharides in human milk have been reported to reduce the development of allergy through modulation of the intestinal microbiota and immune system. Intestinal epithelial cells (IEC) abundantly express galectins, which modulate T cell responses. To this end, human IEC were grown on transwell inserts and apically exposed to 0.5% scGOS/lcFOS together with TLR ligands and co-cultured with CD3/CD28-activated PBMC. We found that galectin-9 (Gal9) is expressed and secreted by IEC upon exposure to TLR9 ligand and scGOS/lcFOS, and supports a T_h1/T_{reg} effector response in the co-culture system. Furthermore, development of T_h1 and T_{reg} cells was enhanced in Gal9-treated PBMC, resulting in increased IL-10 and IFN-γ, but suppressed IL-17 secretion. In mice orally sensitized to whey, while receiving a diet containing Bifidobacterium breve M-16V and scGOS/lcFOS (synbiotics), enhanced serum Gal9 levels were found and immunohistochemistry revealed specific basolateral Gal9 expression by IEC. Increased serum Gal9 concentrations correlated with decreased allergic symptoms. In addition, infants suffering from atopic dermatitis receiving synbiotics showed enhanced Gal9 levels in serum, which coincided with less severe allergic symptoms. These results indicate that dietary supplementation with synbiotics has significant implications for the prevention of allergy through TLR9-induced galectin-9 secretion by IEC.

OR.116. Dysregulation of Allergic Airway Inflammation in the Absence of Microbial Colonization

Tina Herbst[1], Anke Sichelstiel[2], Corinne Schar[1], Koshika Yadava[2], Julia Cahenzli[3], Kathy McCoy[3], Ben Marsland[2], Nicola Harris[1]. [1]École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; [2]Centre Hospitalier Universitaire Vaudois Lausanne, Lausanne, Switzerland; [3]University of Bern, Bern, Switzerland

The incidence of allergic disorders is increasing in developed countries and has been associated with alterations in the commensal bacterial flora. To ascertain the relevance of commensal bacteria upon the development of an allergic response, we utilized a model of allergic airway inflammation in germ-free (GF) mice lacking any exposure to any microbes. Allergic airway inflammation was induced in GF, specific pathogen free (SPF) or recolonized mice by sensitization and challenge with ovalbumin (OVA). Our results show that the total number of airway lymphocytes and eosinophils were elevated in allergic GF mice as compared to control SPF mice, and that this increase could be reversed by re-colonization of GF mice with the complex commensal flora of SPF mice. Exaggerated airway eosinophilia correlated with increased local production of Th2 associated cytokines, elevated IgE production and an altered number and phenotype of conventional dendritic cells (cDC). Regulatory T cell populations and regulatory cytokine levels were unaltered but GF mice exhibited an increased number of basophils and decreased numbers of alveolar macrophages (AM) and plasmacytoid dendritic cells (pDC). These data demonstrate that the presence of commensal bacteria is critical for ensuring normal cellular maturation, recruitment and control of allergic airway inflammation.

OR.117. The Commensal Microbiota Regulates Susceptibility to Allergic Responses to Food

Andrew Stefka, Tiffany Patton, Michael Burrows, Yunwei Wang, Dionysios Antonopoulos, Eugene Chang, Cathryn Nagler. University of Chicago, Chicago, IL

Earlier work from our laboratory implicated the commensal microbiota in the regulation of susceptibility to allergic responses to food. Allergen-specific IgE responses were enhanced in mice unable to signal via TLR4 and in mice treated with a cocktail of broad-spectrum antibiotics. 16S rRNA-based clone library analysis of fecal DNA shows that this antibiotic administration, initiated one week before weaning, enriches for Proteobacteria and greatly reduces the Firmicutes and Bacteroidetes phyla that typically predominate in the gastrointestinal tract. Total serum IgE levels were elevated in unsensitized antibiotic-treated mice. We also find that circulating levels of total IgE increase with age in TLR4-/- but not TLR4WT mice maintained in the same colony, further supporting a role for a bacteria-derived signal in regulating IgE production in unsensitized mice. Moreover Foxp3+ regulatory T cells, sorted using a GFP reporter, from the mesenteric lymph nodes of TLR4-/- mice or antibiotic-treated TLR4WT mice, are greatly impaired in their ability to produce IL-10 in response to TCR stimulation *in vitro*. Ongoing treatment studies with single antibiotics selected from the original cocktail are revealing the bacterial subpopulations that alter IgE and regulatory cell responses in this model and protect against susceptibility to allergic responses to food.

OR.118. Probiotic Administration Promotes Regulatory Responses in the Gut and Counter-regulates Th2 Responses in a Mouse Model of Food Allergy

Elisa Schiavi, Bianca Barletta, Cinzia Butteroni, Silvia Corinti, Monica Boirivant, Gabriella Di Felice. Istituto Superiore di Sanità, Rome, Italy

The immunological mechanisms responsible for the anti-allergic effects of probiotic bacteria are still poorly defined. We tested the effect of a probiotic mixture (VSL#3) administration both in naive mice and in a mouse model of sensitization and anaphylaxis to peanut. Analysis of mesenteric lymph node(MLN)-isolated DC (MLN-DC) from naïve mice receiving for three weeks VSL#3 by oral administration, indicated that probiotic treatment increased the frequency of plasmacytoid DC (pDC, B220+CD11clow), and upregulated the expression of maturation markers on conventional DC (cDC). Moreover, the frequency of IL-10-expressing cDC was increased. These findings were paralleled by an increased frequency of IL-10 producing CD4+ T cells. In mice previously intragastrically sensitized and challenged with peanut to induce *in vivo* anaphylaxis, a three-weeks oral treatment with VSL#3 was able to reduce anaphylaxis symptoms associated with a second challenge. This feature was associated with a significant reduction of IL-13 content and in the number of mast cell infiltrating the jejunum of probiotic treated mice, that also showed an increased tissue content of TGF-β. Furthermore, allergen-specific IgA were increased at the gut level. The ability of probiotics to counter-regulate an established Th2 responses might become an effective strategy in the treatment of type I allergy.

OR.119. Induction of Tregs by Mucosal Administration of Aryl Hydrocarbon Receptor Ligands and Anti-CD3 Monoclonal Antibody to Treat Autoimmunity in Animals and Humans

Howard Weiner[1], Andre da Cunha[1], Henry Wu[3], Ruth Maron[1], Francisco Quintana[1], Yaron Ilan[2]. [1]Brigham and Women's Hospital, Boston, MA; [2]Hadassa Hospital/Hebrew University, Jerusalem, Israel; [3]Novartis Pharma, Basel, Switzerland

We induced Tregs at mucosal surfaces using (1) anti-CD3 mAb and (2) endogenous ligands of the aryl hydrocarbon receptor (AHR). (1) Oral anti-CD3 suppressed EAE by inducing CD4+TGF-b latency associated peptide (CD4+LAP+) Tregs in the MLN which suppressed via TGF-b. Foxp3 Tregs were not increased and no modulation of CD3 occurred. Oral anti-CD3 also suppressed autoimmune diabetes and collagen induced arthritis. In lupus oral/nasal anti-CD3 suppressed established lupus via IL-10 (nasal) or TGF-b dependent (oral) Tregs. In the Ob/Ob model of type 2 diabetes, oral anti-CD3 decreased the metabolic syndrome and adipose tissue inflammation by inducing TGF-b-dependent Tregs. In healthy human subjects oral OKT3 antibody decreased IL-17 and induced IL-10/TGF-b without side effects or HAMA responses. (2) Oral administration of the mucosal non-toxic AHR ligand ITE induced Tregs and also induced tolerogenic DC that promote the differentiation of FoxP3+ Treg in a retinoic acid (RA)-dependent manner. In addition, AHR interacts with c-Maf to promote the induction of Tr1 like cells in both mice and humans and promotes the transactivation of both il10 and il21 promoters. These results identify novel physiologic mechanisms to induce regulatory T cells via mucosal surfaces that are clinically applicable to a variety of immune mediated disorders.

OR.120. TLR4 Licences Mesenteric Lymph Node Dendritic Cells for Induction of Oral Tolerance

Feriel Hacini-Rachinel[1], Mercedes Gomez de Agüero[1], Rémi Doucet Ladevèze[1], Jean-Benoit Le Luduec[1], Stéphane Nancey[1], Philippe Langella[2], Bertrand Dubois[1], Dominique Kaiserlian[1]. [1]INSERM IFR 128 - Lyon Sud, Lyon, France; [2]INRA, Jouy en Josas, France

Oral tolerance (OT) involves dendritic cells (DC) subsets in liver (Gastroenrology 2009,137:1019; Immunity 2008,29:464) and mesenteric lymph nodes (MLN) and regulatory T cells (Tregs) (Blood 2003,102:3295) but the contribution of intestinal microbiota remains controversial. Using a model of CD8 T cell-mediated contact hypersensitivity to DNFB, we show that OT induced by hapten gavage prior to skin sensitization is impaired in germ free as well as in TLR4°/°, but not TLR2°/° or TLR9°/° mice, as compared to conventional and wild type B6 mice. Using bone marrow chimeras we found that TLR4 expression on hematopoietic cells is necessary and sufficient for OT induction. Adoptive transfer of cells from hapten-fed TLR4°/° and B6 mice indicated that TLR4 is not involved in CD8 T cell hyporesponsiveness, a critical event in OT that requires plasmacytoid DC.

Alternatively, TLR4 is essential for the capacity of MLN DC from DNFB-fed mice to transfer OT in B6 recipients. Moreover, TLR4 promotes the activity of the enzyme RALDH2 and the ability of MLN DC to induce Foxp3$^+$ Treg conversion *in vitro*. Taken together, these data underline the crucial role of TLR4 in the tolerogenic function of MLN DC in OT and their ability to generate induced Treg.

Probiotics (3500)
Friday, July 8, 16:15-17:45

OR.121. Regulation of Systemic Immune Responses by Probiotics

Peter Van Baarlen[1], Guido Hooiveld[1], Jerry Wells[1], Edward Nieuwenhuis[3], Michiel Kleerebezem[2]. [1]Wageningen University, Wageningen, Netherlands; [2]NIZO Food Research, Ede, Netherlands; [3]University Medical Centre, Utrecht, Netherlands

We are interested in characterising responses of healthy persons to common microbiota and to investigate the genetic basis of tolerance and inflammatory bowel disease (IBD). *in vivo* responses of healthy humans were studied after consumption of four different species of probiotic Lactobacillus species. We found that human transcriptional responses to all four Lactobacillus species including three different growth stages of the species L. plantarum were different in terms of pathway activation and induced gene regulatory networks. Intruigingly, some modulated pathways play roles in IBD, for example the IL-23 signalling pathway that was induced by L. acidophilus. We concluded that lactic acid bacteria induce pathways and processes that play roles in lipid metabolism, immunity and tolerance, cell proliferation and mucosal homeostasis. Investigating the modulated pathways with known involvement in IBD could contribute to understanding how these pathways are dysregulated in persons suffering from IBD. For this, we are also using a germ-free mouse commensal colonisation model to study how homeostasis is preserved using a combination of histology, transcriptomics and metabolomics. These experiments use a time-series design and show among others clusters of co-expressed genes and pathways regulated during mouse colonisation that are known to be deregulated in certain human intestinal diseases.

OR.122. The Strain-specific Anti-inflammatory Capacities of Lactobacilli is Driven by NOD2-mediated Recognition of a Specific Peptidoglycan-derived Muropeptide

Elise Macho fernandez[1], Veronique Valenti[1], Christoph Rockel[2], Corinna Hermann[2], Bruno Pot[1], Ivo Boneca[3], Corinne Grangette[1]. [1]Institut Pasteur de Lille- Inserm U1019- CNRS UMR8204, Lille, France; [2]University of Konstanz, Konstanz, Germany; [3]Insitut Pasteur, Paris, France

In genetically susceptible individuals, an inappropriate mucosal immune response against the microbiota appears to be the principal mechanism in the pathogenesis of inflammatory bowel disease (IBD). Therefore, manipulation of the luminal contents with probiotics represents an attractive therapeutic option. Beneficial effects, however, were shown to be strain-specific and the precise underlying mechanisms remain often unclear. Since we demonstrated that the protective effect of selective lactobacilli in experimental colitis is NOD2-dependent, we evaluated the effect of purified peptidoglycan (PGN) and showed that it could exert potent anti-inflammatory effects. In addition, such PGN was able to induce regulatory CD103+ dendritic cells as well as expansion of CD4+ FoxP3+ Tregs. More interestingly, the observed anti-inflammatory properties were strain-specific, since they were not obtained with PGN derived from a non anti-inflammatory strain. We hypothesized that specific PGN structures might be the driving force and linked the selective anti-inflammatory properties of the PGN to the presence of a specific NOD2 ligand, which was confirmed to be protective *in vivo* in a NOD2-dependent but MyD88-independent manner. The work presented points out that PGN and derived muropeptides are active compounds in probiotic functionality and might represent new immune intervention tools for IBD.

OR.123. Probiotic-derived Protease Lactocepin Degrades the Pro-inflammatory Chemokine IP-10: Impact on Chronic Intestinal Inflammation

Marie-Anne von Schillde[1], Gabriele Hörmannsperger[1], Carl-Alfred Alpert[2], Hannes Hahne[1], Christine Bäuerl[3], Gaspar Perez Martinez[3], Dirk Haller[1]. [1]Technical University of Munich, Freising, Germany; [2]German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany; [3]Instituto de Agroquímica y Tecnología de Alimentos, Valencia, Spain

Clinical studies revealed protective effects of probiotic mixture VSL#3 in the context of IBD. We previously reported that VSL#3-derived Lactobacillus paracasei (L.p) expresses cell surface proteins that mediate post-translational loss of the pro-inflammatory chemokine IP-10 in intestinal epithelial cells (IEC). The aim of this study was to identify the active probiotic structure triggering the loss of IP-10. Stimulation of TNF-activated IEC with L.p conditioned media (L.p-CM) revealed that secreted proteins of L.p analogously mediate loss of secreted and surface-bound IP-10 in IEC. Differential LC-ESI-MS/MS analysis of chromatographic fractions, active or inactive in mediating IP-10 loss, identified lactocepin, a cell-wall-associated and secreted serine protease, to be the active probiotic structure of L.p. Cell-free incubation and PMSF-inhibitor-studies with L.p-CM revealed that lactocepin directly degrades IP-10. Beside IP-10, lactocepin selectively targets an array of pro-inflammatory chemokines (eg. I-TAC, Fractalkine). Finally, an isogenic mutant of lactocepin-encoding prtP confirmed lactocepin as IP-10 degrading structure. *in vivo*, intraperitoneal injection of sterile L.p-CM into TNF$^{\Delta ARE/+}$-mice resulted in a significant reduction of ileal inflammation attended with reduced IP-10 tissue levels, thus demonstrating physiological relevance of lactocepin. The identification of lactocepin as probiotic structure enables a structure-based evaluation of probiotic bacteria for therapeutical interventions in the context of IBD.

OR.124. Integrated Omics Approach Identified Acetate Produced by Probiotic Bifidobacteria to Protect Host from Enteropathogenic Infection

Shinji Fukuda[1], Hidehiro Toh[1], Koji Hase[1], Kenshiro Oshima[2], Yumiko Nakanishi[1], Kazutoshi Yoshimura[5], Toru Tobe[3], Julie Clarke[4], David Topping[4], Tohru Suzuki[5], Todd Taylor[1], Kikuji Itoh[5], Jun Kikuchi[4], Hidetoshi Morita[6], Masahira Hattori[2], Hiroshi Ohno[1]. [1]RIKEN, Yokohama, Japan; [2]University of Tokyo, Kashiwa, Japan; [3]Osaka University, Suita, Japan; [4]CSIRO, Adelaide, SA, Australia; [5]Gifu University, Gifu, Japan; [6]Azabu University, Sagamihara, Japan

The human gut is colonized with a wide variety of microorganisms, including species, such as those belonging to the bacterial genus Bifidobacterium, that have beneficial effects on human physiology and pathology. Among the most distinctive benefits of bifidobacteria are modulation of host defense responses and protection against infectious diseases. Nevertheless, the molecular mechanisms underlying these effects have barely been elucidated. To investigate these mechanisms, we used mice associated with certain bifidobacterial strains and a simplified model of lethal infection with enterohaemorrhagic Escherichia coli O157:H7, together with an integrated 'omics' approach. Here we show that genes encoding an ATP-binding-cassette-type carbohydrate transporter present in certain bifidobacteria contribute to protecting mice against death induced by E. coli O157:H7. We found that this effect can be attributed, at least in part, to increased production of acetate and that translocation of the E. coli O157:H7 Shiga toxin from the gut lumen to the blood was inhibited. We propose that acetate produced by protective bifidobacteria improves intestinal defense mediated by epithelial cells and thereby protects the host against lethal infection.

OR.125. Lactobacilli Induce Heme Oxygenase Dependent and Independent Immunoregulatory Pathways in GALT

Khalil Karimi, Nalaayini Kandiah, John Bienenstock, Paul Forsythe. McMaster University, Hamilton, ON, Canada

The anti-inflammatory effects of certain commensal and probiotic bacteria have been linked to their ability to induce regulatory T cells (Treg). However many aspects of the mechanisms underlying Treg induction by these bacteria remain obscure. We investigated the ability of a Lactobacillus species to induce Foxp3+ Treg in GALT and the potential role of dendritic cells and hemeoxygenase-1 (HO-1), an immunomodulatory enzyme, in mediating these responses. Lactobacillus feeding lead to a significant increase in CD4+CD25+Foxp3+ cells in GALT. This increase was greatest in the mesenteric lymph nodes and was associated with a marked decrease in TNF and IFNγ production. Dendritic cell IL-10 and HO-1 expression was also increased as was regulatory function of these cells . Treatment of mice with a heme oxygenase inhibitor, abolished the increase in Foxp3+ regulatory T cells but did not prevent the inhibition of IFNγ and TNF production by T cells. In conclusion, lactobacillus feeding induced a tolerogenic environment in GALT and while activity of HO-1 was critical to the enhancement of Foxp3+ regulatory T cells, additional, as yet unknown, pathways were involved in the down-regulation of inflammatory cytokine production by T cells.

OR.126. Probiotics May be Effective in Changing the Profile of Cytokines in Breast Milk

Angela Tafaro[1], Nicola Laforgia[2], Annamaria Laneve[2], Luigi Amati[1], Teresa Capursi[2], Mariella Baldassarre[2]. [1]National Institute of Gastroenterology, Castellana-Grotte, Italy; [2]University of Bari, Bari, Italy

In our perspective case-control study probiotic VSL#3® was administered to pregnant women (VSL#3 M) in the last four weeks of gestation and in the first four weeks of breastfeeding. Pregnant women of control group did not assume the probiotic. The mothers' milk and the newborns'stool were collected at days 2-3 and 30. To analyzed the effectiveness of VSL#3 on the development of immunity in newborns the interleukins levels of IL-6, IL-1β,IL-10,TGF-β1 in the milk and the Immunoglobulin levels in the milk and stool samples were measured. Data show significant higher level of TGF-β1, a key mediator in the development of immune tolerance, than the other IL; in particular the supplement in the diet of VSL#3 result in a significance increase of level of TGF-β1 at T0 and T30 compare controls ($p < 0.001$). In the milk of VSL#3 M group IgA titers were more elevated than in controls. These data indicate that VSL#3 may be a powerful immunomodulator in terms of IL and IgA production. Indeed the first month of life represent a critical period for the maturation of the infant's immune system and, thus, this is a window of opportunity to reduce the risk of disease.

Inflammation and Microbiota (3501)
Friday, July 8, 16:15-17:45

OR.127. Protective Effect of Oral Treatment with Antigens from Parabacteroides Distasonis in Experimental Colitis is Associated with Oral Tolerance Induction

Miloslav Kverka[1], Zuzana Zakostelska[1], Klara Klimesova[1], Tomas Hudcovic[2], Tomas Hrncir[2], Pavel Rossmann[1], Jakub Mrazek[3], Jan Kopecny[3], Elena Verdu[4], Helena Tlaskalova-Hogenova[1]. [1]Institute of Microbiology AS CR, Prague, Czech Republic; [2]Institute of Microbiology AS CR, Novy Hradek, Czech Republic; [3]Institute of Animal Physiology and Genetics ASCR, Prague, Czech Republic; [4]McMaster University, Hamilton, ON, Canada

Intestinal inflammation in inflammatory bowel disease (IBD) is a result of an aberrant host immune response to luminal microbial antigens. Here, we show that oral pre-treatment of BALB/c mice with antigens from the commensal microbe, Parabacteroides distasonis, significantly reduces the severity of intestinal inflammation in dextran sulphate sodium (DSS)-induced model of colitis. This protective effect was significantly reduced when mPd was administered together with the strong mucosal adjuvant, cholera toxin, and could not be achieved by parenteral administration of this

antigen. The oral treatment with membraneous fraction of P. distasonis (mPd) significantly increased the number of regulatory T cells in mesenteric lymph nodes, increased mPd-specific antibodies in serum, and prevented DSS-induced increases in several pro-inflammatory cytokines in the gut. Moreover, the protective effect of oral mPd was neither observed in severe combined immunodeficient mice nor when Tregs were depleted with anti-CD25 antibodies in immunocompetent BALB/c. Our results suggest that components derived from the commensal bacterium, P. distasonis, protect from intestinal inflammation by several mechanisms, including induction of oral tolerance, and therefore may be useful in the development of new therapeutic strategies for chronic inflammatory disorders such as IBD.

OR.128. Dectin-1 Mediated Control of Mucosal Immunity to Commensal Fungi During Colitis

Iliyan Iliev, Courtney Becker, Christopher Reyes, David Underhill. Cedars-Sinai Medical Center, Los Angeles, CA

Mucosal fungal infections are relatively common in Crohn's Disease patients, and antibodies against fungal antigens (ASCA) are a well accepted clinical marker for disease severity. However what fungi populate the intestine and how immunity to them might play a role in inflammatory disease is currently unknown. Fungi are sensed by number of innate immune receptors among which Dectin-1, expressed on myeloid cells, is critical for host defense. We found that commensal fungi populate the murine gut and that Dectin-1$^{-/-}$ mice are more susceptible to experimental colitis characterized by increased infiltration of Th17 and Th1 cells in the colon. Interestingly this pathology was driven by intestinal fungus, and antifungal therapy ameliorated colitis severity in Dectin-1$^{-/-}$ mice. Deep sequencing analysis of the fungal microbiome in murine feces revealed fungal species that are overrepresented in the gut during colitis. Mice supplemented with a specific commensal fungus experienced more severe colitis and augmented Th17 mucosal responses in absence of Dectin-1, while another commensal fungus enhanced Th17 responses in Dectin-1$^{-/-}$ mice but did not further affect the intestinal pathology. The data demonstrate that altered interactions between the fungal microflora and the host mucosal immune system can profoundly influence intestinal pathology.

OR.129. Specific Gut Microbiota Drive Autoimmune Arthritis by Inducing a Breakdown of T Cell Adaptive Tolerance to a Self-antigen in Gut-associated Lymphoid Tissues

Pascal Chappert, Ronald Schwartz. National Institue of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD

Recent studies have demonstrated the profound effect that commensal flora can have on the magnitude of autoimmune T cell responses, as observed in murine models of rheumatoid arthritis (Immunity 2010, 32-1) and multiple sclerosis (PNAS 2010, Epub 20660719). In both cases, the ability of a single member of the gut microflora, segmented filamentous bacteria (SFB), to promote an intestinal subset of Th17 cells has been link to the induction of disease. Transfer of naïve PCC-specific, TCR-transgenic T cells into a CD3ε-/- PCC expressing host leads to a mild form of arthritis developing by 4-6 weeks (PLoS Biol 2006, 11-e340). Using hosts housed with two different flora, we were able to characterize a critical role for a pro-inflammatory microbiota in regulating the incidence and severity of arthritis. Of great interest, we observed chronic activation and impaired tuning of TCR responsiveness of the transferred T cells in the mesenteric lymph nodes of affected hosts. This directly affected their production of IL-2, IL17 and IFN-γ as well as the maintenance of a stable population of pathogenic Th17 cells. Such data suggest that the potency of pro-inflammatory microbiota might actually reside in its ability to relieve T cells from cell intrinsic mechanisms of tolerance.

OR.130. Nod2 Prevents Colitis and Carcinogenesis by Sequestrating Commensals

Aurélie Couturier[1], Thomas Secher[2], Aude Bressenot[3], Anne Delanoye-Crespin[1], Teddy Grandjean[1], Ludovic Huot[1], Laurent Perin-Biroulet[3], Sylvain Normand[1], David Hot[1], Bernard Ryffel[2], Mathias Chamaillard[1]. [1]Institut Pasteur de Lille, Lille, France; [2]University of Orléans, Orléans, France; [3]Nancy-Université, Vandoeuvre-lès-Nancy, France

A concerted inflammation is thought to be involved in maintenance of the epithelial barrier, whereas carcinogenesis is linked to enhanced inflammatory response. Colorectal cancer remains the major cause of mortality in Crohn's disease (CD), but our understanding of the underlying mechanism remains incomplete. Herein we report that genetic ablation of the CD-predisposing Nod2 gene resulted in an increased growth of tumors bearing hyper-activating mutations in the beta-catenin gene in an experimental model of colitis-associated cancer. Nod2-deficent mice showed impaired mucosal healing in the colon that was characterized by increased expression of molecules involved in cancer progression and in T cell homeostasis. Consistently, the Rip2-deficient mice showed a similar susceptibility to colitis and colitis-associated cancer. Importantly, broad-spectrum antibiotherapy improved inflammation resolution, while administration of metronidazole treatment was not effective in Nod2-deficient mice when compared to control animals. In accordance with previous findings, NOD2 deficiency was linked to changes in the composition of the intestinal microbiota. Taken together, our results indicate that NOD2 coordinates epithelialisation of wounds and prevents adenoma formation in the colon by sequestrating certain opportunistic commensals that reside in the gut.

OR.131. Microbial Symbiosis Factor of Enterobacteriaceae is Crucial for Inflammatory Bowel Disease

Kerstin Gronbach[1], Richard Darveau[2], Andreas Schwiertz[3], Henrik Köhler[4], Patrick Adam[1], Erwin Bohn[1], Ingo Autenrieth[1], Julia Frick[1]. [1]University of Tübingen, Tübingen, Germany; [2]University of Seattle, Seattle, WA; [3]University Medical Center, Erlangen, Germany; [4]Institute of Microoecology, Herborn, Germany

In inflammatory bowel diseases (IBD) the mucosal immune system interacts inappropriately with the microbiota. We analyzed whether the

composition of the intestinal microbiota has an impact on IBD development. In children with active Crohn's disease we found a decreased ratio of Bacteroidetes to Escherichia coli. Using a mouse model of IBD we found that a high proportion of Bacteroidetes was associated with protection, whereas a high proportion of E. coli was associated with development of IBD. Intervention studies revealed that treatment of healthy mice with metronidazole followed by E. coli caused development of IBD whereas treatment of mice, prone to colitis, with streptomycin followed by Bacteroides vulgatus prevented IBD. To investigate the role of Lipid A in this context, we tested an E. coli strain altered in a single Lipid A fatty acid. Administration of E. coli JM83 induced IBD whereas mice treated with E. coli JM83ΔhtrBhtrBPg+ mutant strain stayed healthy and application of E. coli JM83ΔhtrBhtrBPg+ was associated with protection from disease. These are the very first results clearly showing not only the proportion of bacteroidetes versus enterobacteriaceae, but rather the type of Lipid A acylation is a decisive factor of the intestinal microbiota to promote IBD.

OR.132. Modulation of the Gut Microbiota Impacts the Development of Colitis-associated Colorectal Cancer

Janelle Arthur[1], Joshua Uronis[1], Ernesto Perez Chanona[1], Ian Carroll[1], Anthony Fodor[2], Christian Jobin[1]. [1]University of North Carolina at Chapel Hill, Chapel Hill, NC; [2]University of North Carolina at Charlotte, Charlotte, NC

Although studies have reported a role for innate sensors in the development of colitis-associated colorectal cancer (CAC), the specific role of the microbiota in this process remains to be determined. To examine the role of microbial composition on CAC development, we treated conventionalized azoxymethane (AOM)/Il10-/- mice with the probiotic cocktail VSL#3 (1 billion cfu/animal/daily). 454 FLX-titanium sequencing revealed that VSL#3 reduced the biodiversity (Shannon diversity $p<0.05$) and altered community composition of the luminal, but not colonic adherent microbiota. These differences were detected at the level of phylum (ANOSIM R=.306, $p<0.001$), class and order (ANOSIM R=.165, $p<0.006$), with VSL#3-treated mice exhibiting a contraction of Bacteroidetes/Bacteroidia/Bacteroidales ($p<0.01$). Importantly, manipulation of the microbiota enhanced tumor penetrance ($p=0.01$), tumor multiplicity ($p=0.002$), and the penetrance of invasive adenocarcinoma ($p=0.001$) in VSL#3-treated mice compared to untreated mice. There was a high abundance of Alistipes sp. in mice fed VSL#3, and this correlated with the presence of high grade dysplasia/invasive adenocarcinoma (Pearson $p<0.05$). The extent of histological inflammation did not correlate with that of dysplasia, suggesting that microbial composition can alter the development of colorectal cancer independently of inflammation. Our results indicate that microbial composition influences the development of colorectal cancer independently of the inflammatory environment.

Vaccines II (3502)
Friday, July 8, 16:15-17:45

OR.133. Phase I "Proof of Principle:" Women Vaccinated with Virosome-Gp41-Derived Antigen Produce Mucosal Antibodies with Antiviral Properties

Frank van Engelenburg[1], Marieke van den Dobbelsteen[1], Rinaldo Zurbriggen[2], Mario Amacker[2], Michael Adler[3], Mark Spengler[3], Fabienne Anjuère[4], Lucia Lopalco[5], Morgane Bomsel[6], Anick Chalifour[7], Sylvain Fleury[7]. [1]Kinesis Pharma B.V., Breda, Netherlands; [2]Pevion Biotech Ldt., Ittigen, Switzerland; [3]Chimera Biotech GmgH, Dortmund, Germany; [4]INSERM UMR U634, Nice, France; [5]San Raffaele Institute, Milan, Italy; [6]Institut Cochin CNRS UMR8104, Paris, France; [7]Mymetics Corporation, Epalinges, Switzerland

We demonstrated that mucosal IgA/IgG antibodies induced by vaccination with rgp41 and modified P1, a peptide containing the MPER and the mucosal receptor binding motif, both derived from HIV-1 HXB2 and coupled to influenza virosomes, protect non-human primates (NHP) against vaginal heterologous SHIV challenges, in the absence of seric neutralizing antibodies. Correlation was observed between induction of HIV-1 transcytosis-blocking cervico-vaginal antibodies and protection. Modified-P1 on virosomes was used as a vaccine (MYMV101) in a double-blind, placebo-controlled Phase I study at CEVAC, involving 24 healthy women randomized in 2 Panels to monitor safety and mucosal immunogenicity: Panel 1: 10ug/dose and Panel 2: 50 ug/dose. In each Panel, 8 subjects received the vaccine and 4 subjects received the placebo through intra-muscular (weeks 0/8) and intra-nasal (weeks 16/24) administrations. The vaccine was safe and well tolerated. All vaccinees showed specific seric IgA and IgG antibodies and specific mucosal IgG in vaginal and rectal secretions (ImperacerTM technology). Gp41-specific mucosal IgA were detected in 70% of subjects from Panel 2. Vaginal antibodies exhibited strong inhibition of HIV-1 transcytosis, confirming previous results from NHP. Further analyses ongoing. This study confirms the safety profile of virosomes and the promising anti-HIV-1 mucosal responses elicited by gp41-derived virosomal vaccine.

OR.134. Prime/boost Genital Immunization with Human Papillomaviral Vectors Preferentially Induces Effector-memory CD8+ T Cells in the Mouse Female Genital Tract by Promoting Local Proliferation of CD8+ T Cells upon Secondary Immunization

Nicolas Cuburu[1], Barney Graham[2], Rhonda Kines[1], Jeffrey Roberts[1], Christopher Buck[1], Douglas Lowy[1], John Schiller[1]. [1]National Cancer Institute, National Institutes of Health, Bethesda, MD; [2]National Institute of Allergy and Infectious Dieases/National Institutes of Health Bethesda, MD

Defining optimal routes of immunization to achieve strong genital CD8+ T cell responses may impact the development of vaccines against genital infections. We previously found that intravaginal instillation of Human Papillomavirus vector (HPV) in mice led to transient expression of a reporter gene restricted to epithelial cells, and primed immune responses against a genetically-delivered antigen. Here we studied the dynamics of the CD8+ T cell responses in the mouse female genital tract after intravaginal HPV prime/boost immunization, using different HPV types in the prime and boost to overcome antibody-mediated neutralization. HPV prime/boost induced 10 times more genital Ag-specific CD8+ T cells than priming alone or than

intramuscular prime/boost with Adenovirus type-5 vector. Most genital Ag-specific CD8+ T cells were intra- or sub-epithelial, displayed effector-memory phenotype and cytotoxic activity, and were detected until 4 months after immunization. Using fingolimod (FTY720), a drug that promotes lymph node retention of lymphocytes, we found that the expansion of genital memory CD8+ T cells upon secondary HPV immunization was due to local proliferation of resident memory CD8+ T cells. These data underscore HPV vectors as attractive gene-delivery platforms to induce long lasting genital CD8+ T cell responses by promoting local proliferation of Ag-specific CD8+ T cells.

OR.135. Dissemination of Primed T Cells Following Intranasal Immunization

Annalisa Ciabattini, Elena Pettini, Fabio Fiorino, Gennaro Prota, Gianni Pozzi, Donata Medaglini. University of Siena, Siena, Italy

T cell priming after nasal immunization was studied by investigating the distribution of antigen-loaded antigen presenting cells (APCs) and primed antigen-specific T cells. Studies were conducted using the model antigen ovalbumin (OVA) plus CpG oligodeoxynucleotides adjuvant. Trafficking of antigen-specific primed T cells was analyzed *in vivo* after adoptive transfer of OVA-specific transgenic T cells in the presence or absence of fingolimod, that causes lymphocytes sequestration within lymph nodes. Antigen-loaded APCs were observed in mediastinal but not in distal lymph nodes. Antigen-specific proliferating T cells were first observed within draining lymph nodes, and later in distal iliac and mesenteric lymph nodes and spleen. The presence at distal sites was due to migration of locally primed T cells as shown by fingolimod treatment that caused reduction of proliferated T cells in non-draining lymph nodes. Homing of nasally primed T cells in iliac lymph nodes was CD62L-dependent, while entry into mesenteric lymph nodes depended on both CD62L and α4β7. Proliferating T cells were activated as shown by the modulation of CD44 and CD45RB. Different prime-boost schedules were tested to identify optimal strategies for boosting nasally primed T cells. These data provide relevant insights for the design of vaccination strategies based on mucosal priming.

OR.136. The Constant Region of the Broadly Neutralizing 2F5 Antibody Plays an Active Role in HIV-1 Affinity and Antiviral Activities: 2F5-IgA Exhibits Improved Antiviral Activities as Compared to its IgG Counterpart

Daniela Tudor[1], Anne-Sophie Drillet[1], Schwartz-Cornil Isabelle[1], Pierre Tuffery[2], Julien Maupetit[2], Morgane Bomsel[1]. [1]Institut Cochin, Paris-Descartes, Paris, France; [2]INSERM U973, Université Paris Diderot, Paris, France

The heavy chain constant region (CH) can affect antibody affinity and specificity independently of avidity, and may modulate its function. Using as model the broadly HIV-1-neutralizing human mAb 2F5, the contribution of CH region to antibody affinity/function was determined. A monomeric 2F5 IgA was constructed from the 2F5 IgG1. As compared to its IgG counterpart, 2F5 IgA (i) binds gp41 and MPER peptides with higher affinities, especially when viral target is inserted in a lipidic membrane; (ii) inhibits better binding and endocytosis of HIV-1 by dendritic cells; (iii) has enhanced HIV-1 neutralizing activity in CD4+ T cells; (iv) blocks more efficiently HIV-1 transcytosis across epithelial cells and normal human rectal mucosa *ex vivo*, but (v) shows reduced Antibody dependent cytotoxicity (ADCC). Both IgA and IgG specific epitope mapped using a peptide library. It allowed correlating the IgG/IgA functional differences to antibody respective specific epitope at the structural level. Whereas both antibodies recognize the same central LDKW, flanking residues differs and were mapped my modeling to different regions of the gp41 trimeric proteins. The CH regions participate actively to the interaction of the antibody with its target and in turn antibody affinity for antigen and antiviral activities. It is therefore crucial for designing mucosal vaccine responses, especially in the context of HIV-1, an infection transmitted mainly sexually at mucosal sites.

OR.137. Role of Protease-activated Receptor in Vaccine-induced Protection Against Helicobacter Infection

Dominique Velin[1], Nathalie Busso[1], Eric Bernasconi[1], Giancarlo Ramelli[1], Michel Maillard[1], Daniel Bachmann[1], Catherine Pythoud[1], Hanifa Bouzourene[1], Pierre Michetti[2], Alexander So[1]. [1]CHUV, Lausanne, Switzerland; [2]La Source-Beaulieu, Lausanne, Switzerland

Background: Despite the proven ability of immunization to reduce helicobacter infection in mouse models, the precise mechanism of protection has remained elusive. Protease-activated receptor (PAR2) has been implicated in inflammatory responses as well as in modulating of various gastric functions. This study explores the role of PAR2 in vaccine-induced protection against helicobacter infection. Methods: Immune responses and vaccine-induced protection to helicobacter were assessed in PAR2 deficient mice (PAR2-/-) and wild type (WT). Infection persistence, cellular responses and gastric pathology were assessed by the rapid urease test, qPCR, flow cytometry and histology. Results: Vaccinated PAR2-/- mice were unable to reduce helicobacter burden following infection. This observation correlated with a reduction in inflammation-induced stomach tissue damage and lower recruitment of CD4+IL-17+ T cells into the gastric mucosa of vaccinated PAR2-/- mice post bacterial challenge. Interestingly, splenic denditic cells (DC) from vaccinated PAR2-/- mice at day 14 post helicobacter infection exhibited a weaker activated phenotype in comparison to their WT counterparts. Finally, adoptive transfer of WT DCs into vaccinated PAR2-/- mice prior to helicobacter challenge was able to enhance vaccine-induced protection. Conclusion: Signaling pathway initiated following PAR2 activation on DCs appear to be critical in the generation of vaccine-induced protection against helicobacter infection.

OR.138. Development of a Universal Influenza Vaccine through Mucosal Immunization

Mingtao Zeng. Texas Technical University Health Sciences Center, El Paso, TX

Influenza is one of the major public health threats which have potential to become pandemic diseases. Transmission of H5N1 influenza virus from the avian populations to humans shows that an effective vaccine against H5N1 influenza is urgently needed. We have recently developed a

candidate influenza vaccine using the relatively conserved matrix protein 2 (M2) of H5N1 avian influenza A virus as an antigen delivered by a detoxified anthrax edema toxin (EF). In this study, we constructed an expression vector for optimal production of the recombinant fusion N-fragment of EF (EFn) and M2 in E. coli. We have shown the immunogenicity of this detoxified anthrax toxin, the fusion EFn/M2 plus anthrax protective antigen (PA), in mice after vaccination. The candidate vaccine was used to immunize animals by intranasal route and elicited robust systemic and mucosal antibody responses against M2. Animals vaccinated with the candidate M2-based vaccine showed both Th1 (IL-2 and IFN-γ) and Th2 (IL-4 and IL-5) cytokine responses as determined by ELISPOT and BD™ Cytometric Bead Array. These data have demonstrated the feasibility of developing a new-generation nasal vaccine which has potential for cross-strain protection as a universal influenza vaccine, using the unique detoxified toxin system.

Regulatory T Cells and Dendritic Cells (3503)
Friday, July 8, 16:15-17:45

OR.139. IL-23 Regulation of the Treg-IgA Response to the Microbiota

Katie Alexander, Ting Feng, Charles Elson. University of Alabama at Birmingham, Birmingham, AL

Purpose: An important pathway describing immune responses involving Regulatory T cells (Tregs), IgA, and the microbiota has recently been described (Cong, et al PNAS 2009). Interlukin-23 (IL-23) regulates the Th17 pathway and has recently been described to inhibit Treg cells in the intestine. In this study we asked whether IL-23 regulates the Treg-IgA reponse to the microbiota. Methods: B6, B6. IL-23p19-/- mice and B6.10bit.Foxp3.gfp reporter mice were used to examine Foxp3 and Tr1 (IL-10) cell levels. The MFB-F11 cell line was used to measure active TGFB at low levels using SEAP detection. ELISA was used to quantify IgA antibodies in pellets. Results: IL-23p19-/- mice had increased levels of lamina propria (LP) Treg cells. LP CD4 T cells produced increased amounts of active TGFB (greater than 2 fold) in the large and small bowel of B6.IL-23p19-/- mice compared to B6 mice. IL-23p19-/- mice had a 2-fold increase in total IgA as well as in flagellin-specific IgA. Conclusions: IL-23 has been at the forefront of current research as a regulator of the Th17 pathway and inhibitor of the protective Treg pathway. These data indicate that IL-23 regulates the Treg-IgA pathway response to microbiota antigens. Further examination into this relationship using Foxp3+gfp and IL-10 reporter mice will help to reveal if a system deficient in IL-23 alone is able to shift T cell differentiation into a profile favoring Foxp3 over RORc and how this mechanism employs IL-10, Foxp3 and TGFB.

OR.140. WASp Alters Regulatory T Cell Induction via Reduced Strength of T Cell Receptor Signaling

Elisa Boden[1], Paul Arnaboldi[1], Christopher Moran[2], Deanna Nguyen[2], David Dunkin[1], Lloyd Mayer[1], Scott Snapper[2]. [1]Mount Sinai Hospital, New York, NY; [2]Massachusetts General Hospital, Boston, MA

Wiskott-Aldrich Syndrome protein (WASp)-deficient (WKO) mice develop spontaneous colitis that is associated with reduced numbers of CD4+CD25+Foxp3+ regulatory T cells (Tregs). Recent unpublished studies in the laboratory have shown defects in de novo induction of Tregs in WKO mice. Here we assessed whether increased antigen dose could overcome defects in Treg induction in WKO T cells using *in vitro* induction of FoxP3+ Tregs from naive (CD4+CD25-) ova-specific transgenic WT or WKO T cells cultured in the presence of TGFβ. WKO T cells were found to have an altered threshold for the maximal induction of Tregs. While peak induction of Tregs occurred in WT at an antigen dose of 0.1 mcg/mL (33%+/-4), WKO T cells demonstrated reduced induction of Tregs at this antigen dose (19%+/-4, $p < .05$). Maximal Treg induction could be rescued in WKO T cells by increasing antigen dose 10-fold. This defect correlated with significantly reduced proliferation, IL-2 and IFNγ production from WKO compared to WT naïve T cells. *In vitro* -induced WKO Tregs were equally effective as WT at suppression of responder T cell proliferation. WASp-deficiency was also found to alter Treg differentiation *in vivo* when WT or WKO transgenic T cells were transferred into WT mice and recipient mice were injected with ova peptide. WKO T cells had a reduced proliferative response to antigen and required a 10-fold increased antigen dose for maximal induction of Tregs *in vivo*. These data suggest that WASp-deficiency reduces the strength of TCR signaling and results in an altered antigen threshold for the maximal induction of Tregs. Further understanding of the signaling pathways responsible for Treg induction may allow for the development of therapies for inflammatory bowel disease that favor increased Treg numbers.

OR.141. A Critical Role of Dietary Vitamin B9 in the Survival of Intestinal Regulatory T Cells and Consequent IgA Responses

Jun Kunisawa, Izumi Ishikawa, Eri Hashimoto, Hiroshi Kiyono. University of Tokyo, Tokyo, Japan

It is generally accepted that dietary factors are involved in the development and regulation of versatile immunological responses at mucosal compartment, but the underlying mechanisms remain to be elucidated. Here we show that vitamin B9 is a survival factor for intestinal regulatory T (Treg) cells and regulates subsequent IgA responses. In vitamin B9-null condition, Treg cells were differentiated from naïve T cells, but differentiated Treg cells failed to survive with the decreased expression of anti-apoptotic Bcl-2. *in vivo* depletion of dietary vitamin B9 resulted in the specific reduction of intestinal Treg cell-mediated regulation, leading to the increased production of IgA-enhancing cytokines (IL-5 and IL-6) and anti-inflammatory IL-10. Oral immunization of dietary vitamin B9 deficient mice with cholera toxin (CT) thus led to the enhancement of CT-specific IgA-mediated vaccine effects without the induction of any signs of inflammation in the intestine. These findings provide new links between dietary vitamin And the mucosal immune system, which can be applied for new strategy in the development of mucosal vaccine by mimicking vitamin B9-mediated intestinal immunity.

OR.143. Characterization of the APC Presenting a Microbial Polysaccharide to Regulatory T Cells

Suryasarathi Dasgupta, Dennis Kasper. Brigham and Women's Hospital and Harvard Medical School, Boston, MA

Polysaccharide A (PSA) from the human symbiont Bacteroides fragilis is a potent molecule that both prevents and cures inflammation in murine models of colitis by inducing IL-10 from CD4+ T cells. Little is known about the cellular pathways used in PSA-induced immunoregulation. Oral gavage with wild-type B. fragilis—but not with a PSA deletion mutant or PBS alone—results in a significant increase in the percentage of CD4+CD25+FOXP3+ regulatory T cells in mesenteric lymph nodes (MLNs) which correlated with the percentage of B220+CD11cint nonconventional DCs. In a colonic inflammation model involving intra-rectal administration of trinitrobenzene sulfonic acid, mice protected by pre-treatment with PSA had a significantly decreased percentage of potentially pathogenic CD11b+CD11c- non-dendritic myeloid cells. In contrast, the proportion of a subset of nonconventional DCs (marked by B220+CD11cintCD11b-Siglec H+) in MLNs was significantly higher in PSA-protected mice than in diseased mice. Under different conditions, these plasmacytoid DCs have been reported to induce regulatory T cells. We have previously described that TLR2 is a receptor for PSA. Here we show that induction of immunoregulatory T cell features by PSA *in vitro* depends on TLR2 expression on DCs and in TLR2 deficient mice PSA fails to increase PDCs subsequently failing to protect mice in the model colitis. This work suggests that PSA-dependent protection from colitis depends on plasmacytoid DCs, which may be critical in generating regulatory features in T cells as well as other molecules involved in PSA-induced immunoregulation.

OR.144. Inflammation Redirects the Differentiation Program of Monocytes from Anti-inflammatory CD11c^{+} CX3CR1^{hi} Macrophages to Inflammatory CD11c^{+} CX3CR1^{int} Dendritic Cells in the Colon Lamina Propria

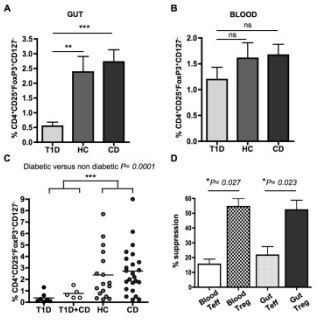
Aymeric Rivollier, JianPing He, Abhi Kole, Vassilis Valatas, Brian Kelsall. National Institutes of Health, Bethesda, MD

Dendritic cells (DCs) and macrophages (MPs) are important for immunological homeostasis in the intestine. We identified five distinct populations of mononuclear phagocytes in the mouse colon. F4/80^{hi} cells account for 80% of mouse colonic lamina propria (cLP) MHC-II^{hi} cells. These cells are CX3CR1^{hi}CD103^{-}CD11b+, and comprised of CD11c^{-} and CD11c^{+} populations, both of which were identified as MPs based on their phenotype by electron microscopy and flow cytometry, their high phagocytic and low naïve T cell stimulation capacities, and by the expression of a series of MP associated genes on microarray analysis. Both CD11c^{+} and CD11c^{-} MP populations constitutively released high levels of IL-10 at least partially in response to intestinal microbiota via an MyD88-independent mechanism. In contrast, CD11c^{+} cells expressing low to intermediate levels of CX3CR1 and F4/80 appear to be DCs based on phenotypic and functional analysis. CD11c^{+}CD11b^{-} DCs are homogeneously CD103^{+} and CD11c^{+}CD11b^{+} DCs are CD103^{+} or CD103^{-}. Ly6C^{hi} monocytes differentiated primarily into anti-inflammatory CD11c^{+}CX3CR1^{hi} MPs in non-inflammatory conditions. In contrast during colitis, Ly6C^{hi} monocytes differentiated into pro-inflammatory CD11c^{+}CX3CR1^{int}DCs, that produced IL-12, IL-23, iNOS and TNFα and massively invaded the cLP. These findings demonstrate the context-dependent differentiation of monocytes into MPs and DCs in the steady state and inflamed colon.

OR.145. Shaping the (Auto) Immune Response in the Gut: The Role of Intestinal Immune Regulation in the Prevention of Type 1 Diabetes (T1D)

Marika Falcone, Chiara Sorini, Ester Badami, Vera Usuelli, Andrea Marioa Bolla, Marina Scavini, Alberto Mariani, Emanuele Bosi. San Raffaele Scientific Institute, Milan, Italy

Environmental factors acting at the intestinal level like viral infections (enteroviruses), reactions to dietary antigens (cow's milk and gluten), and microbiota alterations (low bacteroidetes/firmicutes ratio) have been observed in association with, or as risk factors for, the development of T1D. Those factors could affect T1D by altering the gut immune regulatory system that is crucial to maintain local and systemic immune tolerance. In fact, the gut is the preferential site for extrathymic development and/or expansion of Treg cells by intestinal CD103+CD11c' antigen-presenting cells (APC). We analyzed gut mucosal immunity in T1D patients and found that the percentage of intestinal regulatory CD4+CD25+FoxP3+CD127- T cells was significantly reduced in T1D patients compared to healthy controls (P=0.003). The reduced number of Treg cells was linked to a detective function of intestinal CD11c+ CD103+ APC of T1D patients that were unable to convert CD4+CD25- T cells into CD4+CD25+FoxP3+CD127- Treg cells. Our data suggest that intestinal immune regulation is not only calibrated to tolerate commensal bacteria, food components and intestinal self antigens, but it is also instrumental in maintaining immune tolerance outside the gut and prevent autoimmune diseases like T1D.



Badami E. et al. FIGURE 2

Poster Session: Wednesday, July 6
Authors Present: 13:20-14:30

W.1. Anti-allergic Effects of So-cheong-ryong-tang, a Traditional Korean Herbal Medicine, in an Allergic Rhinitis Mouse Model

Dong-Young Kim[1], Ji-Hun Mo[2], Doo Hee Han[1], Chae-Seo Rhee[1], Chul Hee Lee[1]. [1]Seoul National University College of Medecine, Seoul, Republic of Korea; [2]Dankook University College of Medicine, Cheonan, Republic of Korea

Aim: To evaluate the anti-allergic effects of the Korean herbal medicine So-Cheong-Ryong-Tang (SCRT) in an allergic rhinitis mouse model and to examine the underlying mechanism(s) of its anti-allergic effects. Materials and methods:BALB/c mice were sensitized with ovalbumin (OVA) and alum and then challenged intranasally with OVA. The Korean herbal medicine SCRT (1 g/kg) was given to the treatment group, and multiple parameters of allergic responses were evaluated to determine the effects of SCRT on allergic rhinitis. Results: SCRT reduced allergic symptoms, such as rubbing and sneezing, and eosinophil infiltration into the nasal mucosa. It also suppressed serum total IgE, OVA-specific IgE, and OVA-specific IgG1 levels and increased OVA-specific IgG2a level. SCRT significantly reduced expression of the Th2 cytokine, IL-4; however, the expression of IL-5, IFN-γ, and IL-10 was unchanged in the nasal mucosa of the treatment group (by real-time RT-PCR). In splenocyte culture, levels of both IL-4 and IL-5 decreased, and IFN-γ level increased in the treatment group; however, levels of IL-10 and TGF-β were unaffected by administration of SCRT. Conclusions: This study shows that the Korean herbal medicine SCRT induced anti-allergic effects by decreasing, locally and systemically, the Th2 cytokine IL-4, isotype switching to IgE, and eosinophilic infiltration into the nasal mucosa in an allergic rhinitis mouse model. Thus, SCRT may be useful as a potential therapeutic agent in treating allergic rhinitis.

W.2. FcRn-mediated Absorption of IgG-IgE Immune Complexes

Adam Matson, Lynn Puddington. University of Connecticut School of Medicine, Farmington, CT

The mechanism(s) responsible for the acquisition of maternal antibody isotypes other than IgG are not fully understood. Using a murine model of allergic airway disease (AAD), we demonstrated that allergen-specific IgG1 and IgE are absorbed into the circulation of naïve offspring nursed by allergic mothers (Matson et al., 2007, Matson et al., 2009). Interestingly, the absorption of allergen-specific IgE by breastfed offspring was dependent on the neonatal Fc receptor for IgG uptake FcRn. Although it is generally thought that FcRn does not bind IgE, our data provide compelling evidence that FcRn plays a pivotal role in the acquisition of this antibody isotype. Further investigation demonstrated serum levels of antigen-specific IgE in breastfed pups correlated strongly ($rho = 0.78$, $p < 0.001$) with the concentration of IgG anti-IgE in maternal serum, suggesting a role for anti-IgE autoantibodies in the acquisition of maternal IgE. To further explore this possibility, FcRn-sufficient neonatal mice were fed IgG anti-IgE/IgE immune complexes or isotype control IgG/IgE. Mice fed IgG anti-IgE/IgE immune complexes absorbed IgE efficiently into the systemic circulation; whereas mice fed isotype control IgG/IgE did not. These data suggest a novel mechanism by which FcRn may facilitate the absorption of maternal antibodies other than IgG.

W.3. Metal-allergy Cross-reactions in Mice

Masayuki Kinbara, Yasuhiro Nagai, Teruko Takano-Yamamoto, Yasuo Endo, Shunji Sugawara. Tohoku University, Sendai, Japan

Objective: Patients with metal-allergy often react with several metals in patch-testing. Because the details of metal-allergy remain unclear, it is difficult to judge whether such plural reactions reflect the reactions to individual metals or cross-reactions. We previously found that lipopolysaccharide (LPS) markedly promotes metal-allergy in mice at both the sensitization and elicitation steps. Here, we examined metal-allergy cross-reactions using this mouse model. Methods: A mixture of a metal salt and E. coli LPS was injected intraperitoneally into mice. Ten days later, a metal salt, with or without LPS, was challenged intradermally via ear pinnas, and ear thickness was measured. We used both ultra-pure (>99.99%, UPMs) and low purity (>93%, LPMs) metal salts. Results: In the experiments using UPMs (Ni, Pd, Co, Cr, and Cu), Ni and Pd cross-reacted. However, there were no cross-reactions among Ni, Co, Cr, and Cu. Surprisingly, in the experiments using LPMs, all the tested metals (Ni, Pd, Co, Cr, and Cu) cross-reacted. Discussion: The above results suggest that highly pure metal materials must be used for identifying metal allergens by patch-testing. It should be noted that Ni and Pd belong to the same group (group 10) in the periodic table of elements.

W.4. Rcan1 is Required for the Development of Pulmonary Eosinophilia in Allergic Inflammation in Mice

YongJun Yang[2], TongJun Lin[1]. [1]Dalhousie University, Halifax, NS, Canada; [2]College of Animal Sciences and Veterinary Medicine, Changchun, China

The presence of eosinophils in the lung is often regarded as a defining feature of asthma. Upon allergen stimulation, numbers of eosinophils and their progenitors are increased in both the bone marrow and lungs. Eosinophil progenitors provide an ongoing supply of mature eosinophils. Here, we report that Rcan1 deficiency leads to a near complete absence of eosinophilia in ovalbumin (OVA)-induced allergic asthma and reduced lung inflammation in mice. In the absence of Rcan1, bone marrow cells produce significantly fewer eosinophils *in vivo* and *in vitro* upon IL-5 stimulation. Importantly, Rcan1-/- mice contain significantly reduced eosinophil progenitors in naïve and OVA-challenged mice. Interestingly, bone marrow cells from Rcan1-/- mice are capable of developing into fully mature eosinophils, suggesting that Rcan1 is required for eosinophil progenitor production, but may not be necessary for eosinophil maturation. Thus, Rcan1 represents a novel contributor in the development of eosinophilia in allergic asthma through regulation of eosinophil progenitor production.

W.5. Mother to Child Transfer of IgG and IgA Antibodies Against Dermatophagoides Pteronyssinus

Patricia Macchiaverni[2], Christina Arslanian[1], Josias Frazão[1], Patricia Palmeira[1], Momtchilo Russo[1], Valerie Verhasselt[2], Antônio Condino-Neto[1]. [1]University of São Paulo, São Paulo, Brazil; [2]INSERM U924, Valbonne, France

Background: Experiments in rodents have shown that placental and/or breast milk-mediated transfer of maternal allergen specific IgG to the progeny prevent allergic immune responses and that IgA are associated with immune tolerance. Little is known about maternal transfer of IgG and IgA specific for respiratory allergens in humans. Dermatophagoides pteronyssinus, is an indoor allergen that represent a major cause of asthma. Methods: Blood and colostrum samples were collected from atopic (n=29) and non-atopic mothers (n=48) and cord blood from their respective newborns. We quantified Derp specific IgG and IgG subclasses in paired samples of maternal and cord blood as well as Derp specific S-IgA and IgG in colostrum samples. Results: We found Der p specific IgG and their IgG1, IgG2 and IgG4 subclasses in cord blood. Except for IgG1, their levels were higher in cord blood of newborns from atopic mothers as compared to non-atopic mothers. Der p specific IgA were found in colostrums and levels were similar whatever the maternal atopic status. In addition to IgA, anti-Der p IgG was found in colostrum and their levels correlated with maternal blood anti-Der p IgG. Conclusions: Our observations provide evidence that anti-Der p IgG are efficiently transferred not only through placenta but also through colostrum and that higher levels are found in samples from atopic mother as compared to non-atopic mothers. In addition, anti-Der p IgA is transferred by colostrum. Clinical studies are now required to assess whether anti-Der p IgG and IgA protect the child from allergy as demonstrated in animal studies.

W.6. Oral Administration of Poly-gamma-glutamate Alleviates Atopic Dermatitis in NC/Nga Mice by Modulating IgE Production and the Th1/Th2 Cytokine Balance

Taeyoung Lee[1], Young-Sook Kim[1], Moon-Hee Sung[2], Haryoung Poo[1]. [1]Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea; [2]Kookmin University, Seoul, Republic of Korea

We previously reported that high molecular mass poly-γ-glutamate (γ-PGA) isolated from Bacillus subtilis sp. Chungkookjang could induce the production of Th1 cytokines such as IL-12 and IFN-γ. Here, we investigated the therapeutic effect of γ-PGA in alleviating atopic dermatitis (AD)-like skin disease in an NC/Nga mouse model, and show that this effect is associated with changes in IgE levels and the Th1/Th2 cytokine balance. NC/Nga mice orally dosed with γ-PGA showed dramatic reductions in dermatitis clinical score and scratching behavior; these effects were comparable to those seen in dexamethasone (Dex)-treated mice. Compared to the serum levels in phosphate-buffered saline (PBS) control-treated mice, the anti-AD effects of γ-PGA or Dex were accompanied by decreases in the serum levels of total IgE (PBS: 1,358 ± 458 ng/ml; γ-PGA: 441 ± 120 ng/ml; Dex: 289 ± 162 ng/ml) and IgG1 (PBS: 1,351 ± 140 μg/ml γ-PGA: 550 ± 200 μg/ml; Dex: 289 ± 162 μg/ml), but a slight increase in the level of IgG2a (PBS: 227 ± 116 μg/ml;γ-PGA: 453 ± 187 μg/ml; Dex: 208 ± 85 μg/ml). The splenic level of IL-10 was downregulated in γ-PGA- and Dex-treated mice (PBS: 2,678 ± 691 pg/ml;γ-PGA: 1,269 ± 229 pg/ml; Dex: 1,592 ± 1,270 pg/ml), while those of IFN-γ and IL-12 were increased in γ-PGA treated mice. Oral administration of γ-PGA decreased the histological thickening of the epidermis/dermis and the accumulation of mast cells, and the IL-4 and IL-5 levels significantly decreased in the skin lesions. Collectively, these results indicate that oral administration of γ-PGA may be a potential therapeutic strategy for treating AD.

W.7. Presence of IL-10-independent Pathway for Tolerance Induction in a Murine Model of Intestinal Allergy

Manja Burggraf[1], Haruyo Nakajima-Adachi[2], Satoshi Hachimura[2], Maren Krause[1], Hiroshi Kiyono[2], Stefan Vieths[1], Masako Toda[1]. [1]Paul-Ehrlich-Institut, Langen, Germany; [2]University of Tokyo, Tokyo, Japan

Oral immunotherapy (OIT) has gained attention as a therapy for food allergies. To investigate mechanisms of tolerance induction by OIT, we established a mouse model of intestinal allergy. BALB/c mice were sensitised with Ovalbumin (OVA, a major egg white allergen)/ALUM and fed egg-white diet (EW-diet). During the first seven days of EW-diet, OVA-sensitised mice (OVA/EW mice) developed gastrointestinal symptoms (e.g. weight loss and lethargy), which eventually recovered if the feeding was further continued. Upon *in vitro* stimulation with OVA splenic $CD4^+$ T cells of OVA/EW mice showed less proliferation than OVA-sensitised mice on conventional diet, suggesting induction of systemic tolerance by EW-diet. $CD4^+$ T cells from mesenteric lymph nodes (MLNs) of OVA/EW mice produced the Th2-type cytokine IL-4 and a regulatory cytokine IL-10 in response to OVA. Interestingly, not $CD4^+CD25^+$ T cells, but $CD4^+CD25^-$ T cells in MLNs were the major source of IL-10. OVA-sensitised IL-10-deficient mice on EW-diet presented a more dramatic weight loss, but recovered from their clinical symptoms. In the IL-10 deficiency, induction of systemic tolerance was maintained. These results suggest that IL-10 has a regulatory effect during the development of gastrointestinal symptoms, but not on the resolution and induction of systemic tolerance in our mouse model of intestinal allergy.

W.8. Analysis of Antigen Incorporation and Procession around the Sublingual Mucosa in Sublingual Immunotherapy

Daisuke Shiraishi, Yasuhiro Nagai, Yukinori Tanaka, Yasuo Endo, Hidetoshi Shimauchi, Shunji Sugawara. Tohoku University Graduate School of Dentistory, Sendai, Japan

Introduction: Antigen-specific sublingual immunotherapy is safe and efficient in treating type I allergies. In this study, we analyzed the mechanism of the allergen incorporation in sublingual compartment and characterized cells involved in these phenomenon by immunohistology. Materials and Methods: Ovalbumin (OVA) solution, latex beads, and pHrodo E. coli Bioparticles were applied under tongue of Balb/c mice, and the sublingual

tissue was dissected at 0.5, 1, and 2 hours after administration. Tissue sections were serially cut and subjected to immunohistochemistry using anti-OVA, anti-MHC class II, anti-CD11c antibodies, and specific M cell marker UEA-1 to reveal localization of OVA and its incorporating cells, and antigen processing cells. Results and Conclusion: At 0.5 hours after administration, substantial amounts of OVA were detected at the ductal system in the submucosal tissue. Some MHC class II positive cells were localized at the around of the sublingual ductal system. Some of them were CD11c positive cells. Tissue sections administered with fluorescent particles such as microbes showed that the particulate can enter into UEA-1 positive sublingual ductal cells. These results suggest that M cell-like cells in sublingual ductal system has a role in antigen incorporation in sublingual compartment.

W.9. Infant Gut Microbiota with Dominance of Bifidobacterium Spp. and Bacteroides Spp. is Protective Against Cow's Milk Allergy Despite Immature Ileal T Cell Response

Anne Judith Waligora[2], Bertrand Rodriguez[2], Guenolee Prioult[1], Feriel Hacini-Rachinel[1], Deborah Moine[1], Anne Bruttin[1], Catherine Ngom-Bru[1], Bernard Berger[1], Annick Mercenier[1], Marie José Butel[2]. [1]Nestlé Research Center, Lausanne, Switzerland; [2]Université Paris Descartes, Paris, France

Faecal commensal microbiota in healthy infant displays a large abundance in Bifidobacterium spp. and Bacteroides spp. Although some studies indicated an association between these two genera and allergy, it still remains a subject of debate. Using a gnotobiotic mouse model of cow's milk allergy, we aimed at investigating the impact of an infant gut microbiota mainly composed of Bifidobacterium spp. and Bacteroides spp. on immune activation and subsequent allergic manifestations. The dominance of Bifidobacterium and Bacteroides was preserved in faecal samples of gnotobiotic mice. The transplanted microbiota induced T cell responses in different organs similar to those observed in conventional mice, except in the ileum. Allergic response to whey proteins was then monitored in germ-free, gnotobiotic and conventional mice. Gnotobiotic and conventional mice displayed lower drop of rectal temperatures upon oral challenge with β-lactoglobulin, lower plasma mMCP-1 and anti-β-lactoglobulin IgG1 than germ-free mice. Interestingly, the foxp3 gene was highly expressed in the ileum of gnotobiotic and conventional mice that were protected against cow's milk allergy. This work showed for the first time that a transplanted healthy infant microbiota mainly composed of Bifidobacterium and Bacteroides had a protective impact on food allergy despite altered T cell response in the ileum.

W.10. Staphylococcal Enterotoxin B (SEB) Enhances Allergic Sensitization to Cutaneously Administered Ovalbumin (OVA)

Kirsi Jarvinen-Seppo, Hugh Sampson, Cecilia Berin. Mount Sinai School of Medicine, New York, NY

Toxin-producing S. aureus is found on the skin in ~90% of patients with atopic dermatitis. Because skin has emerged as a potential route of sensitization in food allergy, we examined the adjuvant potential of SEB in the induction of food allergy from cutaneous food exposure. After hair removal, C3H/HeJ mice were exposed to OVA ± SEB or cholera toxin (CT) as a positive control for 7 weekly topical administrations without adhesive dressing. To assess clinical reactivity to an oral feeding of OVA, symptom scores and temperatures were recorded. Serum OVA-specific IgE, IgG1 and IgG2a were measured by ELISA. Surprisingly, OVA alone induced sensitization, which was further enhanced by using an adjuvant. Symptoms scores (median 3) were comparable in mice exposed to OVA+SEB, OVA+CT or OVA alone, but the SEB group reacted to lower dose of OVA (median 25 mg vs. 50 mg) and there was a trend toward a greater drop in body temperature (median 33.3°C vs. 34.9°C). In conclusion, SEB is a potent cutaneous adjuvant and may play a role in clinical food sensitization in subjects with atopic dermatitis. Further studies will address the sensitization potential of SEB when suboptimal doses or weaker allergens such as milk are studied.

W.11. Maintenance of Excess IL-4 Production in Mesenteric Lymph Node Developed IgE-independent Food Allergic Enteropathy

Haruyo Nakajima-Adachi[1], Hiroshi Kiyono[1], Satoshi Hachimura[1], Shuichi Kaminogawa[2], Masafumi Yamamoto[2], Emiko Koike[1], Miran Kihara[1], Mamoru Totsuka[1], Takeshi Makino[3], Ryuichiro Sato[1], Akira Kikuchi[1], Osamu Igarashi[2], Yoko Fujimura[1]. [1]University of Tokyo, Tokyo, Japan; [2]Nihon University, Chiba, Japan; [3]Japan SLC, Inc., Shizuoka, Japan

IL-4 has been shown to behave as both non-inflammatory and inflammatory molecule in the intestinal immune system. To clarify how these two functions of IL-4 are regulated in the induction of food allergic enteropathy, a unique food allergic model of T cell receptor transgenic OVA23-3 mice were employed and compared with DO11.10 mice sensitized with egg-white-diet alone. In OVA23-3 mice, the enteropathy was inhibited by anti-IL-4 Abs and CsA administration, while RAG-2-deficiency had no effect, showing critical regulation by IL-4 and T cells, not IgE. In DO11.10 mice, which did not develop the enteropathy, the highest IL-4 production was obtained in splenic, not mesenteric lymph node (MLN) CD4+T cells by 2 days egg-white-diet feeding, followed by immediate abrogation of the IL-4 response. Conversely, MLN CD4+T cells of egg-white-diet fed OVA23-3 mice showed significantly higher IL-4 production than splenic CD4+T cells. The significant IL-4 response in MLN was maintained even when systemic tolerance was induced on Day 28. Different from DO11.10 mice, the condition maintaining IL-4 production by CD4+T cells was made by instantaneous and excess IL-4 in MLN, not in spleen of egg-white-diet fed OVA23-3 mice. The maintenance would play critical pathological role in the development of IgE-independent food allergic enteropathy.

W.12. Lipopolysaccharide Induces Unidentified Partner Molecules that Possess Nickel Allergy-promoting Activity

Yukinori Tanaka, Masayuki Kinbara, Yasuhiro Nagai, Toshinobu Kuroishi, Yasuo Endo, Shunji Sugawara. Tohoku University Graduate School of Dentistry, Sendai, Japan

Background: Metal ions are known to be contact allergens. Unlike classical haptens, metal ions form reversible coordination complexes with partner molecules. It remains to be clarified the precise mechanism of metal ion transport by potential carrier proteins to antigen presenting cells and the antigenic determinants of metal-protein complexes. We previously reported that lipopolysaccharide (LPS) promotes nickel (Ni) allergy in a murine model. From this observation, we speculate that LPS might induce partner proteins, such as Ni-binding proteins, which might promote Ni presentation. Methods: We collected serum from mice that had received LPS, which was called “LPS-serum.” To examine Ni allergy-promoting activity of LPS-serum, a mixture of 1 μM $NiCl_2$ (lower than minimum allergy-inducing concentration) and fractionated LPS-serum was injected i.d. into ears of Ni-sensitized mice, and ear swelling was measured. Results: The mixture of 1 μM $NiCl_2$ and LPS-serum induced allergic ear swelling. Then, we fractionated the LPS-serum by ion-exchanging column chromatography and by ultrafiltration. Ni allergy-promoting activity of original LPS-serum was retained in the flow-through fraction of anion exchanger at pH 7.5 and in the fraction under 50 kDa. Discussion: Our results implied the presence of putative partner proteins. To identify them, further purification of LPS-serum is underway.

W.13. Cooking Enhances Recognition of Linearized, but Not of Native Ovomucoid

Franziska Roth-Walter[1], Philipp Starkl[1], Michael Gartner[1], Richard Brunner[1], Janis Kinkel[1], Torsten Zuberbier[2], Erika Jensen-Jarolim[1], Tamar Kinaciyan[1]. [1]Medical University of Vienna, Vienna, Austria; [2]Charité - Universitätsmedizin Berlin, Berlin, Germany

Background: Egg-allergy is usually outgrown within few years. Patients in which egg-allergy persist have a larger proportion of IgE against sequential rather than conformational epitopes of egg proteins. Also cooked egg has been reported to be better tolerated than raw egg. Hence, the impact of cooking to the major egg allergen, ovomucoid in its native and linearized form was investigated. Methods: Ovomucoid was linearized and analyzed by CD-spectrometry. 18 Patients sera with egg allergy were tested against native and linearized ovomucoid, cooked and uncooked, by Western Blot & ELISA. Results: CD-spectroanalysis revealed denaturation of ovomucoid upon linearization, however not by cooking. In Western Blot analysis with cooked samples more patients had IgE against linearized than native ovomucoid. In ELISA patients IgE recognized better native than linearized ovomucoid. Upon cooking however, IgE reactivity towards native ovomucoid was significantly reduced, whereas increased to linearized ovomucoid. Conclusion: We provide evidence that cooked conformational intact ovomucoid is better tolerated than “raw” ovomucoid, since IgE binding is impaired. However, cooking of linearized ovomucoid increased IgE binding in ELISA. Hence, dependent on patients IgE against sequential or conformational epitopes of ovomucoid, cooked egg will be rather tolerated-by transient than persistent egg-allergic patients.

W.14. Allergic Lung Disease Induced by Serine or Cysteine Proteases

Esther Florsheim, Ivan Bragatto, Eliane Gomes, Clélia Ferreira, Momtchilo Russo. University of São Pãulo, São Pãulo, Brazil

Intrinsic protease activity is recognized in some groups of airborne allergens. Here we compared lung allergic inflammation induced by serine or cysteine proteases in a murine model of asthma. Subtilisin, a serine protease, was widely used in the detergent industry during the 60s, which coincidently resulted in an increased incidence of occupational asthma. Papain, a cysteine protease associated with occupational allergy in humans, was also investigated. BALB/c mice were sensitized with 5 μg of subtilisin or papain co-adsorbed onto alum subcuteanously on days 0 and 7 and challenged intranasally on days 14 and 21. The development of allergic lung disease was analyzed on day 22. Sensitization and challenge with subtilisin or papain increased total IgE levels (8.8±1.7 μg/mL; 5.1±0.9 μg/mL, respectively) as well as specific IgG1 (958.0 ng/mL; 837.2 ng/mL). In both cases, the basal levels were under detection. The number of inflammatory cells in broncoalveolar lavage (BAL) (6.9x105±0.7; 7.2x105±0.9) was also increased when compared to control groups. Differential cell counts revealed that BAL cells were constituted mainly of macrophages and eosinophils (Mac: 36% and 28%; Eos: 35% and 22%, respectively). In conclusion, our data establish new murine models to study allergic inflammation induced by serine or cysteine proteases.

W.15. Role of Neonatal Natural Killer T Cells in the Downregulation of Post-respiratory Syncytial Virus-infection Associated Dendritic Cell Polarization Towards Plasmacytoid Dendritic Cell upon Exogenous Administration of Osteopontin

Kainath Durre, Brooks Beal, Keith Habeeb, Nagib Qureshi, Mahboob Qureshi. Touro University Nevada College of Osteopathic Medicine, Henderson, NV

A predominant Th2 response is implicated into the pathogenesis of post-RSV infection associated bronchial asthma. Further, osteopontin and NKT cells have been reported to be involved with induction of Th1 and Th2 type host-immune responses, respectively. NKT cell modulation of T helper cell function is associated with NKT cells’ modulation of dendritic cells (DCs) towards plasmacytoid DCs (pDCs). Therefore, in our present studies, using an NKT cell KO (CD1d-/-) mouse model, we have studied the role of osteopontin and NKT cells in modulating the polarization of DCs towards pDC in RSV-infected neonatal lungs. Flowcytometric analysis determined that there was an enhanced NKT cell (CD19-CD3-CD1d+) response in the RSV-infected neonatal lungs compared to that in the infected adult lungs. There was a decrease in the NKT cell response to RSV infection in the neonatal lungs upon concomitant administration of exogenous osteopontin as compared to those without osteopontin. Furthermore, in the absence of NKT cells (CD1d-/-), RSV-induced polarization of DC towards pDC was inhibited upon osteopontin administration. Thus, osteopontin-mediated

downregulation of neonatal pDC response to RSV infection, even in the absence of NKT cells, warrants further investigation into involvement of additional factors in the development of RSV-induced bronchial asthma in later life.

W.16. Oral Immunotherapy with an Immunodominant T Cell Epitope Alleviates Allergic Reactions in a Balb/c Mouse Model of Egg Allergy

Prithy Rupa, Yoshinori Mine. University of Guelph, Guelph, ON, Canada

Allergen-specific T cell epitopes are obvious targets for intervention in allergic disease. T cell epitope peptides given orally may provide a practical way of inducing tolerance and prevent allergy. This study investigates oral immunotherapy (OIT) with T cell epitope peptides of the dominant egg-white allergen Ovomucoid (Ovm) in a Balb/c mouse model of egg allergy. Groups of mice were orally sensitized to Ovm and subsequently administered Ovm T cell epitopes (single-157-171:SP or multiple-(157-171)3:MP peptide) followed by oral challenge with Ovm. Outcomes were measured as clinical signs, serum histamine, antibody activity (IgG; IgE; IgG1, IgG2), cytokines and T-regulatory cells. Clinical signs were less frequent and lower specific IgE in both SP and MP groups ($p \leq 0.05$). However, the SP-treated mice had more IgG2 and less histamine, and IgG1 indicating a type-1 bias ($p \leq 0.05$). Concentration of type-2 cytokine interleukin IL-4 was significantly less in both group and the regulatory cytokine IL-10 was more in the SP-treated mice ($p \leq 0.001$). There was significant increase in percentage of CD4+Foxp3+ and CD4+CD25+ cells in the SP group indicating significant role of T-regs in immune regulation. Thus, OIT with SP significantly reduced subsequent frequency of allergy to Ovm and validates potential use of Ovm T cell epitope as an immunoregulator.

W.17. Mucosal and Systemic Immune Responses Elicited in a Mouse Model of Food Allergy Provide *in vivo* Evidence of Cross-reactivity Between Cow's Milk and Soybean Proteins

Paola Smaldini, Renata Curciarello, Maria Angela Candreva, Silvana Petruccelli, Carlos Alberto Fossati, Guillermo Docena. University of La Plata, La Plata, Argentina

Food allergy animal models have been used to study different aspects of allergic reactions. We used a food allergy mouse model to study *in vivo* the cross-reactivity between cows-milk (CMP) and soy-proteins (SP) BALC/c mice were orally sensitised with cholera toxin plus CMP, and challenged with CMP or SP. Symptoms elicited, plasma histamine level, and humoral, cellular and mucosal response were analysed. Th1-and Th2-associated cytokines and their transcription factors were assessed in splenocytes and at mucosal sites. We found that immediate symptoms elicited in CMP-sensitised mice orally challenged with SP were consistent with an increase in plasma histamine concentration. Serum levels of CMP-specific-IgE and IgG1 antibodies were increased. The same antibodies also recognised SP. Cellular immunity tests showed that splenocytes and MLN cells secreted IL-5 and IL-13 after incubation with CMP or SP. mRNA expression of Th2-associated genes (IL-5, IL-13, and GATA-3) was up-regulated in mucosal samples, indicating a local activation of immune cells. Moreover, sensitised animals exhibited positive cutaneous tests after injection of CMP or SP. In conclusion, we demonstrate that CMP-sensitised mice mount a relevant clinical immune response against SP, which may constitute the basis for the development of a tolerogenic procedure for CMA using soy proteins.

W.18. Clinical Manifestations in Diarrhea-prone Irritable Bowel Syndrome Patients are Associated with Increased Humoral Immunity in the Jejunal Mucosa

Ana Maria Gonzalez-Castro[1], Cristina Martinez[1], Beatriz Lobo[1], Marc Pigrau[1], Mar Guilarte[2], Carmen Alonso[1], Meritxell Guila[1], Ines deTorres[2], Fernando Azpiroz[1], Javier Santos[1], Maria Vicario[1]. [1]Institut de Recerca Vall Hebron, Barcelona, Spain; [2]Hospital Universitary Vall Hebron, Barcelona, Spain

Background: Low-grade intestinal inflammation and abnormal immunological function have been implicated in the pathophysiology of irritable bowel syndrome (IBS). However, the role of B cells and humoral responses in the intestinal mucosa in IBS remains unknown. Methods: Mucosal jejunal biopsies were obtained from healthy volunteers (H; n=12) and age-matched naïve participants meeting diarrhea-IBS Rome II criteria (IBS-D; n=17). The number of B cells (CD20+) and plasma cells were assessed by immunohistochemistry and transmission electron microscopy, respectively. The expression of genes involved in B-cell homeostasis, maturation and immunoglobulin production was quantified by PCR. Abdominal pain, number of bowel movements and stool consistency were also recorded. Results: The number of CD20+ cells and plasma cells (also with ultrastructural signs of activation) were increased in IBS-D group ($P<0.05$). Gene expression of BCMA (B cell maturation antigen), BAFF-R (B cell-activating factor receptor), TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor) and the immunoglobulin heavy chains IGHA, IGHG1, IGHG4 and IGHE were significantly increased in IBS-D respect to H ($P<0.05$). Furthermore, mRNA expression of genes related with immunoglobulin production positively correlated with bowel movements and stool consistency. Conclusion: Enhanced local humoral activity may contribute to the pathophysiology and clinical manifestations in IBS-D patients.

W.19. A Rat Model of Food Allergy Using Intraperitoneal Sensitization and Oral Exposure to Ovalbumin

Maria del Mar Abril Gil, Malen Massot Cladera, Teresa Pérez-Berezo, Sara Ramos-Romero, Cristina Castellote, Francisco Perez-Cano, Margarida Castell, Angels Franch. University of Barcelona, Barcelona, Spain

Various experimental models of rodents have been established in an attempt to provide insights into the pathophysiological and immune mechanisms of human food allergic diseases. However, as yet, none of these methods has been gained widespread acceptance. The purpose of the

present study was to develop a new rat model of food allergy to ovalbumin (OVA). Eight-week-old Lewis rats were firstly sensitized with an OVA emulsion with adjuvant administrated by intraperitoneal route. Fourteen days later rats were given OVA by oral gavage daily for three weeks. Blood samples were obtained weekly in order to analyze the sensitization immune response by means of sera specific anti-OVA antibodies quantification by ELISA. The results of the antibody response at 5 weeks after OVA i.p administration showed that rats receiving daily OVA by oral gavage developed a higher anti-OVA antibody response of IgG1, IgG2a and IgG2b isotypes than those animals which were non-orally challenged. However, serum anti-OVA IgE antibodies were not detected in any of both groups. In conclusion, the protocol here evaluated induces oral sensitization to OVA in Lewis strain rats, however this response is not associated to IgE, the main isotype involved in the allergic reaction.

W.20. **Circulating γδ T Cell**s: Predictors of Atopic Disease in Childhood?

Anna Thysen[1], Jeppe Larsen[1], Klaus Bønnelykke[2], Hans Bisgaard[2], Susanne Brix[1]. [1]Technical University of Denmark, Lyngby, Denmark; [2]Copenhagen University, Copenhagen, Denmark

Atopic diseases are the most common chronic diseases among children and represent a heterogeneous group with little to differentiate clinical presentation into defined disease endotypes. γδ T cells are innate-like cells with different functionalities dependent on tissue distribution and antigen receptor repertoire. In human blood, the main γδ T cell type express the Vδ2 chain and are responsive to phosphoantigens. While γδ T cells with surface Vδ1 chains are primarily found in mucosal tissues. Previous human and murine studies have suggested a role for γδ T cells in the development of atopy. Here, we enumerate γδ T cells in the peripheral blood from a total of 76 eighteen-month-old children enrolled in the Asthma Begins in Childhood birth cohort study. The percentage of γδ T cells within the CD3 positive T cell population do not correlate to a diagnosis of atopic dermatitis (n=17). Nor does a parent history of atopic dermatitis, rhinitis, or asthma influence the level of γδ T cells. Next, we will characterize multiple cytokine responses to phosphoantigen-specific activation of γδ T cells. This will, hopefully, reveal differences in the functionality of circulating γδ T cells in children with future atopic disease.

W.21. Allergen-neoglycoconjugate Complexes of Mannan and Papain Facilitate Uptake by Antigen Presenting Cells

Esther Weinberger, Christina Ramsauer, Peter Hammerl, Josef Thalhamer, Martin Himly, Weiss Richard. University of Salzburg, Salzburg, Austria

Sublingual immunotherapy (SLIT) has been developed as non-invasive alternative to subcutaneous immunotherapy (SCIT) and proved its clinical safety and efficacy in patients suffering from rhinoconjunctivitis to mild asthma for more than fifteen years. After all, one major drawback of this therapy are the considerably high allergen dosages necessary for successful therapeutic outcome. Therefore, enhancing uptake of mucosally applied antigens by targeting C-type lectin receptors of resident oral mucosal dendritic cells (oDCs) using carbohydrate-protein neoglycoantigens has been investigated. Here, we evaluated the potential of delivering the model allergen papain to oDCs based on carbohydrate-mediated targeting of DC-expressed lectins. For this purpose, two different carbohydrates, namely mannan derived from S. cerevisiae and dextran derived from Leuconostoc spp., differing in size and microbial origin were covalently coupled to papain (nCarp1), generating high molecular-weight neocarbohydrate-allergen complexes. Complexes were analyzed by SDS-PAGE and dynamic light scatter (DLS). First experimental results show that, mannan-papain complexes (MN-Pap) elicit superior uptake by RAW 264.7 macrophages compared to papain. Furthermore, the mucosal uptake in a Balb/c mouse model in terms of cross-reacting humoral and cellular immune responses to ensure B and T cell epitope integrity were analysed so far.

W.22. Uptake of Antigen through Intestinal Epithelium Leads to the Generation of CD8+ Regulatory T Cells in the Mesenteric Lymph Node

Paul Arnaboldi, Lloyd Mayer. Mount Sinai School of Medicine, New York, NY

CD8+ regulatory T cells are an important, albeit poorly studied, component of the immune repertoire. We are utilizing a murine model in which antigen-specific CD8+ Tregs are generated to the OVA-derived peptide, SIINFEKL, to identify the sites and cells involved in CD8+ Treg induction. Within 10 min of feeding FITC-labeled SIINFEKL to C57BL/6 mice there is detectable fluorescence in the epithelial layer and lamina propria of the small bowel, but not Peyer's patches. The fluorescent signal quickly moves distally and is no longer observed in the small intestine by 30 minutes post-feeding, indicating that the peptide is cleared from the intestine rapidly. In mice that have been engrafted with transgenic OT-I cells, specific for the SIINFEKL peptide, there is a preferential accumulation of these cells in the MLN and spleen following feeding, suggesting that these are sites of antigen presentation. There is clear site specificity, however, to the generation of CD8 Tregs, as tolerance to the OVA protein can be transferred with CD8+ cells isolated from the MLN, but not the spleen, of SIINFEKL-fed mice. These data suggest that SIINFEKL is taken up from the lumen of the gut through intestinal epithelial cells and transferred to the MLN for presentation to CD8 cells. Thus, there is distinct site specificity with regard to the generation of CD8+ Tregs in response to intestinal antigen that is distinct from that of CD4+ Tregs (which were previously shown to be induced through PPs).

B Cells & Lymphoid Organogenesis

W.23. Evolution of Intestinal T and B Cells from Birth in Human Infants

Claire Gustafson[1], Alison McMahon[1], Dana Higbee[1], Ron Bouchard[2], Paul Jedlicka[1], Edward Janoff[1]. [1]University of Colorado, Aurora, CO; [2]Denver VA Medical Center, Denver, CO

With limited antigen exposure in utero, infants must begin recruitment of mucosal B and T cells soon after birth. We characterized the frequency of B and T cell subsets in histologically normal rectal tissues from 63 children (25 to date) by immunohistochemistry and 3-color immunofluorescence. We identified no plasma cells within one week of birth. However, B cells with cytoplasmic IgA (cIgA), cIgM, and cIgG in the lamina propria (LP) increased over time (all $p < 0.02$). By 12 months, the number of cIg+ cells in infants remained lower than that in 5 adult intestinal tissues (1822 vs. 5450 cells/mm^2; p= 0.0007). The proportion of cIgM+ peaked at one month and declined thereafter (50.0%, 1mo vs. 13.46%, 2mo), while cIgA+ predominated after one month (81.06%, 2mo). We identified CD138+ plasma cells primarily in the LP, but CD20+ B cells were exclusively identified in isolated lymphoid follicles. CD3+, CD4+, and CD8+ T cell densities in LP and epithelium did not increase consistently with age (all $p > 0.09$). Thus, although cIgM+ B cells were predominant in early infancy, progressive accumulation of class-switched cIgA+ cells was not dependent on CD4+ T cell numbers, but the role of subsets, e.g. Tregs, is undetermined.

W.24. Differential Regulation of Isolated Lymphoid Follicle Development in the Small and Large Intestine

David Donaldson, Neil Mabbott. Roslin Institute and Royal School of Veterinary Studies, Edinburgh, United Kingdom

Isolated lymphoid follicles (ILF) represent a dynamic way in which gut lymphoid system responds to environmental challenges. Although most developmental requirements are common to both small (SI-ILF) and large intestinal ILF (LI-ILF), some differences have been noted. In order to study these, the development and maturation of LI-ILF have been characterised using models in which ILF development is promoted by pre-natal LT-β deficiency or blockade. Higher numbers of LI-ILF than SI-ILF were observed at 2 weeks after birth, with only mature ILF present in the large intestine. Maximal LI-ILF development was achieved at 4 weeks, earlier than SI-ILF. Depletion of LT expressing B cells did not block LI-ILF development, but did prevent maturation, and depletion of CD11c+ cells reduced the number LI-ILF, similar to SI-ILF. In studies of germ-free mice, LI-ILF were present and mature in the absence of bacterial stimulation, and in contrast to SI-ILF, were reduced following re-colonisation. Interestingly, IL-23 p19+ cells localise to LI-ILF and IL-23 p19-/- mice have a reduced number of LI-ILF despite similar numbers of SI-ILF. These differences in ILF development and maturation point to factors intrinsic to the large intestinal environment, such as IL-23, as regulators of LI-ILF in the steady state.

W.25. Lactoferrin Triggers TGF-β Signal Pathway Leading to IgA and IgG2b Isotype Switching

Young Saeng Jang[1], Hye Young Seo[1], Woan Sub Kim[2], Pyeung Hyeun Kim[1]. [1]Kangwon National University, Chuncheon, Republic of Korea; [2]Hankyong National University, Anseong, Republic of Korea

Lactoferrin (Lf), an 80 kDa iron-binding glycoprotein, is known to modulate humoral immune response. However, its exact role in Ig synthesis is obscure. In this study, we investigated the effect of bovine Lf on Ig production by mouse spleen B cells. Lf enhanced IgA and IgG2b production while concurrently downregulating other isotypes such as IgG1, IgG3, and IgM. In parallel, Lf increased germ-line transcript α, GLTα, and GLTγ2b but not GLTγ1 and GLTγ2a. As similar to TGF-β1, Lf increased GLα promoter activity, and this was further enhanced by overexpression of Smad3/4 while completely abolished by DN-Smad3. Interestingly, anti-TGFβ1 or soluble TGF-β type II receptor added during the culture did not repress the Lf effect, suggesting that Lf does not stimulate B cells to secrete TGF-β. Finally, we found that Lf increases phosphorylation of Smad3 and nuclear translocation of this molecule. These results suggest that lactoferrin induces IgA and IgG2b production through the Smad3/4-dependent TGF-β1 signaling.

W.26. Antibody Reactivity of Intestinal IgA+ and IgG+ Plasma Blasts in Humans

Julia Benckert[1], Nina Schmolka[1], Markus Zoller[1], Matthias Muellenbeck[1], Andreas Sturm[2], Bertram Wiedenmann[2], Hedda Wardemann[1]. [1]Max-Planck-Institute for Infection Biology, Berlin, Germany; [2]Charité University Hospital, Berlin, Germany

Mucosal antibody responses play a major role in mediating homeostasis with the intestinal flora. Changes in antibody repertoire and reactivity may play a pathogenetic role in the development of intestinal disease like inflammatory bowel disease. However, the antibody reactivity of human intestinal IgA+ and IgG+ plasma blasts has not been determined. By antibody cloning and *in vitro* expression we determined the reactivity profile of single isolated IgA+ and IgG+ plasma blasts from human terminal ileum of healthy donors and of patients with Crohn's Disease. The data show that under healthy conditions about 25% of intestinal IgA and IgG plasma blast antibodies are polyreactive whereas the majority develops in antigen-specific B cell responses. Antigen-specific antibodies are not only generated against enteropathogenic microbes, but also against commensals and self-antigen. In Crohn's Disease less antibodies with specificity for representatives of the commensal flora or for intestinal pathogens are found indicating a biased reactivity in response to intestinal antigens. In summary, the data suggest that antigen-specific immune responses to intestinal microbes including commensals play an important role in intestinal homeostasis and thus provide a basis for understanding deregulated mucosal immune responses in patients with inflammatory bowel disease.

W.27. Mechanisms Underlying RA-induced IgA Isotype Switching in Mice

Seok-Rae Park[1], Goo-young Seo[2], Hyun-A Kim[2], Mi-Ra Lee[2], Mi-Hee Park[2], Pyeung Hyeun Kim[2]. [1]Konyang University, Daejeon, Republic of Korea; [2]Kangwon National University, Chuncheon, Republic of Korea

Retinoic acid (RA) is known to have several activities which lead to potent mucosal IgA response. In the present study, we obtained several evidences that RA can act as a specific IgA isotype switching factor. RA, though less effective than TGF-β1, significantly increased IgA secretion and Ig germ-line α transcripts (GLTα) by LPS-activated mouse spleen B cells, while concurrently decreasing other isotypes secretion and GL transcripts. Based on limiting dilution analysis, RA increased the frequency of IgA secreting B cell clones by two-fold. This was not accompanied by increased numbers of IgA secreting cells/clone. Further, RA increased the activity of GLα promoter reporter by 3-fold and this was further increased by TGF-β1. Herein, RA-induced GLα expression was diminished by a TGF-β receptor I inhibitor (SB431542) and overexpression of DN-Smad3. Nevertheless, site-directed mutagenesis revealed that putative Smad3 binding elements (SBEs) are not relevant to RA-inducible promoter activity. Interestingly, RA-induced GLα transcription and IgA secretion were virtually disappeared by LE540, an antagonist of RA receptor (RAR). In addition, p38 inhibitor (SB203580) and ERK inhibitor (PD98059) diminished RA-induced GLα transcription and IgA secretion. Also, we found that RA can induce p38MAPK/ERK phosphorylation. Taken together, these results suggest that RA induces GLα transcription through diverse pathways including RAR, Smad3, and p38MAPK/ERK, leading to IgA isotype switching.

W.28. Imprints of Embryonic Stromal Cell Markers in a Novel Model of Salivary Gland Ectopic Lymphoneogenesis

Francesca Barone[1], Saba Nayar[1], Michele Bombardieri[2], Davide Lucchesi[2], Debbie Hardie[1], Gordon Proctor[3], Peter Lane[1], Costantino PItzalis[2], Sanjiv Luther[4], Chris Buckley[1]. [1]University of Birmigham, Birmingham, United Kingdom; [2]Queen Mary University, London, United Kingdom; [3]King's College London, London, United Kingdom; [4]University of Lausanne, Epalinges, Switzerland

Re-expression of embryonic stromal cell markers has been shown in cancer associated fibroblasts and inflammatory conditions. In this work we used a novel inducible model of salivary gland inflammation to define this process in salivary glands tertiary lympho neogenesis. Resting salivary glands showed physiological gp38 expression on myoepithelieal cells and negligible expression of CD248 and FAP. Progressive infiltrate organization was observed at day 10-18 post-infection with regression from day 20 Gp38+ fibroblastic reticular cells were detected in T cell areas within inflamed foci. Pixel count and FACS analysis confirmed these data showing positive correlation between gp38 and CD4 (R2=0.99) and concurrent increase in the number of gp38+CD31- and CD45+ cells. High expression of CD248 and FAP was detected in heavily infiltrated glands. CD248+ cells were detected in CD31- linear structures at the infiltrate edges. Negligible CD248 expression was detected inside the aggregates, which were sites of intense FAP expression. Decreased expression of the three markers was observed in the resolving aggregates at day 23. We have found that re-acquisition of the embryonic stromal cell phenotype in different fibroblast cell types is a critical feature of stromal cell activation during inflammation.

W.29. Ectopic Lymphoneogenesis in a Novel Murine Model of Salivary Glands Sialoadenitis is Characterized by Asynchrony in the Lymphangiogenetic and Angiogenetic Program

Francesca Barone[1], Saba Nayar[1], Michele Bombardieri[3], Davide Lucchesi[3], Debbie Hardie[1], Gordon Proctor[2], Costantino PItzalis[3], Chris Buckley[1]. [1]University of Birmigham, Birmingham, United Kingdom; [2]King's College London, London, United Kingdom; [3]Queen Mary University, London, United Kingdom

Defective drainage of inflammatory cells in chronic inflammation is suggested to play a role in persistence. Secondary lymphoid organs are characterized by concordance in time and response to stimuli between lymphatic and vascular systems. Here we use an inducible model of resolving ectopic lymphoneogenesis, in replication deficient adenoviral infected murine salivary glands, to evaluate this relationship in ectopic lymphoneogenesis. We demonstrated progressive acquisition of lymphoid features in aggregates starting at day 10 post-infection with regression of the foci at day 23. Accordingly, FACS analysis of collagenase digested tissue showed increase in the CD45+ component up to day 18 p.i. with progressive decrease from day 20. The percentage of gp38-CD31+ vascular endothelial cells was increased between day 10 and 15 but overall stable throughout the inflammatory process. We observed a significant decrease in gp38+CD31+ lymphatic vascular cells from day 10 to 15, with a slight increase at day 20 coinciding with aggregate regression. Scarce Lyve expression was observed by immunofluorescence in the inflamed glands that were instead characterized by formation of CD31+ high endothelial venules and up-regulation of CD31-gp38+ cells within the inflammatory foci. Here we demonstrate that in ectopic tertiary lymphoneogenesis the relationship between lymphatics and endothelial vasculature is altered until resolution occurs.

W.30. Bacterial Gelatinase Mediates Loss of Mucosal Barrier Function and Contributes to Intestinal Inflammation

Natalie Steck[1], Micha Hoffmann[1], Irina Sava[1], Dagmar Krüger[1], Hannes Hahne[1], Sandra Kim[2], Michael Schemann[1], Balfour Sartor[2], Dirk Haller[1]. [1]Technische Universität München, Freising, Germany; [2]University of North Carolina, Chapel Hill, NC

The activation of matrix metalloproteases (MMP) plays an important role in the pathogenesis of IBD. We investigated the influence of a commensal-derived protease, Gelatinase (GelE) from Enterococcus faecalis, on the development of intestinal inflammation. Monoassociation of IL-10 deficient mice with GelE producing E. faecalis strain OG1RF revealed a significantly higher inflammation compared to the colonization with GelE lacking strains TX5264 (ΔGelE) and TX5266 (ΔfsrB). Purified GelE significantly decreased barrier function of distal colon segments from susceptibility models for intestinal inflammation (IL10-/-, Rag2-/- and TNFΔARE/Wt). E-Cadherin, as important barrier and differentiation marker, was reduced after GelE exposure explaining the decrease in barrier integrity. We could indentify cleavage sites for GelE in the sequence of recombinant murine E-Cadherin suggesting the possibility for a GelE mediated degradation. Furthermore GelE impaired barrier function of intestinal epithelial cells *in vitro*. GelE specificity was evaluated using concentrated supernatant from E. faecalis strain OG1RF, the mutant strains ΔGelE and ΔfsrB, as well as

the GelE reconstituted strains TX5439 (ΔGelE complementation) and TX5266.01 (ΔfsrB complementation) and the MMP Inhibitor Marimastat, which inhibited GelE activity. Our data show, that a protease produced by commensal gut bacteria, might contribute to the development of intestinal inflammation through impairing mucosal barrier functions.

W.31. Differential Immunomodulatory Effects of Lactobacilli in Th2 Immune Responses

Chiung-Hui Huang, Pei-Chi Lynette Shek, Hongmei Wen, Peng Yian Chua, I-Chun Kuo, Kaw Yan Chua. National University of Singapore, Singapore

Lactobacilli have been used for managing allergic diseases but the species-specific immunomodulatory effects on hosts remain unclear. We evaluated the effects of Lactobacillus rhamnosus GG (LGG) and lactobacillus plantarum (Lp) as well as recombinant LGG (rLGG) and Lp (rLp) expressing a major mite allergen Blo t 5 (Bt5) in the suppression of Th2 immune responses. Mice were fed with live lactobacilli for five consecutive days per week over three weeks before or after a Th2 skewed epicutaneously sensitization with Bt5 in the prophylactic or the therapeutic model respectively. In the prophylactic model, rLGG or rLp but not wildtype lactobacilli enhanced Bt5-specific IgG2a and reduced Bt5-specific IgE production. Both rLGG and rLp suppressed the Th2 cytokines production by Bt5 stimulated splenocytes. In the therapeutic model, rLGG suppressed Bt5-specific IgE and enhanced Bt5-specific IgG2a production. However, rLp suppressed the Bt5-specific IgE with no enhancement on Bt5-specific IgG2a. Wild-type LGG enhanced Bt5-specific IgG2a but could not suppress Bt5-specific IgE. Therapeutic treatments with rLGG or rLp did not suppress Th2 cytokine production. Our preliminary results showed that rLGG and rLp exhibited differential immunomodulatory effects *in vivo* and recombinant lactobacilli were more efficient than wild-type lactobacilli in the suppression of allergen specific Th2 immune responses.

W.32. The Enteric Microflora-dependent Differentiation of Villous M Cells in the Distal Ileum of Mice is Markedly Increased in the Absence of B Cells or IgA

Kathryn Knoop, Betsy Butler, Nachiket Kumar, Ifor Williams. Emory University, Atlanta, GA

The differentiation of intestinal epithelial cells into M cells specialized for antigen sampling is a RANKL-dependent process that predominantly occurs in the follicle-associated epithelium overlying lymphoid aggregates such as intestinal Peyer's patches. A smaller number of M cells are found on the villous epithelium of wild type mice. The average density of villous M (vM) cells in wild type BALB/c mice as detected by whole mount UEA-I staining was 0.07 vM cells/villus. In wild type mice the density of vM cells was relatively constant along the length of the small intestine. In sharp contrast, the density of vM cells in the last 3 cm of the ileum increased to 10-17 vM cells/villus in mice lacking B cells (JH null and μMT strains) or IgA. These distal ileum vM cells were functional M cells, as evidenced by the enhanced uptake of 500 nm fluorescent beads from the lumen of isolated intestinal loops into the lamina propria. This expansion of vM cells in the distal ileum was completely blocked by oral ampicillin treatment for 3 days, indicating that escape of the commensal enteric flora from the normal homeostatic control of secretory IgA was one factor contributing to the expansion of vM cells. Treatment of JH null mice with neutralizing anti-RANKL for 4 days also blocked the differentiation of vM cells in the distal ileum, indicating that RANKL also contributes to the differentiation and/or survival of these vM cells. The increase of vM cells in the distal ileum of mice when secretory IgA is absent supports a model in which the commensal microflora and RANKL coordinately regulate the extent of vM cell differentiation in the small intestine. Supported by grants from the NIH and the Gates Foundation.

W.33. TLR4/IRF3-dependent Signaling Determines Innate Resistance to Kidney Infection

Hans Fischer[1], Nataliya Lutay[1], Bryndís Ragnarsdóttir[1], Manisha Yadav[1], Klas Jönsson[2], Alexander Urbano[2], Ahmed Al Hadad[1], Sebastian Rämisch[1], Petter Storm[1], Ulrich Dobrindt[3], Ellaine Salvador[3], Diana Karpman[1], Ulf Jodal[4], Catharina Svanborg[1]. [1]Lund University, Lund, Sweden; [2]Biomedical Sciences Institutes, Singapore, Singapore; [3]Julius-Maximilians-University Würzburg, Würzburg, Germany; [4]University of Gothenburg, Gothenburg, Sweden

The host innate immune system identifies and fights invading pathogens while tolerates commensal microflora. However, the mechanisms governing pathogenic/normal flora discrimination remain poorly understood. We describe here a new, TLR4/IRF3 signalling pathway which provides a molecular basis for fimbriae-specific pathogen distinguishing at mucosal epithelium. This pathway triggers the innate immune response to P-fimbriated E. coli, which is the main cause of kidney infections, urosepsis and associated morbidity. Pathogenic but not commensal E. coli induces a TLR4-dependent TRAM, CREB, Fos and Jun phosphorylation and p38 MAPK-dependent nuclear translocation of IRF3 and activation of IRF3/IFNβ-dependent antibacterial effector mechanisms. This signaling pathway is crucial for the antimicrobial host defence since Irf3-/- mice infected with uropathogenic E. coli showed severe tissue pathology. Relevance of this pathway for human disease was supported by polymorphic IRF3 promoter sequences, differing between highly disease-prone patients and asymptomatic bacterial carriers or controls. IRF3 promoter activity was reduced by the disease-associated genotype, consistent with the pathology in IRF3-/- mice. The genetic and functional studies identify crucial pathway for pathogen discrimination at the mucosal surface. Genetic variation in IRF3 influences individual susceptibility to kidney infection and might serve as a new tool for early diagnosis and therapy.

W.34. Upper Airways Bacterial Microbiota Profiling in a Case/Control Study of Early Onset Wheezing Infants and Healthy Controls from the Tropics of Ecuador

Paul Cardenas[1], Philip Cooper[2], Michael Cox[1], Miriam Moffatt[1], William Cookson[1]. [1]Imperial College, London, United Kingdom; [2]Universidad San Francisco de Quito, Quito, Ecuador

Introduction: The hygiene theory has previously identified a relationship between living in rural areas and the acquisition of protective environmental factors against the development of asthma and atopy. In a pilot study carried on a Tropics living population in Ecuador, we found a correlation between particular bacterial species and early onset wheezing infants. We have therefore conducted a larger case/control study. Materials and Methods: Oropharynx swabs were taken from 25 non-infectious early onset wheezing infants and 25 healthy controls (average age 7.4 months). During at least 1 month prior sampling, subjects didn't receive antibiotics or present any respiratory infection. Pyrosequensing of the 16S ribosomal RNA gene was done and sequences were analysed using QIIME. Results: 77919 high quality sequences were obtained and these were classified in 1280 OTUs. Firmicutes was the most frequent and diverse phylum with Streptococcus being the most common genus. In the phylum Bacteroidetes, Porphyromonas was more prevalent in the healthy children whilst Prevotella was more prevalent in cases. In Firmicutes, Staphylococcus was more prevalent in cases and Veillonella in controls. Discussion: Comparisons between the microbiota of the oropharnyx mucosa of healthy and wheezing infants revealed a differences in both numbers and types of bacterial species.

W.35. Lung Sensitization by Candida Albicans Protects from Pseudomonas Aeruginosa-induced Lung Injury Independently of Quorum Sensing

Jean-Baptiste Mear[4], Eric Kipnis[5], Helene Tiesset[1], Samir Jawhara[4], Cecile Rouyer[3], Daniel Poulain[5], Mathias Chamaillard[2], Karine Faure[5], Benoit Guery[5]. [1]University Lille Nord de France, Lille, France; [2]Institut Pasteur de Lille, Lille, France; [3]Centre National de la Recherche Scientifique, Lille, France; [4]Institut National de la Sante et de la recherche Medicale, Lille, France; [5]CHRU de Lille, Lille, France

Objectives: Pseudomonas aeruginosa is often isolated with Candida albicans in ICU patients airways. Prior lung mucosal sensitization with C. albicans in mice attenuated subsequent P. aeruginosa-induced lung injury. We explored whether this effect was due to C. albicans modulating Quorum Sensing (QS)-dependant P. aeruginosa virulence through the QS molecule farnesol or C. albicans filaments as decoy targets for P. aeruginosa. Methods: Strains were: C. albicans Ca-SC5314 and P. aeruginosa PaO1 as reference, Ca-ATCC10231 as naturally farnesol deficient, and CaΔEfg1 as non-filamentous mutant. *In vitro* effect of 1µM farnesol on PaO1 QS-dependent virulence factors was assessed. A co-culture killing assay was performed to assess C. albicans filaments as P. aeruginosa targets. Mucosal sensitization effect was tested with all. C. allbicans strains on P. aeruginosa-induced lung injury assessed through lung permeability and bacterial burden at 48h.Results: *In vitro*, farnesol significantly decreased P. aeruginosa QS-dependant virulence factor secretion ($p<0.01$). Candida filaments were confirmed as P. aeruginosa targets ($p<0,05$ vs. CaΔEfg1). Importantly, lung sensitization with farnesol-deficient or non-filamenting Candida strains still improved lung injury ($p<0,05$) and bacterial clearance ($p<0,05$).

W.36. Comparing Pathogenic and Commensal Lung Bacteria: Distinct Immunological Properties of Asthma-associated Proteobacteria

Jeppe Larsen, Hanieh Musavian, Tariq Butt, Susanne Brix. Technical University of Denmark, Copenhagen, Denmark

Recent studies have found asthma-associated changes in the composition of the lung microbiome. Lungs of asthmatics are more frequently colonized with pathogenic proteobacteria as compared to healthy individuals. On the contrary, commensal bacteroidetes, particularly Prevotella spp., are usually absent in asthmatics but predominantly present in healthy lungs. We investigated whether pathogenic proteobacteria comprise immunological properties that could augment asthma immunopathology. To this end, primary murine lung epithelial cells and leukocytes were stimulated with asthma-associated proteobacteria (Haemophilus spp. and Moraxella spp.) or commensal bacteria (Prevotella spp.) associated with healthy lungs. Asthma-associated proteobacteria demonstrated greater potency for the induction of TSLP, MIP-2 and TNF-α in lung epithelial cells compared to the commensal bacteria. Furthermore, the proteobacteria induced higher production of MIP-2 and TNF-α, but similar IL-5 levels, in lung leukocytes. The reduced inflammatory potential of the commensal bacteria could not be ascribed to increased IL-10 production. Furthermore, the TSLP and MIP-2, but not TNF-α, production in lung epithelial cells was dependent on TLR2 signaling. Combined our results demonstrate that asthma-associated proteobacteria exhibit intrinsic immunological properties different from commensal bacteria of healthy lungs. The pathogenic proteobacteria could contribute to asthma pathogenesis and immunopathology by driving type-2 inflammation via TSLP in a TLR2-dependent manner.

W.37. Probable Causes of Ileal Injury in Two Mice Models of Microbial Sepsis and the Protective Role of Phytic Acid

Hossam Ebaid. King Saud University, Riyad, Saudi Arabia

This study aims to determine the causes of ileal injury in two models of sepsis and the possible protective role of phytic acid. Results showed an increased inflammatory and lymphocytic cells' influx associated with apoptotic index decrease. The inflammation was accompanied by hyper-mucus secretion, villar atrophy, necrosis and desquamation with each infection and was much severe in LPS. Most enterocytes of the infected mice lost their microvillar brush border and had destructed organelles. The morphometric studies recorded significant decrease in all examined measures after four weeks of the onset of the experiment with both models. Phytic acid had the ability to attenuate ileal injury in the two models of sepsis after four weeks of its administration where its supplementation can greatly minimize the histopathological and cytological complications and morphometric alterations resulted from bacteria or its endotoxin. Its administration for four weeks was better for inducing its ameliorative effect via increasing mucus secretion, decreasing apoptotic index, attenuating the inflammatory and lymphocytic cells' count or increasing the renewal of the crypt cells and villar epithelial cells proliferation.

W.38. ECM33 Gene Contributes to the Damage of Engineered Human Oral Mucosa Following its Contact with Candida Albicans

Mahmoud Rouabhia, Abdelhabib Semlali, Pranab Mukherjee, Mahmoud Ghannoum. University of Laval, Quebec, QC, Canada

Candida albicans ECM33 gene encodes a glycosylphosphatidylinositol-linked cell wall protein that is important for cell wall integrity and interaction with host tissue. This gene is critical for Candida pathogenesis. The aim of this study was to investigate the role of ECM3 gene on Candida pathogenesis. For this purpose, an engineered human oral mucosa was used to investigate the interaction between human engineered tissue and ECM33 gene positive and negative Candida strains. Our results demonstrated that Ecm33 mutant have attenuated damaging effect on tissue structure as compared to parental strains. This was confirmed by the reduced level of LDH, and Bax and Bcl2 gene expression and protein production by EHOM following infection with ecm33 mutant. In contrast, all Candida strains including ecm33 mutant decreased basement membrane protein (laminin 5 and type IV collagen) production. The results stress the contribution of ECM33 gene to the virulence of C. albicans through tissue damage and cell apoptosis but not laminin 5 and type IV collagen deregulation. We propose that ECM33 gene/protein may be suitable targets for future control of Candida pathogenesis. Financially supported by grants from the ISERC and the NIH/R01-DE017486-01A1.

W.39. Interaction of Sulfate-reducing Bacteria with Human Intestinal Epithelial Cells Results in Production of Human Monocyte Chemotactic Factor

Alessandra Abalo[1], Carolina Souza[1], Christien Oliveira-Silva[1], Felippe Ribeiro[1], Rachel Soler[1], Giani Santoro[1], Vinicius Cotta-de-Almeida[1], Robson Coutinho-Silva[2], Claudia Mara Lara Melo Coutinho[3]. [1]IOC/FIOCRUZ, Rio de Janeiro, Brazil; [2]UFRJ, Rio de Janeiro, Brazil; [3]UFF, Niterói, Brazil

Sulphate-reducing bacteria (SRB) are anaerobic organisms which are found colonizing the human gut and that have been associated with development of chronic inflammatory bowel diseases. In this work, we investigated *in vitro* interactions between SRB and human intestinal epithelial cells and the consequences of this interaction. Human intestinal epithelial cell line HCT8 were cultivated alone or in presence of SRB (107 total bacteria/bottle). After 3 h or 24 h of co-incubation, supernatants or scraped culture were collected and immediately injected in the selective VMNI medium and then degassed with N2. After 24h, only medium containing scraped culture became black, as an indicator of H2S production. In addition, we evaluated the monocytes migration properties using the system of modified Boyden chamber. We found a marked human monocyte chemotactic response towards supernatants collected after 3 h of co-incubation (336.3±40.4 cells/10HPF -high power field, n=3) and after 24 h (384.0±62.9 cells/10HPF, n=3), as compared to supernatant recovered from control HCT8 culture (130.0±1.52 cells/10HPF, n=3). We suggested that SRB needs HCT8 interaction to survive in aerobic medium, and this interaction induces intestinal epithelial cells to produce chemotactic factors for human monocyte. These data suggest that SRB might be involved in the pathogenesis of IBD.

W.40. Rat Microbiota Modulation by a Long-term Cocoa-enriched Diet

Malen Massot Cladera, Teresa Pérez-Berezo, Maria del Mar Abril Gil, Angels Franch, Margarida Castell, Francisco Perez-Cano. University of Barcelona, Barcelona, Spain

During the last decade, the interest in cocoa has increased due to its high content in flavonoids, compounds with recognized antioxidant activity as well as immunomodulatory action. The aim of this study was to evaluate the effects of a flavonoid-rich diet on intestinal microbiota composition and IgA-coating bacteria of rats. To achieve this purpose, 3 week-old Wistar rats were randomized into 2 dietary groups: one was fed standard chow whereas the other group received a cocoa diet (0.2% w/w polyphenol content) in pelleted chow for 6 weeks. Faecal samples were collected before and after the nutritional intervention. Microbiota composition was detected by fluorescence in situ hybridisation (FISH) techniques coupled to flow cytometry analysis (FC) using 16S rRNA probes conjugated to fluorocrome complementary to specific genus as lactobacillus, bacteroides, bifidobacterium, staphylococcus, streptococcus and clostridium. IgA-coating bacteria were detected with fluorescent-labelled anti-rat IgA and flow cytometry analysis in faecal homogenates. After the intervention with the cocoa diet, there was a significant decrease in the proportion of Bacteroides, Clostridium and Staphylococcus genus. Moreover cocoa decreased IgA-coating bacteria proportion in faeces (p 0.05). In conclusion, cocoa diet modifies the intestinal microbiota and exerts a modulatory action on the IgA-coating bacteria in rats.

W.41. TLR9 Mediates Oral Bacteria-induced IL-8 Production in Gingival Epithelial Cells

Youngsook Kim, Ah-ram Jo, Yong-Joon Cho, Jongsik Chun, Byung-Moo Min, Youngnim Choi. Seoul National University, Seoul, Republic of Korea

Previously, we reported that various oral bacteria differently regulate IL-8 production in gingival epithelial cells. The aim of this study was to characterize the pattern recognition receptor(s) that mediate bacteria-induced IL-8 expression. Among ligands that mimic bacterial components, only CpG oligonucleotides enhanced IL-8 expression as determined by ELISA and real-time RT-PCR. Both normal and immortalized human gingival epithelial (HOK-16B) cells expressed TLR9 intracellularly. HOK-16B cells were stimulated with eight strains of four oral bacterial species in the absence or presence of 2% human serum. In the absence of human serum three out of four species enhanced IL-8 expression, but only one species did in the presence of human serum. Treatments with an agent to block acidification or a TLR9 antagonist inhibited IL-8 induction by bacteria. Genomic DNA (gDNA) isolated from these bacteria showed differential abilities to induce IL-8 depending on the species and strains of bacteria. However, the observed immunostimulatory activity of the gDNA could not be linked to CpG motif content. The IL-8 inducing ability of bacteria had better correlation with invasive ability than the immunostimulatory activity of DNA. In conclusion, invasive oral bacteria induce IL-8 from gingival

epithelial cells through TLR9. Supported by the NRF of Korea (#2010-0029512).

W.42. Host Microflora and Bordetella Bronchiseptica Interactions During Respiratory Infection

Laura Weyrich, Sarah Muse, Olivier Rolin, Jihye Park, Eric Harvill. Pennsylvania State University, University Park, PA

As we begin to understand respiratory tract microflora composition and their role in disease, we can also begin to characterize the mechanisms underlying microflora changes and the interactions between host microflora and pathogens. Using a robust mouse model of the respiratory pathogen Bordetella bronchiseptica, we monitored microflora in the nasal cavity before and after infection. Initially, displacement of culturable host microflora was observed three days post-inoculation, and 16S RNA sequencing of these microorganisms verified that Staphylococcus, Kytococcus, Enterobacter, and Bacillus species were all eliminated, suggesting that B. bronchiseptica infection actively displaces nasal cavity flora. To observe changes in unculturable microbes, a metagenomic sequencing approach identified the displacement of several Gram-positive species. Next, virulence factor mutants of B. bronchiseptica were screened to identify the bacterial mechanism behind host microflora displacement. Mutants defective in the Type III Secretion System, the Type VI Secretion System, or the master virulence regulator BvgAS were not able to clear microflora, suggesting these virulence factors work synergistically to cause displacement. Interestingly, closely related human pathogens, Bordetella pertussis and Bordetella parapertussis, do not cause clearance of culturable nasal cavity flora, suggesting host microflora displacement may be a pathogen specific event essential for early respiratory tract colonization.

W.43. Toll-like Receptors and Chemokines are Differently Expressed in Enterocytes Isolated from Different Regions of the Small Intestine from Mice

Stine Metzdorff[1], Annette Christensen[1], Tine Licht[2], Hanne Frøkiær[1]. [1]University of Copenhagen, Frederiksberg, Denmark; [2]Technical University of Denmark, Mørkhøj, Denmark

It is well established that commensal microbiota contributes to homeostasis of the intestine. Different bacteria species are located in the various part of the intestine, and the density of the gut microbiota is lowest in the proximal part of the small intestine and it increases to the distal part. Enterocytes lining the intestine serve as a physical barrier against the gut bacteria, and enterocytes express toll-like receptors and other pattern recognition receptors (PRR) that respond to microorganisms. Through PRR signaling, the enterocytes play a key role in maintenance of the intestinal homeostasis and in defense against pathogens. We hypothesized that the expression of PRRs depends on which region of the small intestine the enterocytes are located. Expression analysis of genes encoding PRR and chemokines in enterocytes, isolated from different part of the small intestine from mice, revealed substantial differences between enterocytes; Tlr2, Tlr4 and Cxcl1 were strongest up-regulated in the distal part, whereas Tlr3 and Cxcl2 were most strongly expressed in the proximal part. These findings reflect different functions or different microbial communities in the different parts of the intestine. Influence of the local gut microbiota composition as well as the influence of signaling through specific receptors is currently investigated.

W.44. Intestinal Epithelial Dysplasia and Adenocarcinoma in Salmonids is Associated with Inflammation

Torfinn Moldal[1], Erling Koppang[2], Magne Kaldhusdal[1], Ivar Hordvik[3], Ole Bendik Dale[1]. [1]Norwegian Veterinary Institute, Oslo, Norway; [2]Norwegian School of Veterinary Medicine, Oslo, Norway; [3]University of Bergen, Bergen, Norway

Salmonids are indigenous carnivores, but plant components are increasingly used in their feeds. Several studies have explored the effects of plant proteins on the intestinal health of salmonids in early stages of their feeding period. Salient findings include shortening and widening of mucosal folds, infiltration of inflammatory cells and altered expression of certain cytokines. Cancer is reportedly a rare condition in salmonids, but intestinal epithelial dysplasia and adenocarcinomas with metastasis was recently described in brood stock fed a commercial diet containing plant ingredients. The aim of the present study was to characterize the inflammation associated with these enteric lesions and metastatic tumours. Tissue was routinely fixed in formalin and processed for histological examination or prepared for detection of T cells and antigen-presenting cells by immunohistochemistry. Our results show high numbers of these inflammatory cells in intestines with epithelial dysplasia and adenocarcinomas, but few such cells in tissues with metastatic tumours or within the tumours themselves. These findings support a pathogenesis similar to the inflammation-dysplasia-carcinoma sequence in humans with inflammatory bowel disease, suggesting salmonids fed plant components may be a suitable animal model for future research.

W.45. Cytotoxic Effects of Methanolic Fractions of Ornithogalum Cuspidatum in Caco-2 Cell Line, Model of Human Colonic Carcinoma

Behzad Baradaran, Jafar Majidi, Samira Valiyari, Minoo Baradaran, Rasool Estakhri. Immunology Research Center,Tabriz University of Medical sciences, Tabriz, Islamic Republic of Iran

Introduction: Colorectal cancers are the third leading cause of cancer-related death in the Western world. Herbal medicines with apoptotsis induction effects are one of the promising therapeutic ways in cancer treatment. In this study cytotoxic effect of the methanolic fractions of Ornithogalum cuspidatum “an Iranian natural plant” have been assayed on cancer cell line Caco-2, model of Human colonic carcinoma.Material and Methods: Cancer cell line, Caco-2 was treated with different concentrations of O. Cuspidatum methanolic fractions in various times . MTT assay and Trypan blue exclusion assay was used for measurement of the cytotoxicity and cell viability in 24 hours and in different concentrations of methanolic

fractions. ELISA and Flowcytometry methods were used to study apoptotsis in 24 hours and in different concentrations of methanolic fractions. Results: Apoptosis of caco-2 cells were begun from 200µg/ml of 40% fraction and in 60%, 80% and 100% fractions were begun from 200µg/ml. These fractions induced mostly apoptosis but Flocytometry showed a little necrosis in cell death. Conclusions: The results obtained show that increasing of treatment time and fraction concentration result the increase in cytotoxic effects of O.cuspidatum methanolic fractions. Therefore the effects of O.cuspidatum methanolic fractions depend on treatment times and concentrations.

W.46. Secretory Leukocyte Protease Inhibitor Binds to Neisseria Gonorrhoeae Outer Membrane Opacity Protein and is Bactericidal

Ona Barauskas[1], Morris Cooper[2], Melissa Roberts[2], Gary Jarvis[1]. [1]VA Medical Center San Francisco, San Francisco, CA; [2]Southern Illinois University, Springfield, IL

Secretory leukocyte protease inhibitor (SLPI) is the predominant serine protease inhibitor on mucosal epithelial sufaces including the genitourinary tract and has been shown to have antimicrobial activity. In this study, we investigated the interaction of SLPI with six strains of Neisseria gonorrhoeae. Whole bacteria ELISA assays and far-western blot analyses revealed that SLPI binds to the gonococcal outer membrane opacity (Opa) protein. The identity of Opa was confirmed using trypsin digests and mass spectrometric analyses. Bactericidal assays showed that SLPI is bactericidal in a dose-dependent manner for gonococci expressing the Opa protein, but not for Opa-negative gonococci, and that anti-SLPI antibody significantly reduced SLPI bactericidal activity. Epithelial cells from both the reproductive and intestinal tracts constitutively expressed SLPI, but to variable degrees, with the reproductive tract cells expressing significantly greater amounts than the intestinal cells. Expression of SLPI by cells was not affected by gonococcal cell adherence or invasion. We conclude that although SLPI binds to gonococci and is bactericidal, gonococci of the Opa-negative phenotype are resistant to its bactericidal effect, and that variations in the levels of SLPI expression on the genitourinary tract mucosa in combination with the gonococcal Opa phenotype may influence the clinical course of gonorrheae.

W.47. Orthotopic Induction and Surveillance of Colorectal Tumors in Mice Using Live Colonoscopy

Ehud Zigmond[2], Zamir Halpern[1], Steffen Jung[2], Chen Varol[1]. [1]Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel; [2]Weizmann Institute of Science, Rehovot, Israel

Colorectal carcinoma (CRC) research has profited much from the availability of murine models, including mice harboring targeted mutations, tumor models based on induction of chronic colonic inflammation, usually with simultaneous administration of carcinogens, and the orthotopic implantation of tumor cells by surgical approaches. These models often suffer from prolonged windows of establishment, poor reproducibility and their association with major side effects. The recent technical advance in *in vivo* mouse endoscopy now allows for repetitive high-resolution evaluation of the colon in living mice. Utilizing this system we have developed a method for sub-mucosal orthotopic induction of CRC. Single administration of the murine CRC cell-line MC38 into the colonic sub-mucosa of C57BL/6 mice resulted in a highly reproducible, gradual progressive growth of CRC, reaching a tumor blocking 95% of the intestinal lumen in less than 3 weeks. We show that this minimal invasive technique circumvents the side effects associated with other murine CRC models, is highly controllable, and importantly allows for the side-by side comparison of adjacent genetically distinct tumors within the same mouse. We currently use this system to investigate the interactions between CRC and specific intestinal lamina propria mononuclear phagocyte subsets.

W.48. Cigarette Smoke Induces Dendritic Cell Oxidative Stress, Suppresses Th1 Cytokine Generation and Promotes Tumor Spread in Mice

Robert Vassallo, Paula Walters. Mayo Clinic, Rochester, MN

Cigarette smoke-induced oxidative stress profoundly alters dendritic cell functions. The purpose of this study was to determine the relative role of oxidative stress in the induction of tumor spread *in vivo*, and determine the relative oxidative stress induced by cigarettes marketed by tobacco companies with the claim of reduced carcinogenicity (Eclipse cigarettes). To determine whether cigarette smoke promotes tumor development *in vivo*, C57B6 mice were divided into a cigarette smoke challenged group (3 hours daily for 4 weeks) and a control group. Following 4 weeks of exposure, both groups of mice were challenged with B16F10 melanoma tumor cells by tail injection. Twenty one days following tumor challenge (and either continued cigarette smoke exposure or air exposure), mice were sacrificed and tumor burden was determined by enumeration. Mice exposed to cigarette smoke developed significantly greater tumor metastases compared with control mice. Determination of heme-oxygenase-1 (HO-1) levels as a surrogate of oxidative stress in the lungs of mice showed upregulation of the protein only in cigarette smoke exposed mice, which correlated with tumor burden. Incubation of dendritic cells to extracts prepared from presumed reduced harm cigarettes potently induced cellular HO-1 protein levels, and suppressed interleukin-12p70 generation which was at least equivalent to that observed with cells incubated with control cigarette smoke extract. Cigarette smoke promotes lung tumor spread which correlates with the induction of oxidative stress. Extracts prepared from proposed reduced harm cigarettes induce equivalent or greater dendritic cell dysfunction. Development of relevant animal and *in vitro* models are essential to objectively determine relative immune toxicity of novel cigarettes marketed as potential reduced harm products.

W.49. Increased Treg, but Not Th17, Cells in Patients with Gastric Adenocarcinoma

Ana Aguinaga-Barrilero[1], Alberto Gutiérrez-Calvo[2], Inmaculada Lasa[2], José Martin-Villa[1]. [1]Universidad Complutense de Madrid, Madrid, Spain; [2]Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Madrid, Spain

Background: The role of Treg and Th17 cells in tumor growth has been actively investigated in recent years. Aim: We wished to investigate both populations in a group of patients with gastric adenocarcinoma. Methods: Enriched T cell populations were achieved from blood or gastric tissue (tumor and tumor-free) samples from 21 patients (14 with intestinal type and 7 with diffuse type disease). As a control, blood samples from 23 healthy subjects were used. CD4, CD8, CD25, FOXP3 and IL-17 markers were measured by cytometry. Results: A significant increase in the percentage of CD4+CD25+ cells was found in T cell populations obtained from tissue samples, when comparing tumor with tumor-free gastric tissue in patients (9±5.7% vs 4±3%; p=0.014). Likewise, intestinal type tumor shows higher FoxP3 and CD4+CD25+FoxP3+ percentages (7.3±4.6% and 2.7±2.4%, respectively) when compared to tumor-free tissue (4.5±4.8% and 1.6±2.6%; p=0.036 and p=0.028,respectively). Finally, the FoxP3+/IL17+ ratio is higher in blood samples of patients (2.2±2.1) than in control subjects (1.1±1.6; p=0.025). No significant differences were found for CD4+ or CD8+ IL-17-producing cells. Conclusions: Treg, and not Th17, cells seem to play a key role in tumor progression, more so in the intestinal type of gastric cancer.

W.50. Higher LAP+/CD8+LAP- (**Latency TGFβ**-Associated Peptide) Ratio in the T Cell Population of Patients with Early Gastric Cancer

Ana Aguinaga-Barrilero[1], Alberto Gutiérrez-Calvo[2], Adela López[2], José Martin-Villa[1]. [1]Universidad Complutense de Madrid, Madrid, Spain; [2]Hospital Universitario Príncipe de Asturias, Madrid, Spain

Background: Two different immunoregulatory T cell subpopulations have been recently described: CD4+LAP+ in humans and CD8+LAP+ in a mice EAE model. Aim: To investigate the presence of both subpopulations in patients with gastric adenocarcinoma. Methods: Enriched T cell populations were achieved from blood or gastric tissue (tumor or tumor-free, TF) samples from 20 patients, 6 with early (ES) and 14 with advanced disease-stage (AS). Blood samples from 23 healthy donors (HD) were used as control. CD4, CD8, LAP (inactive membrane TGFβ), FOXP3 and IFN-γ markers were measured by cytometry. Results: A novel CD8+LAP+ subpopulation is reported for the first time in humans. Its frequency is increased in patients, whether in blood (HD 2.8±2.8; ES 7.1±9.7; AS 4.9±8.3) or tissue (TF 5.3±7.8; ES 6.6±4.5; AS 3.6±3.1). Likewise, the LAP+/CD8+LAP- ratio (a measure of the immunosuppression exerted on CTLs) is also increased in patients (blood: HD 0.14±0.14; ES 0.44±0.6; AS 0.24±0.4, tissue: TF 0.3±0.35; ES 0.37±0.12*, AS 0.2±0.3 *p<0.05 vs AS). The results suggest that disease progression is accompanied by a decreased LAP membrane expression and an increased active TGFβ secretion, promoting tolerance to the tumor. Conclusions: LAP measurement may be useful to evaluate the immune status of cancer patients, in blood or tissue.

W.51. Tumor Development in Mouse Model of Colitis-associated Carcinoma Under Different Microbial Conditions

Klara Klimesova[1], Miloslav Kverka[1], Zuzana Zakostelska[1], Pavel Rossmann[1], Tomas Hudcovic[1], Tomas Hrncir[1], Jakub Mrazek[2], Jan Kopecny[2], Helena Tlaskalova-Hogenova[1]. [1]Institute of Microbiology, Prague, Czech Republic; [2]Institute of Animal Physiology and Genetics, Prague, Czech Republic

Disruption of the balance between gut mucosa and commensal bacteria is associated with the onset of inflammatory disorders. The aim of this study was to evaluate the impact of bacterial load on colon cancer development in azoxymethane (AOM)/dextran sodium sulfate (DSS) induced colitis-associated neoplasia. We induced neoplasia in mice reared under conventional and germ-free conditions and in conventionally reared antibiotic-treated mice. Cytokine milieu was determined by multiplex analyzer (Luminex) in supernatants after cultivation of tissue fragments from tumors and tumor-free parts of the intestine. Regulatory T cells in spleen, mesenteric lymph nodes and Peyer's patches were determined by FACS analysis. To compare the gut microbiota composition we used 16S rRNA gene analysis of stool samples. We found that animals with reduced microbiota burden developed fewer tumors than control animals. The incidence of colonic adenocarcinoma in conventionally reared, antibiotic-treated and germ-free mice was 82.6%, 55% and 20%, respectively. Neoplastic transformation of cells was linked with accumulation of beta-catenin in nucleus and with increased staining for iNOS. We found higher levels of pro-inflammatory cytokines IL-1 beta, IL-6 and IL-17 in supernatants from tumors. We observed increased number of Foxp3+ T cells in Peyer's patches of tumor bearing mice. Moreover, we documented dramatic decrease in microbiota diversity in both groups of AOM/DSS-treated mice. We conclude that intestinal homeostasis and process of colitis-associated carcinogenesis are substantially influenced by the presence of commensal bacteria and gut microbiota composition.

W.52. Down-regulation of the Polymeric Immunoglobulin Receptor in Ulcerative Colitis and in Experimental Murine Colitis-associated Cancer

Maria Bruno, Aubrey Frantz, Eric Rogier, Gavin Ellis, Cathryn Kurkjian, Razvan Arsenescu, Charlotte Kaetzel. University of Kentucky, Lexington, KY

Secretory IgA antibodies, which are transported into gut secretions by the polymeric immunoglobulin receptor (pIgR), enhance intestinal homeostasis by neutralizing bacteria and regulating inflammation. Inflammatory bowel diseases, including Crohn's disease (CD) and ulcerative colitis (UC), result from a dysregulated mucosal immune response to the gut microbiota in genetically susceptible individuals. Colitis-associated cancer (CAC) is the leading cause of death in UC. We previously reported that expression of pIgR is reduced in sporadic colon cancer and in the colonic mucosa of CD patients. We now demonstrate that colonic pIgR expression is also decreased in UC patients. To test the hypothesis that inflammation-associated down-regulation of pIgR expression is a risk factor for CAC, we measured pIgR expression in isolated colonic epithelial cells in a murine model of CAC, in which a single injection of the carcinogen AOM is followed by multiple cycles of DSS treatment and recovery. Expression of pIgR was reduced during recovery from inflammation and during the transition to epithelial dysplasia and tumorigenesis. We conclude that optimal expression of pIgR is important for intestinal homeostasis, and reduction of pIgR expression may be an early biomarker for CAC development. Supported by the

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W.53. Syntetic Dansil-C-glucoside BLF501 Protects Intestinal Epithelium from Chemotherapic-induced Mucositis

Diego Cardani[1], Giuseppina Dusio[1], Barbara la Ferla[1], Fabrizio Marcucci[2], Andrea Balsari[1], Daniela Olivero[1], Francesco Nicotra[1], Cristiano Rumio[1]. [1]Università Degli Studi di Milano, Milan, Italy; [2]Istituto Superiore di Sanità (ISS), Rome, Italy

Anti-cancer therapeutic strategies based on radiotherapy and chemotherapy are effective in the treatment of malignant disease but present a plethora of side effects including oral and gastrointestinal mucositis. Anthracyclines like doxorubicin are among the most widely used chemotherapeutic agents but they cause mucositis onset (especially combined with 5-fluorouracil); events like apoptosis and inflammation contribute to the onset of mucositis. We evaluate the activation of SGLT1 by a new molecule, BLF501 in order to assess its ability to inhibit the onset of the intestinal mucositis in a mouse model of mucositis induced by doxorubicin and 5-fluorouracil. Previously we showed that the activation of SGLT-1 by an high dose of D-glucose is capable of inhibiting pro-inflammatory pathways (J Immunol. 2008,181:3126) and proapoptotic pathways associated with oxidative stress. To test the possible protective activity of BLF501 with increased potency and lower dosage against mucositis from chemotherapy we examined morphology of the small intestine, expression of junctional proteins in the gut epithelium, proliferative activity and systemic levels of pro-inflammatory cytokines. Results assess that BLF501 protects small intestine from the damage from chemotherapy while maintaining the proliferative capacity of cells of the crypts ensuring cell turnover to allow the maintenance of the epithelial functionality.

W.54. Correlation Between Total and Specific IgA and IgM Antibody Against Gliadin and Transglutaminase in Patients with IBD

Aristo Vojdani. Immunosciences Lab, Inc., Los Angeles, CA

Several studies used salivary IgA antibodies against gliadin and tissue transglutaminase (TG) as a convenient non-invasive screening method for gluten sensitivity and celiac disease. To evaluate the utility of saliva for IBD patients, samples were collected from healthy controls and patients with gastrointestinal disorders. Using ELISA, total secretory, gliadin, and TG IgA and IgM were measured. At ± 2 SD above the mean of controls, IgA was elevated in 63% and reduced in 16% of patients with GI disorders, while only 13% and 6% of controls showed high or low SIgA respectively. All individuals with low SIgA showed elevation in SIgM. Therefore, to assess an association between total and specific SIgA, α-gliadin and TG antibodies were measured in clinical specimens with low, normal or high SIgA. Majority of patients with high IgA showed high levels of IgA against gliadin, TG, or gliadin+TG. Interestingly, gliadin and TG antibodies were also detected in 7 out of 13 healthy controls with high total SIgA but were not detected in patients or controls with low SIgA. In specimens with low SIgA, about 50% showed elevation in gliadin and TG IgM antibody. Assessment of SIgA and SIgA+IgM antibody against gliadin and TG in saliva is an excellent method for evaluating loss of mucosal immune tolerance and immune reaction against dietary proteins and peptides.

W.55. Heterogeneity in IgG and IgA Response Against an Array of Wheat Antigens and Peptides in Patients with Celiac and Crohn's Disease

Aristo Vojdani. Immunosciences Lab, Inc., Los Angeles, CA

It was recently demonstrated that intestinal T cells from celiac disease (CD) patients respond to a wide heterogeneous array of peptides. In this study we extended this heterogeneity to humoral immune response to various wheat proteins and peptides in patients with celiac and Crohn's disease. A magnitude of IgG and IgA antibodies were measured simultaneously against α-, γ-, and ω-gliadin, glutenin, wheat germ agglutinin (WGA), gluteomorphin, prodynorphins, transglutaminase (TG) and gliadin-bound TG in sera from CD patients, Crohn's disease patients and healthy control subjects. In comparison to controls, in CD patients IgG reacted most against TG, prodynorphin, wheat extract, and α-, γ-, and ω-gliadin, while IgA was most reactive against wheat, then TG, glutenin and other peptides. In Crohn's IgG was more prevalent against wheat and WGA, then TG, prodynorphin, α-and γ-gliadin, while IgA was detected foremost against prodynorphin, then TG and α-gliadin. These results showed a substantial heterogeneity in the magnitude of IgG and IgA response against various wheat antigens and peptides. Measurements of IgG and IgA antibodies against an array of wheat peptides along with gliadin-TG, TG and WGA can enhance not only the sensitivity and specificity of serological assays for CD but may also detect silent CD or its overlap with IBD.

W.56. The Effect of Gliadin and Various Bacterial Strains on Intestinal Mucosa: Study in Germ-free Rats

Ludmila Tuckova[1], Renata Stepankova[1], Jana Cinova[1], Giada De Palma[2], Olga Kofronova[1], Miloslav Kverka[1], Yolanda Sanz[2]. [1]Institute of Microbiology, Prague, Czech Republic; [2]Institute of Agrochemistry and Food Technology, Valencia, Spain

Background: Celiac disease (CD) is a chronic disorder of small intestine induced by dietary wheat gluten/gliadin proteins in genetically predisposed individuals. The overgrowth of potentially pathogenic bacteria could contribute to CD pathogenesis. Results:We studied the effects of gliadin and various intestinal bacterial strains on mucosal barrier integrity, gliadin translocation, and cytokine production. Changes in gut mucosa were assessed in the intestinal loops of inbred Wistar-AVN rats, that were reared under germ-free conditions, in the presence of intestinal enterobacteria and bifidobacteria isolated from CD patients and healthy children. Gliadin fragments alone or together with the proinflammatory cytokine IFN-γ significantly decreased the number of goblet cells in the small intestine. This effect was more pronounced in the presence of Escherichia coli CBL2 and Shigella CBD8. Shigella CBD8 and IFN-γ induced the highest mucin secretion and the greatest impairment in tight junctions and, consequently,

translocation of gliadin fragments into the lamina propria. The number of goblet cells in small intestine increased by the simultaneous incubation of Bifidobacterium bifidum IATA-ES2. B. bifidum IATA-ES2 also enhanced the production of chemotactic factors and inhibitors of metalloproteinases, which can contribute to gut protection. Conclusion: Our data support the involvement of intestinal bacteria in early stages of CD pathogenesis.

W.57. IL-21 Production is Positively Regulated by IL-15 in Celiac Disease Mucosa

Massimiliano Sarra[1], Maria Laura Cupi[1], Ivan Monteleone[1], Antonio Di Sabatino[2], Paolo Gentileschi[1], Pierpaolo Sileri[1], Giovanna Del Vecchio Blanco[1], Eleonora Franzè[1], Micaela Cretella[1], Omero Alessandro Paoluzi[1], Gino Roberto Corazza[2], Francesco Pallone[1], Giovanni Monteleone[1]. [1]University of Tor Vergata, Rome, Italy; [2]IRCCS Policlinico, San Matteo, University of Pavia, Pavia, Italy

Celiac disease (CD)-associated immune response is characterized by excessive production of IL-21, a cytokine supposed to activate detrimental pathways in the gut. In this study, we analyzed IL-21 expression at single cell-level in mucosal cells and examined how IL-21 production is regulated in CD. Duodenal intra-epithelial (IELs) and lamina propria lymphocytes (LPLs), isolated from patients with active and inactive CD and healthy controls, were analyzed for cell markers, cytokines and transcription factors by flow-cytometry. Additionally, CD4+ LPLs were stimulated with IL-15 in the presence or absence of wortmannin, an AKT inhibitor, and then analyzed for active AKT and IL-21/IFN-γ by flow-cytometry. A neutralizing IL-15 ab was added to organ cultures of duodenal biopsies taken from active CD biopsies or peptic-tryptic digest of gliadin (PT)-stimulated inactive CD biopsies. High IL-21 was seen in CD4+ and CD4+/CD8+ IELs and LPLs of active CD patients as compared to controls. IL-21-producing cells co-expressed preferentially IFN-γ and to a lesser extent Th17-related cytokines. Treatment of CD4+ LPLs with IL-15 activated AKT thus leading to enhanced synthesis of IL-21. Consistently, blockade of IL-15 in cultures of CD biopsies reduced IL-21. These findings delineate a novel mechanism by which IL-21 is regulated in CD mucosa.

W.59. Characterization of IgA Interactions with Transferrin Receptor CD71 at the Apical Surface of Enterocytes From Celiac Patients and in Caco2 Cells

Lebreton Corinne[1], Sandrine Ménard[1], Juliette Abed[1], Mériem Garfa-Traoré[4], Ivan Moura[2], Georgia Malamut[3], Nadine Cerf-Bensussan[1], Martine Heyman[1]. [1]Université René Descartes, Paris, France; [2]Université Paris Diderot, Paris, France; [3]AP-HP, Paris, France; [4]IFR94, Paris, France

Objectives: In CD, permeability to gliadin peptides occurs mainly along a transcellular transport pathway depending on secretory IgA (SIgA) and the transferrin receptor CD71, overexpressed at the apex of enterocytes. Molecular interactions between IgA and CD71 have been investigated. Methods: In duodenal biopsies from active celiac patients and in Caco2 cell line, SIgA binding to apical CD71 was characterized using confocal microscopy, fluorescence resonance energy transfer (FRET), duolink™ confocal microscopy, flow cytometry and immuno-precipitation. Intracellular traffic was checked by co-localisation with EEA1 (early endosomes) or LAMP-2 (lysosomes). Results: In CD biopsies, FRET indicated molecular interactions between epithelial IgA, CD71 and a third partner, transglutaminase 2 (tgase 2) at the brush-border membrane of enterocytes. In Caco2 cells, flow cytometry demonstrated that CD71 is highly expressed and bind specifically SIgA. IgA was co-immunoprecipitated with CD71 and tgase 2 in cell lysates and interactions between molecules were confirmed by duolink™ imaging. Conclusions: In biopsies from active CD patients and the colon cancer cell line Caco2, two situations where CD71 is highly overexpressed, IgA interact strongly with CD71/tgase 2 at the apex of enterocytes. This is likely to play a role in the retro-transport of IgA/gliadin immune complexes observed in active celiac disease.

W.60. Increased IL-21, but not IL-17 Production in the Small Intestine is Characteristic for Pediatric Celiac Disease

Marieke van Leeuwen, Dicky Lindenbergh-Kortleve, R. de Krijger, Hankje Escher, Janneke Samsom. Erasmus Medical Center, Rotterdam, Netherlands

Celiac disease (CD) is an inflammatory response to gluten, resulting in small intestinal villous atrophy and infiltration of T cells. Currently, the contribution of the T helper 17 derived cytokines IL-21 and IL-17 to the onset of disease is unclear. Therefore we studied the role of IL-21 and IL-17 in a cohort of pediatric CD patients. Using immunohistochemistry, we observed high numbers of IL-21 producing cells in the small intestine of pediatric CD patients compared to healthy controls. IL-21 was produced by CD4+ T cells that did not secrete IL-17 as no significant increase in the number of IL-17 producing cells was observed. To examine the specificity for CD, biopsies from pediatric IBD patients were taken as a control. In IBD increased numbers of both IL-21 and IL-17 secreting T cells were observed. The localization of IL-21 producing cells differed between IBD and CD. In CD, IL-21 producing cells were randomly distributed along the lamina propria, whereas in IBD biopsies the IL-21 producing cells were restricted to infiltrates within crypt abscesses. These results suggest that an IL-17 independent increase of IL-21 production by CD4+ T cells is characteristic for pediatric CD. The underlying mechanism inducing IL-17-independent IL-21 production in CD is currently being investigated.

W.61. Increased Numbers of Circulating Foxp3+T Cells in Adult and Refractory Celiac Disease

Marieke van Leeuwen[1], M. du Pré[1], L. de Ruiter[1], H. Raatgeep[1], G. Tack[2], C. Mulder[2], Hankje Escher[1], Janneke Samsom[1]. [1]Erasmus Medical Center, Rotterdam, Netherlands; [2]VU University Medical Center, Amsterdam, Netherlands

Celiac disease (CD) is a chronic inflammation of the small intestine that develops due to gluten intolerance. The disease is driven by inflammatory gluten-reactive T cells that may develop as a result of defects in regulatory T (Treg)-cell subsets. Recently, we have established that CD62LnegCD38+ expression on T cells identifies circulating mucosal T cells. The aim of this study was to determine whether alterations in the

naturally occurring CD62LhiFoxp3+ T cell population or in mucosally-induced CD62LnegCD38+Foxp3+ Treg-cells could be involved in the pathogenesis of CD. Using flow cytometry we investigated T cell subsets in peripheral blood from pediatric CD patients as well as adult patients with active CD, treated CD and refractory CD (RCD). Healthy children and adults were used as controls. No differences in the number of mucosal CD62LnegCD38+Foxp3+Treg-cells could be detected between any of the groups analyzed. A higher percentage of circulating CD62LhiFoxp3+T cells was observed in adult patients with active CD, treated CD and RCD in comparison with healthy adults. In contrast, pediatric CD patients and healthy children had a similar percentage of Foxp3+T cells. These data demonstrate that adult CD and RCD are associated with increased numbers of circulating naturally occurring Foxp3+Treg but not mucosally-induced Foxp3+Treg.

W.62. Gliadin Induces IL-**1β Production in Peripheral Blood Mononuclear Cell (PBMC) and Monocytes Obtained from Celiac Patients**

Lenka Palova-Jelinkova, Klara Danova, Ludmila Tuckova. Czech Academy of Sciences, Prague, Czech Republic

Background: Celiac disease (CD) is an inflammatory disorder of the small intestine triggered in susceptible individuals by ingestion of wheat gluten. Inflammasomes are cytoplasmic caspase-1-activating protein complexes that promote the inflammation and secretion of the proinflammatory cytokines IL-1β and IL-18. As cytokines associated with Th1 and Th17-mediated pathology including IL-1β and IL-18 have been implicated in the pathogenesis of CD, we have investigated the IL-1β production and inflammasome activation in innate immunity cells stimulated with gliadin. Principal findings: We have found that peptic digest of gliadin induced increased dose-dependent secretion of mature IL-1β from peripheral blood mononuclear cell (PBMC) and monocytes obtained from CD patients compared to healthy donors. The gliadin-triggered secretion of IL-1β was markedly abolished by caspase-1 inhibitor Z-YVAD-fmk. In contrast, monocyte-derived dendritic cells from CD patients and healthy donors as well as mouse bone marrow derived dendritic cells failed to produce IL-1β after beeing stimulated with gliadin alone or in combination with LPS. Conclusion: The gliadin induced IL-1β production and possible inflammasome activation in PBMC and monocytes from CD patients may provide new insight into the role of innate immunity cells in celiac disease pathogenesis.

W.63. A Mouse Model of CD71- and IgA-dependent Absorption of Food Antigens by the Intestinal Epithelium

Juliette Abed[1], Lebreton Corinne[1], Gaël Champier[1], Armelle Cuvillier[1], Michel Cogné[1], Christophe Dugave[2], Nadine Cerf-Bensussan[1], Martine Heyman[1]. [1]Université René Descartes, Paris, France; [2]CEA, Gif sur Yvette, France

Background: Secretory IgA avoid uncontrolled absorption of luminal antigens. In celiac disease however, IgA/gliadin immune complexes are abnormally absorbed via the epithelial transferrin receptor CD71. A mouse model was set-up to reproduce this abnormal retro-transcytosis of IgA/antigen complexes. Methods: BalbC mice were backpacked with OVA-specific IgA B cell hybridoma obtained from α1-knock-in (α1KI) mice producing chimeric human IgA. IgA interaction with epithelial CD71 was characterized by flow cytometry and bi-photonic microscopy. Permeability to 3H-OVA was analyzed in mice treated with tyrphostin-A8, a drug promoting apical CD71 expression in enterocytes. Results: In tyrphostin-A8-treated-backpacked mice, OVA- specific IgA secreted in the intestinal lumen favored intestinal absorption of intact OVA (1.03pmol/90mn.cm2) as compared to control mice (0.18 pmol/90mn.cm2; $p<0.0001$). The transport was IgA-dependent and needed apical expression of CD71 in enterocytes. Conclusion: Ectopic expression of CD71 in enterocytes promotes permeability to IgA-dependent antigen transport, a phenomenon likely to alter mucosal immune response.

W.64. High Prevalence of Antibodies to Deamidated Gliadin in Down Syndrome Patients without Celiac Disease

Oivi Uibo, Anu Kaldmaa, Tiia Reimand, Kaja Metsküla, Tamara Vorobjova, Koit Reimand, Raivo Uibo. University of Tartu, Tartu, Estonia

Down syndrome (DS) is frequently associated with *celiac* disease (CD), but far more with immune reactivity against gluten or gliadin. In present study we aimed to investigate in DS patients antibodies to deamidated gliadin (ADGA) supposing that these antibodies are pathogenetically more significant for CD than antibodies to unmodified gliadin (AGA). Altogether 137 DS patients (63 females, mean age 11 y), including 5 with CD, were studied. IgA and IgG type ADGA were determined by GAF-3X assays (Euroimmune), IgA and IgG type AGA and IgA type antibodies to recombinant human tissue transglutaminase (ATGA) by in-house enzyme immunoassays. In a subset of patients the mRNA tight junction protein 1 (TJP1) expression was evaluated in small bowel biopsy samples by RT-PCR. Our results showed similarly high prevalence of ADGA (26%) and AGA (41%) in DS, in spite of absence of CD or ATGA in most cases. TJP1 mRNA expression did not differ between DS patients and controls. Conclusion: ADGA are frequent in DS without CD. High prevalence of AGA and ADGA is probably not related to changes in intestinal mucosal integrity but rather to general immune disturbances in DS patients.

W.65. Bacterial Motility and Chemotaxis Mediate Initiation Events in the Squid-Vibrio Symbiosis

Caitlin Brennan, Natacha Kremer, Benjamin Krasity, Margaret McFall-Ngai, Edward Ruby. University of Wisconsin, Madison, WI

The symbiosis between bioluminescent vibrio fischeri and the hawaiian bobtail squid, Euprymna scolopes, begins anew each generation. Colonization is initiated as symbionts migrate from mucus-bound aggregates on the light-organ surface to epithelium-lined crypts deep within its tissue. To understand the roles of chemotaxis and flagellar motility during initiation we used complementary genetic and imaging techniques. Analysis of mutants that exhibited motility defects in soft agar revealed that, while flagellar motility is essential, chemotaxis mediates efficient colonization of juvenile squid. Secondly, by observing GFP-labelled V. fischeri cells, we identified three stages at which chemotaxis likely mediates

colonization. Recently, we observed another, novel role for motility during initiation. RNA-Seq analysis of host cells upon initial contact with wild-type V. fischeri identified marked up-regulation of expression of eslbp2, a gene encoding a lipopolysaccharide-binding protein. In contrast, exposure to a flagellated, non-motile mutant resulted in eslbp2 levels comparable to squid not exposed to V. fischeri. Thus, flagellar motility and chemotaxis play roles in both activating the host immune response and mediating the migration into the deep crypts. The complexity observed during initiation of the squid-vibrio symbiosis continues to illuminate general mechanisms underlying host-microbe interactions and serves as a natural, experimentally tractable mutualism model.

W.66. Resemblance of Fine Specificity of Iga Anti-**gliadin Antibodies Between Patients with Celiac Disease and Humanized α1KI Mice**

Daniel Sánchez[1], Michel Cogné[2], Helena Tlaskalova-Hogenova[1], Thomas Mothes[3], Ludmila Tuckova[1]. [1]Academy of Sciences of the Czech Republic, Prague, Czech Republic; [2]Université de Limoges, CNRS, Limoges, France; [3]University Hospital Leipzig, Leipzig, Germany

Gliadins, and mainly α-gliadins possessing various sequences such as aa 31-49, aa 56-88 (33-mer), aa 57-68, aa 69-82, are critical in induction of immune response or toxic reaction leading to the development of celiac disease (CLD). The role of IgA anti-gliadin antibodies (IgA AGA) is unknown. For this reason, we prepared several humanized monoclonal IgA AGA using transgenic α1KI mice. Using Pepscan technique with overlapping decapeptides of α-gliadin we observed a robust similarity between the specificity of humanized mouse monoclonal IgA AGA and IgA AGA from patients with active CLD. The common immunodominant region included several sequential epitopes localized in the N-terminal part of α-gliadin (QFQGQQQPFPPQQPYPQPQPFP, aa 29-50, and QPFPSQQPYLQL, aa 47-58). Notably, IgA AGA secreted by clones 8D12, 15B9, 9D12, and 18E2 had significant reactivity against sequences localized in the 33-mer - LQLQPFPQPQ (aa 56-65) and PQLPYPQPQPFL (aa 69-80). Humanized mouse monoclonal IgA AGA having known specificity are suitable as standard in ELISAs to detect serum IgA AGA of CLD patients and for studying the AGA pathogenic role in CLD, especially for analyzing the translocation of complex of specific IgA antibodies and individual gliadin peptides through enterocyte barrier.

W.67. Analysis of TCR Rearrangements in RCDII Intra-epithelial Lymphocytes Pleads for their Origin from Immature T Cells Blocked at an Early Stage of Differentiation

Nicolas Montcuquet[1], Kheira Beldjord[2], Elodie Vidal[2], Elizabeth Macintyre[2], Georgia Malamut[1], Christophe Cellier[1], Bertrand Meresse[1], Nadine Cerf-Bensussan[1]. [1]INSERM, Paris, France; [2]Hôpital Necker-Enfants-Malades, Paris, France

Type II refractory CD (RCDII) is a major complication of celiac disease, now considered as an intraepithelial T cell lymphoma. RCDII is characterized by phenotypically aberrant intra-epithelial lymphocytes (IELs), which lack surface CD3-T cell receptor (TCR) complexes (sCD3-) but contained intracellular CD3epsilon and other CD3 chains. Two hypotheses were proposed to explain RCDII sCD3- abnormal phenotype. First, they may derive from mature T cells which have lost their TCR after chronic stimulation. Alternatively, they may be immature T cells blocked at an early stage of differentiation. In order to gain insight into the origin of RCDII sCD3- IELs, we have analyzed the characteristics of their TCR rearrangements. V(D)J rearrangements of TCR gamma, delta and beta genes were analyzed on biopsies and/or CD3- IELs lines from 28 RCDII patients. Evidence of functional rearrangements of the clonal TCR was only found in 9 out of 28 RCDII patients. In the 19 other patients, clonal TCR rearrangements were either incomplete or out of frame, and incompatible with the expression of a functional surface TCR. The majority of abnormal sCD3- IELs in RCDII exhibit TCR rearrangements suggestive of an early block in T cell differentiation. Analysis of the underlying mechanism(s) is in progress.

W.68. Internalization of the Non-immunodominant p31-43 but Not the Immunodominant p57-68 Gliadin Peptide by Celiac Myeloid Dendritic Cells (DCs) in an *in vitro* Co-culture Transwell Model with Caco2 Cells

Antonio Di Sabatino[1], Laura Rovedatti[1], Cristina Ubezio[1], Paolo Biancheri[1], Patrizia Vaghi[1], Maria Rescigno[2], Gino Roberto Corazza[1]. [1]University of Pavia, Pavia, Italy; [2]European Institute of Oncology, Milano, Italy

Background and aim: DCs are able to open tight junctions and sample luminal antigens. Gluten peptides induce DC maturation and migration. By using an *in vitro* co-culture transwell model with Caco-2 cells, which resembles the intestinal barrier, we investigated the role of celiac DCs in sampling the non-immunodominant (p31-43) and immunodominant (p57-68) gliadin peptides, the latter supposed to trigger innate immunity. Methods: Peripheral myeloid DCs from HLA-DQ2+ celiac disease (CD) patients were co-cultured in a transwell model with Caco-2 cells and a fluorescent peptide (p31-43 or p57-68). Transwell membranes were analyzed by confocal microscopy. The same experiments were performed using peripheral myeloid DCs from HLA-DQ2+ and HLA-DQ2- healthy controls. Purity of DCs was confirmed by flow cytometry. Results: At confocal, myeloid celiac DCs colocalized with p31-43 but not p57-68 at the apical side of Caco-2 cell monolayer. DCs from both HLA-DQ2+ and HLA-DQ2- control groups colocalized with none of the peptides and laid at the basolateral side of Caco-2 cell monolayer. Conclusions: Celiac myeloid DCs are able to internalize the non-immunodominant but not the immunodominant gliadin peptide, strengthening the key role of innate immunity in CD. Since the presence of HLA-DQ2 gene is not sufficient for peptide sampling, other mechanisms are likely involved in this process.

W.69. Anandamide Metabolism in Celiac Disease

Antonio Di Sabatino[1], Natalia Battista[2], Paolo Biancheri[1], Laura Rovedatti[1], Monia Di Tommaso[2], Cristina Ubezio[1], Mauro Maccarrone[2], Gino Roberto Corazza[1]. [1]Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy; [2]University of Teramo, Teramo, Italy

Background and aim: The involvement of the endocannabinoid system in chronic inflammatory bowel disorders has been already described. In order to investigate potential changes of the major endocannabinoid anandamide (AEA) metabolism in celiac disease (CD), we examined the activity of enzymes responsible for AEA synthesis and degradation. Methods: Duodenal biopsies were collected from 7 untreated CD patients, 7 CD patients on gluten-free diet, and 7 control subjects. 24h-organ cultures were performed culturing biopsies from treated CD patients with or without the peptic-tryptic digest of gliadin (PT-gliadin). AEA synthesis through N-acylphosphatidylethanolamine phospholipase D (NAPE-PLD) activity and AEA hydrolysis by fatty acid amide hydrolase (FAAH) were assayed on mucosal tissue homogenates. The cannabinoid (CB)1 and CB2 receptor expression was detected by immunoblotting and confocal microscopy. Results: NAPE-PLD activity was significantly higher in active CD mucosa or when biopsies were incubated with PT-gliadin. On the contrary, FAAH activity did not change both in *in vivo* and *ex vivo* experiments. While CB1 expression was enhanced in untreated CD patients in comparison to treated CD and controls, no difference was observed in CB2 expression. Conclusions: These findings, which are in agreement with a previous report showing higher AEA levels in active CD mucosa, strengthen the involvement of AEA metabolism in CD.

W.70. Food Modifications by Industrial used Transglutaminase Induces Allergenic and Autoantigenic Proteins

Anja Gräfe, Maria Bode, Karl Skriner. Humboldt University and Free University, Berlin, Germany

Background: In former east-Berlin allergies and the amount of celiac disease patients increase since food from the west-Berlin is available. Methods: Sera form wheat-allergic and celiac disease patients were used to study the autoantibody response to cereal proteins and 34 Eragrostis. tef-(teff)-varieties. Results: So far in teff no immunogenic gluten could be detected, although 17% of celiac patients after consumption respond with disease-specific symptoms. Proteins were purified from 34 different varieties of teff, commercially available teff flour, and various cereal varieties. Several immunoreactive proteins in all teff varieties were found after transglutamination. Similarly, we could prove IgE-mediated reaction to teff by immunoblot. Wheat allergic and celiac disease sera were tested with industrial transglutaminase-modified wheat, teff and showed that the transglutamination was essential or influenced the reactivity of allergenic proteins and detected new autoantigens present in celiac disease patient sera. Moreover, it was shown that the modification of cereal extracts with industrial transglutaminase under certain conditions can inhibit but also increase the immunoreactivities in wheat-allergic and celiac disease patient sera. Conclusion: Transglutaminated teff should not be eaten from wheat-allergic and celiac disease patients. Industrial-transglutaminase is essential for rendering cereal proteins and Eragrostis tef to be autoantigenic and allergenic.

W.71. Human Mucosal Mast Cells Produce Multiple Chemokines

Katrin Feuser, Stephan Bischoff, Axel Lorentz. University of Hohenheim, Stuttgart, Germany

Mast cells exert their biological functions by releasing mediators inter alia chemokines in response to activation. Chemokines are small proteins with important immunoregulatory functions. Here, we analyzed the whole chemokine profile produced by human mucosal mast cells isolated from intestinal tissue. Mast cells were cultured in the presence of SCF with or without IL-4. We detected mRNA expression for CCL1, CCL2, CCL3, CCL3L1, CCL4, CCL4L1, CCL5, CCL7, CCL13, CCL18, CCL19, CCL20, CCL23, CCL25, CCL28, CXCL2, CXCL3, CXCL4, CXCL5, CXCL8, CXCL10, CXCL14, CXCL16, XCL1, and CX3CL1. Furthermore, we detected mRNA expression for the chemokine receptors CCR6, CCR7, CXCR1, CXCR2, CXCR3, CXCR4, XCR1, and CX3CR1. The expression of CCL2, CCL3, CCL4, CCL5, CCL7, CCL18, CCL20, CXCL2, CXCL3, CXCL8, and XCL1 was more than 4-fold up-regulated following mast cell activation by IgE receptor crosslinking. Moreover, expression and release of CCL2, CCL3, CCL4, and CXCL8 was up to 10-fold up-regulated and most pronounced for CXCL-8 with up to 8 pg/1000 cells in response to sequential challenge of mast cells by priming with IL-4, stimualtion with SCF, and activation by IgE receptor crosslinking in comparison to IgE receptor crosslinking alone. In summary, human mucosal mast cells are a potent source of multiple chemokines.

W.72. Role of Thymic Stromal Lymphopoietin (TSLP) Signaling in Intestinal Immune Homeostasis

Ilaria Mosconi, Joanna Massacand, Nicola Harris. École Polytechnique Fédérale, Lausanne, Switzerland

TSLP is a cytokine constitutively expressed by intestinal epithelial cells (IECs) which has been hypothesized to contribute to intestinal inflammation and homeostasis. To address its role in intestinal homeostasis we utilized TSLP receptor (TSLPR) deficient mice. TSLPR-/- mice exhibited enlarged caecal patches and colonic isolated lymphoid follicles (ILF). This observation did not correlate with defects in SIgA production or changes in the bacterial microflora. Instead we observed significant changes in immune cell populations of both the lymphoid tissues and intestinal lamina propria (LP) of TSLPR-/- mice: we found a higher proportion of interferon-γ (IFNγ) and tumor necrosis factor-α (TNFα) producing-CD8+ T cells and of IFNγ and interleukin (IL)-17 producing-CD4+ T cells. Although TSLP has been reported to regulate dendritic cell production of IL-12p40, no differences in IL-12p40 within the serum or intestine were noted. Gene expression data indicated an upregulation of CXCL11, together with other genes known to be associated with inflammatory or metabolic pathways, and typically associated with intestinal macrophages and/or IECs. Preliminary data indicates that functional TSLPR is expressed by intestinal macrophages, but not IECs. Future experiments are aimed at elucidating the impact of TSLP-TSLPR interactions in modulating macrophage function and the impact of enhanced CXCL11 on ILFs formation.

W.73. Peyer's Patch Dendritic Cells Sample Antigens by Extending Dendrites through M Cell-specific Transcellular Pores

Hugues Lelouard, Mathieu Fallet, Béatrice de Bovis, Stéphane Méresse, Jean-pierre Gorvel. CNRS/INSERM, Marseille, France

Peyer's patches (PP) of the small intestine are antigen sampling and inductive sites for the establishment of the mucosal immunity. Luminal antigens are transported from the mucosal surface of PP to the subepithelial dome (SED) through the specialized epithelial M cells of the follicle-associated epithelium. Among the SED resident dendritic cells (DC), which are ideally situated for taking up these antigens, LysoDC express high levels of lysozyme and display a strong phagocytic activity. However, the mechanisms by which LysoDC capture luminal antigens *in vivo* are currently unknown. Here we show that LysoDC extend dendrites through M cell-specific transcellular pores to reach the gut lumen. M cell cellular adhesion molecules are recruited at the site of the transcellular migration where they form a channel surrounding the DC extension. Transcellular dendrites scan the M cell apical surface and the gut luminal content, taking pathogenic bacteria in the lumen before retracting back to the SED. This new sampling mechanism improves our understanding of the mucosal immune response initiation and offers a new alternative for the specific targeting of mucosal vaccines.

W.74. Interleukin-13 Induced Airway Inflammation and Chemokine Production is Mediated by CD11b+ Cells

Margaret Crapster-Pregont, Janice Yeo, Douglas Kuperman. Northwestern University, Chicago, IL

Interleukin (IL)-13 is a critical mediator of allergic inflammation. It has been difficult to identify the important IL-13-responding cell type. We studied the effects of airway delivery of IL-13 alone as compared to a mixture of IL-13 and IFN-γ on airway inflammation and chemokine production. IL-13 induced allergic inflammation and IFN-γ inhibited the pro-inflammatory effects of IL-13. We used ELISA and RT-PCR to survey the effects on chemokines. We observed a restricted pattern of chemokines that were induced by IL-13 and inhibited by IFN-γ (CCL11, CCL17, CCL22, CCL24, and CCL4). This pattern matched that of the allergic inflammation. Therefore, we speculated that the cell type that produces the above chemokines was likely to be an important IL-13 responding cell type. Based on the scientific literature, we hypothesized that dendritic cells mediate the pro-inflammatory effects of IL-13. We used mice that express the simian diptheria toxin receptor under control of the CD11b promoter. As compared to controls, the effects of IL-13 on allergic airway inflammation and chemokine production were either largely inhibited or completely ablated by CD11b cell depletion. We conclude that CD11b+ cells, likely dendritic cells, are important mediators of the effects of IL-13 on allergic airway inflammation.

W.75. The Role of Migrating Intestinal Dendritic Cells in Ankylosing Spondylitis

Lotta Utriainen, Dawn Firmin, Vuk Cerovic, Pamela Wright, Iain McInnes, Simon Milling. University of Glasgow, Glasgow, United Kingdom

Ankylosing Spondylitis (AS) is a chronic inflammatory disease that affects the axial skeleton and other tissues. Over 95% of AS patients express the MHC class I molecule HLA-B27, and rats transgenic for the human HLA-B27 and β2 microglobulin genes (HLA-B27-tg rats) develop a multisystem inflammatory disease, offering a model for studying AS. Intestinal inflammation and the commensal mirobiota in the gut are important in disease development. We hypothesise that the HLA-B27 protein alters the functions of the DCs that migrate from the intestine, and that these cells drive disease. We therefore collected migrating intestinal lymph DCs (L-DCs) by thoracic duct cannulation. Flow cytometric analysis of L-DCs showed that whereas the surface expression of MHC II, CD103, CD80 and CD86 are similar on L-DCs from both wild type (WT) and HLA-B27-tg rats, L-DCs from transgenic rats express higher levels of CD25, a marker for DC activation. Additionally, unlike L-DCs from WT rats, L-DCs from HLA-B27-tg animals lack the CD172a-negative population, which is involved in the maintenance of oral tolerance. These results indicate that L-DCs from HLA-B27-tg are altered, as hypothesised. Future experiments will determine whether HLA-B27-tg DCs drive inflammatory pathology, and further explore the functional consequences of their altered phenotype.

W.76. CCR5 and CCR6 Play Central Roles in Adenovirus Expressing Flt3 Ligand-induced NALT Dendritic Cell Migration and S-IgA Ab Responses in Mucosal Effector Tissues

Yoshiko Fukuyama, Shinichi Sekine, Ryoki Kobayashi, Rebekah Gilbert, Kohtaro Fujihashi. University of Alabama at Birmingham, Birmingham, AL

Our previous studies showed that an adenovirus expressing Flt3 ligand (Ad-FL) as nasal adjuvant activated $CD11b^+$ dendritic cells (DCs) enhanced antigen (Ag)-specific immune responses in both mucosal and systemic compartments. In this study, we examined the expression of chemokine receptors by $CD11b^+$ DCs and determined their roles in cell migration and induction of Ag-specific mucosal immunity. C57BL/6, $CCR5^{-/-}$ and $CCR6^{-/-}$ mice were nasally immunized three times at weekly intervals with ovalbumin (OVA) plus Ad-FL. The kinetic flow cytometry analyses revealed that early expansion of $CCR5^+$ and $CCR6^+$ $CD11b^+$ DC populations in NALT and cervical lymph nodes of C57BL/6 mice given Ad-FL. Significantly increased numbers of both CCR5- and CCR6- but not CCR7-expressing $CD11b^+$ DC subsets were seen in submandibular glands and nasal passages in a time-dependent manner. Thus, both $CCR5^{-/-}$ and $CCR6^{-/-}$ mice given nasal Ad-FL plus OVA showed reduced numbers of $CD11b^+$ DCs. Further, OVA-specific S-IgA Ab responses in saliva and nasal washes were significantly diminished in $CCR5^{-/-}$ and $CCR6^{-/-}$ mice when compared with C57BL/6 mice. These results show that the CCR5 and CCR6 play important roles in DC migration and subsequent induction of Ag-specific S-IgA Ab responses. Supported by NIH grants DE12242 and AG025873.

W.77. IFN-γ **Secreted by CD103+ Dendritic Cells Leads to IgG Class Switching in the Mesenteric Lymph Node in the Absence of** Vitamin A

Sun-Young Chang[1], Jae-Hoon Chang[1], Hye-Ran Cha[1], Soo Jung Han[1], Jae-Ouk Kim[1], Sang-Uk Seo[1], Hyun-Jeong Ko[2], Mi-Na Kweon[1]. [1]International Vaccine Institute, Seoul, Republic of Korea; [2]Kangwon National University, Chuncheon, Republic of Korea

Although the induction mechanism of SIgA has been well studied, that of IgG in the mucosal compartments is not well understood. In the current study, vitamin A deficiency was convincingly shown to be associated with increased IgG in serum and intestinal fluid. We found increased numbers of IgG-secreting B cells in the lamina propria of the small intestine and mesenteric lymph node (MLN) of vitamin A-deficient (VAD) mice. Of note, IFN-γ secreted by MLN DCs was significantly augmented in VAD mice unlike control mice, and CD103+ DCs were main subsets to secret IFN-γ. The aberrant increase of IgG in VAD mice can be ascribable to IFN-γ, since IFN-γ knockout VAD mice have normal IgG levels and the addition of recombinant IFN-γ increased IgG production by B cells co-cultured with MLN DC from IFN-γ knockout VAD mice. Oral feeding of antibiotics resulted in significant reduction of IgG, indicating a critical role for altered commensal bacteria for IgG class-switching recombination in the absence of vitamin A. Collectively, vitamin A deficiency provokes the generation of IFN-γ-secreting CD103+ DCs, which may be a critical regulator for IgG generation in the MLN.

W.78. CD83 Regulates DC Immune Responses through a Homotypic Interaction

Jennifer Bates, Jason Hackney, Elizabeth Luis, Jiabing Ding, Irene Tom, Zora Modrusan, Lino Gonzalez, Lauri Diehl. Genentech, Inc., South San Francisco, CA

CD83 is a well-conserved type-1 membrane protein of the Ig superfamily found primarily on the surface of mature dendritic cells (DCs). Soluble CD83 has immunosuppressive activity, however, the function of CD83 on DCs and its putative ligand remains unknown. We have identified a CD83 homotypic interaction that elicits anti-inflammatory effects on DCs. Treatment with soluble CD83 or anti-CD83 antibody during DC maturation resulted in decreased expression of surface activation markers and secretion of proinflammatory cytokines, such as IL-12p40. Knockdown of surface CD83 expression, or truncation of the cytoplasmic region, abrogated the response to CD83 treatment demonstrating that CD83 homotypic interaction mediates inhibition of inflammation. MAPK and mTOR signaling function downstream of this repression as CD83 treatment inhibits phosphorylation of mTOR and p38α, which are necessary for surface activation marker expression and IL-12p40 production. CD83 immunosuppression is pivotal in maintaining the balance between tolerance and immunity, as mice overexpressing CD83 at the mucosal surface are more resistant to colitis, leading to weight retention and decreased serum cytokine levels. Thus, a CD83 homotypic interaction regulates DC immune response, preventing inappropriate inflammation and promoting tolerance.

W.80. Homeostatic Signals of the Gut are Lost in Affected Areas of Ulcerative Colitis Patients Driving Dendritic Cells into a Pro-inflammatory Phenotype

David Bernardo Ordiz[1], Sara Vallejo-Díez[2], Hafid Al-Hassi[1], Elizabeth Mann[1], Beatriz Martínez-Abad[2], Enrique Montalvillo[2], Cheng Tee[1], Aravinth Murugananthan[1], Ailsa Hart[3], Luis Fernández-Salazar[2], José Garrote[2], Eduardo Arranz[1], Stella Knight[1]. [1]Imperial College London, London, United Kingdom; [2]Universidad de Valladolid, IBGM-CSIC, Valladolid, United Kingdom; [3]St. Mark's Hospital, London, United Kingdom

Background/aims: In health, the gut microenvironment controls the phenotype and function of human dendritic cells (DC) driving them into a "tolerogenic" phenotype. We studied the effect of conditioning DC with the tissue microenvironment of ulcerative colitis (UC) patients. Methods: Cell-free supernatant (SN) from cultured colonic biopsies (from affected and unaffected areas of UC patients and healthy controls) were used to condition human blood enriched DC from healthy volunteers. Phenotype and function of DC was determined by flow cytometry and mixed leukocyte reactions respectively. Results: Healthy-SN-conditioned DC acquired a "gut-like" phenotype assessed by depletion of skin homing markers, a lower stimulatory capacity for allogeneic T cells and acquisition of a gut-homing profile on the stimulated T cells. SN from unaffected areas of UC patients induced a similar phenotype on DC. However, DC conditioned with affected-UC-SN failed to acquire a restricted gut-homing profile. Moreover, those conditioned DC did not decrease their stimulatory capacity and primed responding T cells with a skin homing profile through an IL-6 and IL-13 dependent mechanism. Conclusion: Homeostatic signals of the gut are lost in affected areas of UC patients driving DC, through an IL-6 and IL-13 dependent mechanism, into a pro-inflammatory not gut-restricted phenotype.

W.81. The Effects of Glucagon-like Peptide 2 on the Phenotype and Functions of Blood Enriched Dendritic Cells

Cheng Tee[1], David Bernardo Ordiz[1], Aravinth Murugananthan[1], Elizabeth Mann[1], Simon Peake[1], Katharina Wallis[2], Simon Gabe[2], Stella Knight[1], Hafid Al-Hassi[1]. [1]Imperial College London, London, United Kingdom; [2]St. Mark's Hospital, London, United Kingdom

Introduction: Glucagon-like peptide 2 (GLP-2), an intestinal growth factor reduces mucosal inflammation in murine studies by decreasing pro-inflammatory cytokines1. This anti-inflammatory characteristic however has not been studied in humans. Method: Human blood enriched dendritic cells (DC) from healthy volunteers were cultured in-vitro for 24 hours with GLP-2. Phenotype and function of DC were then assessed by flow cytometry. Ongoing intracellular cytokine production was measured by accumulation during a 4-hour incubation in monensin (n=5) and analysed for statistical significance. Results: GLP-2 conditioning downregulated HLA-DR intensity (p=0.007) and increase in CD14 expression (p=0.006) on DC, compared with basal control culture although these changes were not accompanied by an increased phagocytic capacity (p=NS). GLP-2 treatment also resulted in the reduction of cytokine IFNγ (p=0.003) and IL-12 (p=0.043) production but there were no changes in IL-10 and IL-17a (p=NS). Conclusion: The phenotypic findings suggest an immunomodulatory effect of GLP-2 on DC. The decrease in the ongoing production of IFNγ and IL-12 indicates that GLP-2 exerts its anti-inflammatory function via Th1 pathway. Further human studies are in progress to determine the effects of GLP-2 on the immune responses in the intestinal mucosa. Reference: 1.Ivory et al. (2008). Am J Physiol Gastrointest Liver Physiol 295:G1202-

G1210.

W.82. The Complexity of the Murine Microbiota Influences Recruitment of Immune Cells in Early Life
Matilde Kristensen[1], Tine Licht[1], Anders Bergström[1], Stine Metzdorff[2], Hanne Frøkiær[2]. [1]Technical University of Denmark, DTU, Søborg, Denmark; [2]University of Copenhagen, Frederiksberg, Denmark

Early life microbiota is assumed to be involved in maturation of our immune system, and several studies focus on the bacterial ability to stimulate immune development and homeostasis. We have tested the immune maturing properties of Lactobacillus acidophilus NCFM *in vivo* by comparing mouse pups mono-colonized with L. acidophilus NCFM with conventionally colonized pups. Flow cytometric analyses of splenocytes of the two different groups revealed no significant difference in the ability of the colonizations to mature B-cells, T cells and dendritic cells. However, recruitment of neutrophils to the spleen was significantly lower in the neonatal mono-colonized mice when compared to conventionally colonized animals. Up to five fold higher levels of CD11b+ neutrophilic cells were observed in spleens of conventionally colonized pups compared to mono-colonized pups on post-natal day 4 and 7. Thereafter, the number of neutrophilic cells decreased, and the levels in the two groups were equalized at post-natal-day 35. To reveal mechanisms involved in neutrophilic recruitment to the infant spleen, qPCR analyses have been used to characterize genes in intestinal tissues and spleen anticipated to be involved in the process. Immune histochemical stainings of tissues from spleens and livers have further been used to characterize neutrophilic influx in these organs.

W.83. Selective Transmission of HIV-1 by Vaginal Dendritic Cells
Ruizhong Shen, Holly Richter, Lesley Smythies, Philip Smith. University of Alabama at Birmingham, Birmingham, AL

Worldwide, the heterosexual route is the prevalent mode of HIV-1 transmission. However, many critical issues regarding the immunobiology of HIV-1 transmission in genital mucosa are poorly understood. We recently identified mucosal dendritic cells (DCs) as mediators of HIV-1 entry in the small intestine. However, the role of vaginal mucosal DCs and the “gate-keeping” mechanism that favors the transmission of R5, rather than X4, HIV-1 during acute infection have not yet been defined. Using isolated human vaginal mucosal mononuclear cells (MNLs) and explanted vaginal mucosa, we show that human vaginal DCs (a) consist of a significantly higher proportion of myeloid cells, (b) take up HIV-1 more efficiently compared to monocyte-derived DCs, (c) are the first vaginal MNL target cells to take up HIV-1, (d) disseminate HIV-1 locally and systemically, (e) transmit HIV-1 in trans to CD4+ T cells and (f) preferentially take up and transfer R5, rather than X4, virus to reporter cells. These findings indicate vaginal DCs play a critical role in HIV-1 heterosexual transmission and R5 virus selection.

W.84. Human Gastric Stroma Limits the Th1 Response to H. Pylori Through Inhibition of Dendritic Cell Maturation and IL-12 Secretion
Diane Bimczok, Jayleen Grams, Richard Stahl, Ken Waites, Lesley Smythies, Philip Smith. University of Alabama at Birmingham, Birmingham, AL

Gastric dendritic cells (DCs) initiate the adaptive immune response to Helicobacter pylori, as we recently reported. However, the extent to which the microenvironment of the mucosa shapes the function of gastric DCs in human H. pylori infection is unknown. To recapitulate DC development in the gastric mucosa and elucidate the effects of stromal products on DC function, monocyte-derived DCs were generated in the presence of culture supernatants derived from isolated human gastric stroma. The presence of gastric stromal factors during DC development potently inhibited subsequent H. pylori-induced DC maturation and IL-12p70 secretion. Consequently, the ability of stromal factor-treated DCs to induce CD4 T cell IFN-γ secretion was abrogated, but could be restored by adding rhIL-12 to the DC-T cell co-cultures. We next analyzed the composition of gastric stromal supernatants and detected PGE_2 and TGF-β, both of which have regulatory effects on antigen-presenting cells. Addition of PGE_2, but not TGF-β, during DC generation recapitulated the down-modulated DC phenotype profile induced by gastric stromal factors. In conclusion, stromal PGE_2 inhibition of DC function may be a novel homoeostatic mechanism for the gastric mucosa that limits the initial Th1 response to H. pylori and potentially contributes to mucosal permissiveness for chronic H. pylori infection.

W.85. Modulation of Peyer’s Patch Dendritic Cell Populations and Cytokine and Chemokine Gene Expression by Low Dose LPS
John Guan, Nikhat Contractor. Pfizer, Collegeville, PA

Dendritic cells (DC) in Peyer’s patch (PP) play a crucial role in the induction of oral tolerance and immunity to infectious agents. However, little is known about the modulation of PP DC populations in response to systemic inflammation. We report the effects of intraperitoneal (i.p.) injection of low dose LPS (2.5 mg LPS per kg body weight) on dendritic cell populations and immune-related gene expression in mouse PP. Gene expression was determined for 2h, 4h, 8h and 24h after LPS injection. Inflammatory cytokine and chemokine genes were upregulated 2-4h post LPS, and returned to homeostatic levels 24h post LPS. Total cell yield and DC subsets in PP were evaluated 24h post LPS challenge. Low dose LPS injection resulted in decreased (69.49±4.48%, $p<0.05$) total lymphocytes in the PP. DC subsets were also reduced: CD11c+CD11b+CD8-DC (75.67±7.64%, $p<0.05$), CD11c+CD11b-CD8+DC (91.13±2.52%, $p<0.05$) and CD11c+CD11b-CD8- DC (75.76±2.92%, $p<0.05$). By contrast, plasmacytoid DC were unaltered. These data demonstrate differential regulation of dendritic cell loss in PP after LPS injection. In addition, these data characterize the local intestinal response to systemic low dose LPS injection, and provide a useful model for investigating the effects of dietary components on mucosal immune responses under mild inflammatory conditions.

W.86. CD45 on Innate Immune Cells is Required for the Development of T Cell Transfer and Anti-CD40 Colitis

Asanga Samarakoon, Manisha Dosanjh, Jennifer Cross, Pauline Johnson. University of British Columbia, Vancouver, BC, Canada

CD45 is a leukocyte specific tyrosine phosphatase that regulates TLR induced proinflammatory cytokine production in dendritic cells. Since dendritic cells have been implicated in both tolerogenic and inflammatory responses in the gut, we sought to investigate the effect of CD45 on innate immune cells in mucosal immunity. Colitis was induced in RAG-1 null and CD45RAG-1 null mice using the T cell transfer model or by the injection of anti-CD40 antibodies. In T cell-induced colitis, CD45RAG-1 null mice have delayed wasting disease compared to the RAG-1 mice but have equivalent inflammation in the colon assessed by leukocyte infiltration into the lamina propria and by histopathology. Conversely, in anti-CD40-induced colitis, preliminary data showed no significant differences in weight loss between the RAG-1 null and CD45RAG-1 null mice, but CD45RAG-1 null mice had reduced leukocyte infiltration in the lamina propria and cell analysis revealed differences in dendritic cell and macrophage populations in the CD45RAG-1 null mice compared to the RAG-1 null mice. This demonstrates a disconnect between weight loss and intestinal inflammation in the two mouse models of colitis and implicates a role for CD45 in regulating both responses. Here we will present data showing how CD45 affects both intestinal and systemic inflammatory responses.

W.87. Dendritic Cells Mediate the T Cell Retention in the Interfollicular Region of Peyer's Patches

Naoko Shibata, Takashi Obata, Yoshiyuki Goto, Izumi Ishikawa, Shintaro Sato, Jun Kunisawa, Hiroshi Kiyono. University of Tokyo, Minato-ku, Japan

To achieve the immunological function of Peyer's patches (PPs) to induce antigen-specific immune responses against orally immunized antigens, PPs exhibit rigidly organized micro-architecture composed of all of necessary immunocompetent cells. It remains to be investigated how the distinct cell localization is maintained in the PPs. In this study, we show that conventional dendritic cells (cDCs) are essential for the retention of naïve CD4+ T cells in the interfollicular region (IFR) of PPs. Temporal depletion of cDCs by treating diphtheria toxin (DT) receptor transgenic mice with DT resulted in the abolishment of IFR structure in the PPs while B cell follicle and germinal center in the PPs, and micro-architecture of mesenteric lymph nodes were normal. Additionally, stromal cells in the PPs of cDC-depleted mice expressed normal levels of CCL19 and CCL21, which was reported to be involved in the maintenance of IFR structure. Kinetics analysis of adoptively transferred CD4+ T cells indicated that depletion of cDCs was associated with the impaired retention of T cells, but not immigration of T cells into the IFR of PPs. These data collectively revealed a unique and specific function of PP cDCs in the retention of naïve T cell in the IFR.

W.88. Factors from Inflamed Human Intestinal Mucosa Act on Dendritic Cells to Uncouple the Imprinting of Gut Tropism from FoxP3+ T Cell Generation

Theodore Sanders, Neil McCarthy, Nichola Gellatly, James Lindsay, Andrew Stagg. Blizard Institute of Cell and Molecular Science, London, United Kingdom

In the healthy intestine, dendritic cells (DC) control the balance between effector and regulatory T cell (Treg) responses to commensal bacteria. Production of retinoic acid (RA) allows intestinal DC to imprint T cell gut tropism via expression of α4β7 integrin and CCR9, whilst enhancing generation of FoxP3+ Treg. We hypothesize that this pathway is dysregulated in inflammatory bowel disease (IBD), uncoupling imprinting of gut tropism from Treg differentiation. DC were generated from monocytes with GM-CSF and IL-4 in the presence or absence of medium conditioned by colonic biopsies from IBD patients or healthy controls. Exposure of DC to medium conditioned by inflamed tissue (ICM), but not to medium conditioned by non-inflamed tissue, significantly increased their ability to induce α4β7 on CD4+ T cells responding in a mixed leukocyte reaction. Paradoxically, ICM reduced the DC production of RA as determined by the frequency of ALDH1A+ cells. FoxP3 expression was reduced in T cells stimulated by ICM-DC. These data suggest that factors within the inflamed intestinal mucosa act on DC to enhance generation of gut-tropic T cells, without increasing RA production, and reduce differentiation of FoxP3+ putative Treg. We propose that this uncoupling of homing and Treg generation perpetuates inflammation in IBD.

W.89. Infliximab Response in Crohn's Disease is Associated with Increased CD103+ Mucosal Dendritic Cells and Altered Myeloid Antigen Presenting Cell Dynamics

Neil McCarthy, James Lindsay, Andrew Stagg. Barts and the London School of Medicine & Dentistry, Queen Mary University of London, London, United Kingdom

Introduction: Murine gut homeostasis depends on the balance between monocyte-derived pro-inflammatory dendritic cells (DC) and integrin $CD103^+$ 'regulatory' DC, while activation of these subsets can be influenced by TNFα, which is a key cytokine in Crohn's disease (CD). Although anti-TNF antibodies are therapeutic in CD, their effect on human gut DC is unknown. Method: $CD11c^+HLA\text{-}DR^+$ cells ($CD14^-$ DC and $CD14^+$ monocyte/macrophages; MoMφ) were analysed by flow-cytometry in blood and disaggregated biopsies of non-involved colon from 7 patients with moderately active ileo-colonic CD. (CDAI median:inter-quartile range; 263:194, CRP 25:39mg/L), both pre- and post-infliximab therapy (IFX) and compared with healthy controls (n=12). Results: Circulating 'gut-homing' monocytes (integrin $\beta7^+$) were selectively depleted in CD patients (median reduction 63% $\beta7^+$:$\beta7^-$ 33%; p=0.007). IFX induced clinical remission in all patients (CDAI <100, CRP <6mg/ml) and MoMφ numbers in non-involved colon were reduced from median 527 to 185 cells mg^{-1} (p=0.031), while $\beta7^+$ 'gut-tropic' monocytes increased 2-fold in blood (p=0.004). In non-involved colon, the proportion of myeloid DC expressing CD103 (a marker of regulatory DC) was significantly increased after IFX (median 25%

increase; p=0.012). Conclusion: Infliximab increases CD103+ 'regulatory' DC in the CD colon and blocks mucosal recruitment of pro-inflammatory monocytes, which may support mucosal healing.

W.90. Nicotine Improves Symptoms in Murine Oxazolone-induced Colitis by Inhibiting Migration of Plasmacytoid DC through Alpha7 Nicotinic Acetylcholine Receptors

Makoto Kadowaki, Yuya Kanauchi, Takeshi Yamamoto. University of Toyama, Toyama, Japan

Some clinical trials and epidemiologic studies revealed improvements in symptoms of ulcerative colitis after smoking and nicotine patch treatment. However, mechanisms underlying the ameliorating effect of nicotine remain poorly understood. We have previously reported that nicotine ameliorates murine oxazolone (OXZ)-induced colitis through alpha7 nicotinic acetylcholine receptors (α7nAChRs). In addition, we have shown that mRNA of α7nAChRs was expressed in plasmacytoid dendritic cells (pDCs) of OXZ colitis mouse colon. The aim of this study was to investigate a pathophysiological role of pDCs and α7nAChRs on pDCs in ulcerative colitis model. Result: α7nAChRs mRNA was expressed in mouse bone marrow-pDC cells (BMpDC), but not BM conventional DC (BMcDC). Neither CD80, CD86 nor MHC class II expression in BMpDC was affected with nicotine in the induction of pDC maturation by CpG-DNA. On the other hand, CCL21-induced migration of mature BMpDC was inhibited by nicotine and α7nAChRs agonist GTS-21, and their antagonist methyllycaconitine blocked the inhibitory effect of nicotine and GTS-21. Conclusion: These data suggest that the activation of α7nAChRs reduces pDC-mediated antigen presentation to naive CD4 T cells by inhibiting pDC migration from colon to MLN, thereby alleviating the symptoms in OXZ colitis.

W.91. Selective Increase in Pro-inflammatory Cytokine-producing SIRP-α$^+$CX$_3$CR1$^+$E-Cadherin$^+$ Dendritic Cells in Inflamed Intestinal Mucosa and Lymph Nodes of Patients with Crohn's Disease

Nobuyasu Baba, Manuel Rubio, VuQuang Van, Marc-André Racicot, Carole Bergeron, Fanny Bergeron, Carole Richard, Geneviève Soucy, Raymond Lahaie, Ramses Wassef, Edmond-Jean Bernard, Raja Tamaz, Benoît Panzini, Marika Sarfati. Univérsité de Montréal (CRCHUM), Montreal, QC, Canada

Dysregulated dendritic cells (DCs) responses to commensal flora contribute to Crohn's disease (CD) pathogenesis in genetically predisposed individuals. *in vivo* administration of CD103$^-$SIRP-α (CD172a)$^+$ DCs elicits a Th17-biased chronic inflammatory bowel disease in mice, demonstrating their colitogenic function. We here searched for the human counterpart of murine pathogenic SIRP-α$^+$ DCs in peripheral blood mononuclear cells (PBMC), lymph nodes (LN) and lamina propria cells from intestinal mucosal samples of patients with CD and subjects without IBD (control donors). First, SIRP-α$^+$ (CX$_3$CR1$^+$E-Cadherin$^-$CD103$^-$DC-SIGN$^-$HLA-DR$^+$) cells were present in PBMC. These include the three monocytes subsets and the migratory CD14^{dim}Slan$^+$ DCs/monocytes. Second, SIRP-α (MFI) was over-expressed on Slan$^+$ DCs in CD blood. Third, the frequency of SIRP-α$^+$HLA-DR$^+$ DCs that co-expressed CX$_3$CR1 and E-Cadherin was augmented in the inflamed CD tissues when compared to symptomless CD regions or control tissues. Finally, SIRP-α$^+$HLA-DR$^+$ DCs were the major source of spontaneous TNF-α and IL-1β production, whose frequency was increased in inflamed over non-inflamed CD or control tissues. In conclusion, SIRP-α over-expression on migratory blood Slan$^+$ DCs combined to an increased frequency of cytokine-producing SIRP-α$^+$CX$_3$CR1$^+$E-Cadherin$^+$HLA-DR$^+$ DCs in inflamed intestinal CD sites may contribute to disease pathogenesis and open novel therapeutic avenues for CD patients. This work was supported by the Crohn Colitis Foundation of Canada and Canadian Institutes of Health Research.

W.92. **Intestinal CD103+ Dendritic Cells Defined by SIRPα Expression may Play Distinct Roles in the Induction of Appropriate Immune Responses to Antigens**

Charlotte Scott, Aude Aumeunier, Allan Mowat. University of Glasgow, Glasgow, Scotland

The intestinal immune system is continually challenged by a variety of antigens, including commensal bacteria, food proteins and invasive pathogens. It is essential to discriminate between the different types of antigens so that either protective immunity or tolerance is induced appropriately. Dendritic cells (DC) that express CD103 (αEβ7 integrin) are considered the archetypal intestinal DC involved in the induction of tolerance, but the DC involved in active immunity are less well characterised. Mucosal CD103+ DC migrate from the gut wall, present intestinally derived antigens to naïve T lymphocytes in the mesenteric lymph node and produce retinoic acid that drives the generation of FoxP3+ regulatory T cells. Murine intestinal CD103+ DC are heterogeneous in nature and therefore the different subsets of CD103+ DC may be involved in tolerance and active immunity. Our findings indicate that CD103+ DC can be separated based on the expression of signal regulatory protein α (SIRPα), a receptor with several immunomodulatory roles. Our preliminary experiments demonstrate that SIRPα+ and SIRPα- subsets respond differently to TLR ligation with respect to costimulatory molecule expression and anti-inflammatory cytokine (IL-10) production. We hypothesise that these subsets may play distinct roles in the induction of tolerance or protective immunity in the gut.

W.93. Small Intestinal CD103+ DC are a Heterogeneous Population with Distinct Functions

Aude Aumeunier, Calum Bain, Charlotte Scott, Allan Mowat. University of Glasgow, Glasgow, Scotland.

CD103 expressing dendritic cells (DC) play a crucial role in inducing tolerance in the gut. Two subpopulations of CD103+ DC can be identified based on the expression of CD11b and CD8α molecules, but their respective functions in the small intestinal lamina propria (LP) remain unclear. Neither of

the two subpopulations express TLR nor respond to TLR stimulation *in vitro*, but CD11b+ cells uniquely express CD172α and produce IL10 *in vivo*. Preliminary data suggest that both subsets may be capable of antigen presentation when assessed *in vitro*, although there are differences in their overall potency and ability to prime CD4+ or CD8+ T cells. Both subsets express CCR7, as well as the retinoic acid generating enzyme ALDH. Those results indicate that CD103+ LP DC are a heterogeneous population whose two main subsets may have overlapping, as well as distinct functions. Although both may be capable of acquiring intestinal antigen and migrating to the draining mesenteric lymph node to imprint gut homing properties on naïve T cells, there may be subtle differences in the nature of the response generated by each subset. It will be important to confirm these ideas directly and to explore how the subsets change in inflammatory conditions.

W.94. CCR6 and CCL20 Promote the Association of Lamina Propria Dendritic Cells with the Small Intestine Epithelium

Leroy Wheeler[1], Jeremiah McDole[1], Keely McDonald[1], Ifor Williams[2], Mark Miller[1], Rodney Newberry[1]. [1]Washington University School of Medicine, St. Louis, MO; [2]Emory University School of Medicine, Atlanta, GA

Dendritic cells (DCs) are potent antigen presenting cells instrumental in guiding immune responses. Murine small intestine DCs have been described within the gut associated lymphoid tissue (GALT), the lamina propria (LP), and closely associated with the intestinal epithelium. The epithelia associated DCs (EA-DC) are uniquely positioned for initial encounters with luminal antigens, however the factors promoting the association of LP DCs with the epithelium are not known. We observed that while the LP contains four populations of DCs (CD103+CX3CR1-CD4-, CD103-CD4+CX3CR1+, CD103-CX3CR1- CD4+, and CD103-CX3CR1-CD4-) only two of these associate with the epithelium (CD103+, CX3CR1-CD4- and CD103-CX3CR1+CD4+). Flow cytometry revealed that CCR6 was expressed at similar levels in EA- and LP-DCs, which was lower than that seen in Peyer's patch (PP) DCs. We observed that in the non-PP bearing intestine, CCL20 was predominantly expressed by epithelial cells, and in the absence of CCR6, EA-DCs, but not LP-DCs, were significantly decreased. Wildtype bone marrow (BM) chimeric mice receiving both wildtype and CCR6-/- BM showed equivalent numbers of wildtype and CCR6-/- DCs in the LP, but decreased CCR6-/- DCs closely associating with the epithelium, thus confirming the requirement of CCR6 expression by DCs to associate with epithelium.

W.96. Characterization of Swine Pulmonary Dendritic Cells and their Response to Influenza Virus

Marquet Florian, Bouguyon Edwige, LeGoffic Ronan, Schwartz-Cornil Isabelle, Bertho Nicolas. INRA, Jouy-en-Josas, France

Swine is a natural host of influenza virus. Swine influenza strains can possibly contaminate humans too, as observed from the last human influenza pandemic (panH1N1). Once infected, swine present identical symptoms as human, such as anorexia, pyrexia, cough, fever and nasal discharge, and their cytokine responses in bronchoalveolar lavage are also identical. It has been recently shown in mice (Aldridge, PNAS 2009) that dendritic cells (DC), and among them inflammatory TNF/iNOS-producing DC (Tip DC), are partly responsible both for the virus clearance and for the inflammatory pathology induced by influenza lung infection. In a previous study (Marquet, PlosONE 2011) we have described the different DC subpopulations present in pig skin, which resemble human DC skin subpopulations, and we identified swine inflammatory skin DC. Here, we describe in the lung the counterparts of skin DC subpopulations, adding the bronchoalveolar macrophages. We then analyzed their *in vitro* responses to infection with different influenza viruses. All together these data confirm the swine as a model of choice for the study of normal and pathologic immune responses against influenza infection.

W.97. Defining Anatomical Localisation and Subsets of the Murine Mononuclear Phagocyte System Using Integrin Alpha X (ITGAX/CD11c) and Colony Stimulating Factor 1 Receptor (CSF1-R/CD115) Expression Fails to Discriminate Macrophages from Dendritic Cells

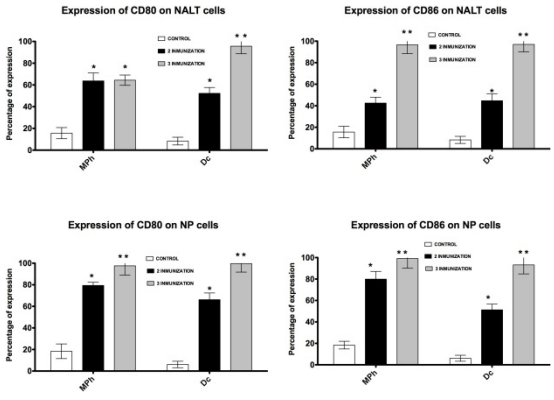
Barry Bradford, David Hume, Neil Mabbott. University of Edinburgh, Roslin, United Kingdom

The murine mononuclear phagocyte (MNP) system is a diverse population of cells, including both dendritic cells (DC) and macrophages. Expression of the integrin alpha X (ITGAX/CD11c) is commonly used to identify classical DC, and similarly expression of colony stimulating factor 1 receptor (CSF1-R/CD115) to identify macrophages. We have characterised the expression of both of these markers using a variety of transgenic mouse models. We confirmed previous observations of CD11c expression in anatomically defined subsets of MNPs in secondary lymphoid organs, including all MNPs identified within lymphoid germinal centres and mucosal intraepithelial region. The majority of MNPs in the intestinal lamina propria and lung express CD11c, and all expressed the CD115-eGFP (MacGreen) transgene. All CD11c expressing cells express CD115-eGFP regardless of location, suggesting CSF1-dependent haematopoietic derivation. To confirm this we have undertaken bone marrow adoptive transfer experiments using precursors sorted via CD115-eGFP expression and presence or lack of Gr-1 immunoreactivity. Our data reveal that CD11c expression alone does not define classical dendritic cells. These results suggest more cautious interpretation of CD11c-dependent experimentation and direct equation with uniquely DC-mediated activities, particularly in the functioning of non-lymphoid MNPs within the intestinal lamina propria.

W.99. Effect of Intranasal Immunization with Total Extract of Naegleria Fowleri Plus CT on the Activation of Dendritic Cells and Macrophages of NALT, NP and CN

Marco Aurelio Rodriguez-Monroy[1], Maria Concepción Peña-Juarez[3], Oscar de Jesus Nieto-Yañez[3], Maria Margarita Canales-Marinez[2], Arturo Contis-Montes de Oca[3], Maricela Carrasco-Yepez[3], Saul Rojas-Hernandez[3], Xochitl Liliana Olivares-Lopez[3], Lucio Rivera-Santiago[3]. [1]Universidad Nacional Autonoma de Mexico, Tlalnepantla, Mexico; [2]Unam Fes Iztacala, Tlalnepantla, Mexico; [3]ESM-IPN, Mexico City, Mexico

Naegleria fowleri is a free-living ameboflagellate that can cause primary amebic meningoencephalitis (PAM) in humans. On the other hand, it has been shown that CT used as an adjuvant enhances the protective immune response when administered with an antigen. In previous studies we have achieved 100% protection by immunizing mice with extracts of N. fowleri plus CT, but the role of dendritic cells (DC) and macrophages in the NALT, nasal passages (PN) and cervical-nodes (CN) was not have been fully described. The aim of this work was to characterize the effect of intranasal immunization with CT and total extract of N. fowleri in the activation of DC and macrophages from NALT, NP and CN in PAM. After the first, second and third immunization, mice were challenged with a lethal dose of live trophozoites N. fowleri, cells from NALT, NP and NC were obtained. By flow-cytometry and immunohistochemistry, the effect on the expression of CD80 and CD86 on the surface of DC and macrophages and migration of DC was determined respectively. Our results show that in the third immunization there is a 90% increase in the expression of CD80 on dendritic cells from NALT and NP, while CN was observed 60%.



W.100. Modulation of the Dendritic Cell Phenotype by Different M. Tuberculosis PAMPs

Jonas Søndergaard, Susanne Brix. Technical University of Denmark, Lyngby, Denmark

One of the key events in the immune response against M. tuberculosis (MTB) is recognition of this pathogen at lung mucosal tissues by dendritic cells (DCs). MTB consists of a range of different pathogen associated molecular patterns (PAMPs) that are recognized by DCs. Here we have analysed the response to these different MTB PAMPs and correlated the results to the response induced by the complete bacterium. In our system, certain PAMPs of the Toll-like receptor family: peptidoglycan and gDNA, are able to induce IL-12 production by the DCs, leading to a high IFN- γ and a low IL-17 production by naïve CD4+ T cells in co-culture studies. On the contrary, the complete bacterium does not lead to IL-12 production and causes a much lower IFN-γ/IL-17 ratio. Two non-canonical PAMPs from the C-type lectin receptor family: ManLAM and PIM6 were found to down-regulate the IL-12 production in DCs, and thereby inducing a DC phenotype closer to the one induced by the complete bacterium. Furthermore, these CLRs also inhibits up-regulation of antigen-presenting and co-stimulatory abilities of the DCs. Investigations are on-going to determine which pathways are involved in the crosstalk between these different MTB PAMPs.

W.101. Plasmacytoid Dendritic Cells Respond Differently to R5 and X4 HIV-1

Jonas Søndergaard, Susanne Brix. Technical University of Denmark, Lyngby, Denmark

An initial defective immune activation upon HIV infection may be a hallmark for the evasion properties of this virus. Plasmacytoid dendritic cells (pDCs) are present at sites of infection, and are central in eliciting anti-viral immune responses, as these cells recognize the virus, produce type I interferons that limit viral replication, and concurrently induces maturation of myeloid dendritic cells, NK cells and T cells. Classically the early infecting HIV-1 strains are mainly of the CCR5 (R5)-tropic type, while later stages of HIV-infection consists of mainly CXCR4 (X4)-tropic strains. Here, we have tested if HIV-1 strains with different tropism differentially modify type I interferon production and activation markers in human pDCs. In human blood-derived pDCs, we observed that the R5 virus induces IFN-α, but only 1/3 the amount of the X4 strain. Moreover, the level of CD40, CCR7 and PD-L1 expression is reduced by the R5-strain compared to the X4-strain. These data suggest that in the early stages of infection (mainly R5 HIV-1) the pDC response is not strong enough to clear the virus, and that in late stages of infection (mainly X4 HIV-1), the pDC response is too vigorous, and thus may add to immune cell exhaustion.

W.103. HSV-2 Prevents Dendritic Cell Maturation, Induces Apoptosis through Dysregulation of Akt Signaling and Triggers the Release of Inflammatory Cytokines to Escape Innate Immunity and Promote HIV Infection

Martha Stefanidou[1], Janie Trepanier[1], Sara Rosenbaum[1], Ana Fernandez-Sesma[2], Betsy Herold[1]. [1]Albert Einstein College of Medicine, Bronx, NY; [2]Mount Sinai School of Medicine, New York, NY

We hypothesize that HSV-2 interferes with dendritic cell (DC) maturation to escape mucosal immunity, which may contribute to increased susceptibility to HIV and enhanced HIV replication in HSV-2+ populations. Immature monocyte-derived DCs were exposed to HSV-2 or LPS. While LPS induced maturation characterized by increased CD80 and CD86 expression, HSV-2 infected DCs without triggering maturation. HSV-2 exposed DCs failed to mature upon subsequent (or simultaneous) treatment with LPS. To determine whether this reflected apoptosis, DCs were exposed to HSV-2, uv-inactivated virus or LPS and analyzed for annexin V and 7AAD. HSV-2 (live and uv-inactivated) triggered an increase in the number of

early and late apoptotic cells, whereas LPS had little effect. Surprisingly, we found that both wildtype and uv-inactivated HSV-2 rapidly and persistently induced activation of Akt, which is critical for DC activation and survival. Blockade of Akt signaling with wortmannin resulted in decreased HSV-induced apoptosis. Despite these aberrant responses, HSV-2 induced the release of TNFα and IL-6 into culture supernatants, which were sufficient to activate HIV-1 replication in latently infected U1 cells. Together, these findings demonstrate that HSV-2 has paradoxical effects on DCs (stimulating cytokine release, but inducing apoptosis), which could promote HIV infection.

W.104. Directed Antigen Targeting *in vivo* Identifies a Role for CD103+ DCs in Tolerogenic and Immunogenic T Cell Responses

Monika Semmrich[1], Marcus Svensson[1], Maud Plantinga[2], Heli Uronen-Hansson[1], Allan Mowat[4], Ulf Yrlid[3], Bart Lambrecht[2], William Agace[1]. [1]Lund University, Lund, Sweden; [2]University Hospital Gent, Gent, Belgium; [3]University of Gothenburg and Swedish Foundation for Strategic Research, Gothenburg, Sweden; [4]University of Glasgow, Glasgow, Scotland

CD103+ dendritic cells (DC) represent a major subset of migratory DC in peripheral tissues, however, the function of these cells in immune responses *in vivo* remains largely undefined. To gain a further understanding of the *in vivo* priming potential of these cells we coupled Ovalbumin to the anti-CD103 antibody M290 (M290.OVA). M290 selectively targeted CD103+ DCs *in vivo* and was internalized by these cells. Intra-peritoneal injection of M290.OVA induced OVA-specific CD8+ and CD4+ T cell proliferation in mesenteric lymph nodes (MLN) of WT but not CD103-/- mice or mice depleted of CD11c+ cells demonstrating that T cell priming was dependent on CD103+ DCs. Primed CD8+ and to a lesser extent CD4+ T cells expressed the gut homing receptors α4β7 and CCR9 however responding CD4+ T cells failed to efficiently differentiate into FoxP3+ Tregs. Similarly intra-tracheal administration of M290.OVA demonstrated a role for lung CD103+ DCs in the induction of CD8+ and CD4+ T cells responses in mediastinal LN. Intra-peritoneal or intra-tracheal administration of M290.OVA alone induced T cell tolerance. In contrast, administration of M290.OVA with adjuvant induced CTL and Th cell differentiation. Together, these findings suggest that CD103 targeting may be useful strategy for regulating immune responses.

W.105. Human MAIT Cells are Xenobiotic Resistant, Tissue-targeted, CD161+ IL-17 Secreting T Cells

Mathilde Dusseaux[1], Emmanuel Martin[1], Nacer Serriari[3], Isabelle Peguillet[2], Virginie Premel[2], Delphine Louis[2], Maud Milder[2], Lionel Le Bourhis[1], Claire Soudais[1], Emmanuel Treiner[3], Olivier Lantz[1]. [1]INSERM, Paris, France; [2]Institut Curie, Paris, France; [3]INSERM, Amiens, France

Mucosal associated invariant T (MAIT) cells are very abundant in humans and have anti-microbial specificity but their functions remain unclear. MAIT cells are CD161+IL-18Rαhi and either CD4-CD8- (DN) or CD8αβint T cells. We now show that they display an effector-memory phenotype (CD45RA-CD45RO+CD95hiCD62Llo) and their chemokine receptor expression pattern (CCR9intCCR7-CCR5hiCXCR6hiCCR6hi) indicates preferential homing to tissues and particularly the intestine and the liver. MAIT cells can represent up to 45 % of the liver lymphocytes. They produce IFN-γ and Granzyme-B as well as high levels of IL-17 after PMA+ionomycine stimulation. Most MAIT cells are non-cycling cells (less than 1% are Ki-67+) and express the multidrug resistance transporter (ABCB1). As expected from this phenotype, MAIT cells are more resistant to chemotherapy than other T cell populations. These features might also allow MAIT cells to resist the xenobiotics potentially secreted by the gut bacteria. We also show that this population is cytotoxic against commensal bacteria infected cells. Together with their already known abundance and anti-microbial reactivity, the gut-liver homing characteristics, high expression of ABCB1 and ability to secrete IL-17 probably participate to the anti-bacterial properties of MAIT cells.

W.106. IL-17-producing Human Invariant Natural Killer T Cells: Possible Implications on Inflammatory Responses

Lucia Moreira-Teixeira[1], Resende Mariana[2], Maryaline Coffre[3], Odile Devergne[1], Jean-Philippe Herbeuval[1], Olivier Hermine[1], Elke Schneider[1], Lars Rogge[1], Frank Ruemmele[3], Michel Dy[1], Anabela Cordeiro[2], Maria Leite-de-Moraes[1]. [1]CNRS, Paris, France; [2]IBMC, Porto, Portugal; [3]INSERM, Paris, France

CD1d-reactive invariant natural killer T (iNKT) cells have been implicated in a number of pathologies. Given the scope of their immunoregulatory activities mediated through distinct cytokine patterns, it has been proposed that this functional diversity originates from distinct iNKT subpopulations. Here we report that human CD161+ iNKT cells are intrinsically endowed with the capacity to generate IL-17, but require TGF-beta, IL-1beta and IL-23 to carry out this potential. IL-17-producing iNKT cells are already present in cord blood but, in contrast to peripheral blood iNKT cells, they cannot generate interferon-gamma. These cells respond to aryl hydrocarbon receptor stimulation, express IL-23 receptor and RORC, like conventional T helper 17 cells from which they differ by their restricted ability to co-produce IL-22. Having established that the inflammatory environment drives human iNKT cells to produce IL-17 we searched for IL-17-producing iNKT cells in patients with chronic inflammatory disorders and we found that iNKT cells from a fraction of Crohn's disease patients were already capable of producing high levels of both IL-17 and IL-22 without the addition of exogenous cytokines. In conclusion, IL-17 production by human iNKT cells depends on two critical parameters, namely an intrinsic program and a pro-inflammatory environment.

W.108. Roles for MyD88 in the Recruitment of Gut CD103+ Dendritic Cells into Draining Mesenteric Lymph Nodes

Karin Fredriksson, Emma Persson, Bengt Johansson-Lindbom, William Agace. Lund University, Lund, Sweden

Intestinal homeostasis and induction of systemic tolerance to fed antigens (i.e. oral tolerance) relies on steady-state migration of small intestinal

lamina propria dendritic cells (DCs) into draining mesenteric lymph nodes. CD103+ DCs are the major migratory antigen-presenting cell population in the small intestine and appear to play a central role in initiating immune responses to soluble luminal antigens. Here we demonstrate that the steady-state mobilization of CD103+ DCs into the MLN is governed by the Toll-like receptor (TLR) signaling adaptor molecule MyD88 and occurs independently of TNF-α. Likewise, TLR signaling through the adaptor molecule TRIF and downstream production of type I interferon are not required. Experiments on bone marrow chimeras show that steady-state CD103+ DC migration relies on MyD88 signaling in both hematopoietic and non-hematopoietic cells. Notably, the levels of IL-18 and IL-1β, both of which signal through Myd88, are reduced in the small intestine of Myd88-/- mice, indicating potential roles for TLR-dependent and -independent Myd88-signaling in CD103+ DC egress from the non-inflamed intestinal mucosa.

W.109. Epithelium and Intraepithelium Gut Mucosae Cross-talk in the Inflammatory Process of an Experimental Model of Secondary Immunodeficiency

Graciela Calabrese, Sofia Olmos, Maria Roux. University of Buenos Aires, Buenos Aires, Argentina

Previous work has shown that dietary antigen induces a specific increase in: number of gamma-delta T cells in the lamina propria and in the intraepithelium, as well as TNF-alpha; suggesting an inflammatory process. In this context, we studied IL17 expression and the transcriptional factor Foxp3 in our experimental model. Western Blot and RT-PCR were used to analyze IL17 expression in intestinal epithelium cells (IECs) from wellnourrished rats (C60), and from renourrished ones: weaning rats lost 25% of initially body weight and were refed with a 20% casein diet during 21 days (R21). Confocal microscopy studies were performed on intraepithelial lymphocytes for CD4, CD8 and gamma-delta T cells expressing either IL17 and/or Foxp3. An increase of IL17 was found in IECs (2.6 R21 vs 1.5 C60, OD/μg) accompanied by an increase in IL17 mRNA. The intraepithelial lymphocytes population gamma-delta-IL17+ T cells were increased in R21 vs C60, while CD8 population was the only one expressing Foxp3.Therefore, we may conclude that: 1)CD8+Foxp3 lymphocytes are the main Treg cell population in our model but also 2) gamma-delta+IL-17+ population contribute to the perpetuation of the inflammation as it is known that gamma-delta cells are involved strongly in the IL-17 production.

W.110. Bronchial Fibroblasts Modulate CD4+T Cells Phenotype Towards Th17 in Asthma

Lionel Loubaki, Sophie Plante, Eric Jacques, Jamila Chakir. Laval University, Quebec, Canada.

Background: In asthma, CD4+T cells are selectively recruited into the bronchial mucosa. CD4+ T consist of different subset that express lineage specific transcription factors and play different roles either in initiating and supporting the development of immune response, but also in orchestrating and regulating them. Objectives: The aim of our study was to evaluate the effect of T cells-bronchial fibroblasts interaction on CD4+T cell phenotype. Methods: Human bronchial fibroblasts were isolated from mild steroid naïve asthmatics and non atopic healthy controls. CD4+T cells were purified from the peripheral blood of healthy and asthmatic subjects. Co-culture of confluent healthy (HF) or asthmatic bronchial fibroblasts (AF) with T cells were performed. CD4 + T cell total RNA was purified and GATA-3, Foxp3 and RORc expression was detected by quantitative PCR. Th17 (IL-17,IL-22) lineage specific cytokines profile were also evaluated. Results: Co-culture of T cells with bronchial fibroblasts significantly stimulated RORc in astmatic T cells only whereas, Foxp3 and GATA-3 was not affected in both asthmatic and healthy T cells. IL-6 and IL-23 expression either by AF and HF were also significantly increased by the coculture when, TGF-β expression was not affected. In CD4+ T cells, IL-17 and IL-22, Th17 lineage specific cytokines were significantly increased by the coculture with AF. Conclusion: Interaction between bronchial fibroblasts and T cells seems to promote specifically Th17 cells profile in asthma. These results suggest that cellular interaction particularly between T cells and fibroblasts may play a pivotal role in the regulation of the inflammatory response in asthma.

W.111. **CD8αα+ TCRαβ+ T Cells Appear at Extra**-intestinal Sites in Mice with Experimental Colitis: Investigations on the Possible Molecular Mechanisms Involved

Nina Dickgreber, Christel Zufferey, Antonia Bünter, Silvia Rihs, Leslie Saurer, Christoph Mueller. University of Bern, Bern, Switzerland

Intestinal intraepithelial lymphocytes (IEL) mainly consist of T cells, which differ in their phenotypic composition from circulating T cells. One important cell type with proposed regulatory functions are CD8αα+CD8β-TCRαβ+ IEL. Parabiosis experiments showed that these cells are resident in the small intestinal epithelium under homeostatic conditions. By using transgenic mice that contain self-specific CD8αα+TCRαβ+ IEL but no conventional CD4+ and CD8+ T cells, we showed a substantial increase in CD8αα+TCRαβ+ T cells in peripheral blood, lymph nodes, spleen and colon after colitis induction by transfer of naïve CD4+CD45RBhi T cells. The appearance of CD8αα+TCRαβ+ T cells at extra-intestinal sites during colitis was accompanied by increased proliferation and changes in homing marker expression. In particular, mRNA levels of CXCR3, the leukotriene B4 receptor BLT1 and sphingosine-1-phosphate-receptor-1 were increased in CD8αα+TCRαβ+ T cells recovered from colon, spleen and lymph nodes of colitic mice compared to small intestinal CD8αα+TCRαβ+ IEL from healthy mice. Interestingly, CD8αα+TCRαβ+ T cells were also detectable at extra-intestinal sites in a chronic dextran sodium sulfate (DSS) induced colitis model. To further investigate the factors involved in the appearance of CD8αα+TCRαβ+ T cells at extra-intestinal sites and the accumulation in the colon, we will now analyse the distribution of CD8αα+TCRαβ+ IEL in mice deficient for CXCR3 or BLT1.

W.112. Intestinal Epithelial Cells Modulate CD4 T Cell Responses via the Thymus Leukemia Antigen

Danyvid Olivares-Villagomez, Holly Scott Algood, Kshipra Singh, Vrajesh Parekh, Kaithlyn Ryan, M. Blanca Piazuelo, Keith Wilson, Luc Van Kaer. Vanderbilt University, Nashville, TN

The thymus leukemia (TL) antigen is a non-classical MHC class I molecule primarily expressed on intestinal epithelial cells (IEC) that interacts with CD8αα, which is primarily expressed by intestinal intraepithelial lymphocytes. The close proximity of cells expressing these two molecules suggests that TL-expressing IEC can regulate CD8αα-expressing lymphocytes. Here, we report that TL influences the cytokine profile and pathogenic potential of CD4+ T cells expressing CD8αα. We show that TL+ IEC regulates IL-17 production by CD4+ T cells. Consequently, TL expression enhances development of colitis in an adoptive transfer model and confers resistance to Citrobacter rodentium infection by allowing the generation of an adequate Th17 response. Thus, TL expressed on IEC modulates CD4+ T cell responses in the intestinal mucosa.

W.113. Gastric Epithelial Cells Induce Th17 Development During Helicobacter Pylori Infection

Ellen Beswick[1], Iryna Pinchuk[2], Rachel Earley[1], Victor Reyes[2]. [1]University of New Mexico Health Sciences Center, Albuquerque, NM; [2]University of Texas Medical Branch, Galveston, TX

Gastric epithelial cells (GECs) express class II MHC, act as antigen presenting cells (APCs), and influence $CD4^+$ T cell responses during H. pylori Hp infection. Hp elicits GEC responses that include production of proinflammatory cytokines, upregulation of class II MHC and costimulatory molecules that provide signals for $CD4^+$ T cell activation. Recently, the proinflammatory $CD4^+$ phenotype, Th17, were detected in the Hp infected gastric mucosa. Since GECs produce inflammatory cytokines and act as APCs during Hp infection, we hypothesized that the GEC response to Hp infection induces development of Th17 cells in the gastric mucosa. In order to examine this, we investigated Hp-induced GEC production of IL-6 and TGF-β, the cytokines required for Th17 development. These cytokines induced naive $CD4^+$ T expression of RORγ and IL-17A in culture with infected GECs. IL-21, which may play a role in Th17 development, was also found in cultures. Surprisingly, B7-H2 blocking on GECs increased Th17 development. Th17 proliferated in culture and class II MHC was crucial in Th17 proliferation as determined by blocking class II MHC on GECs. These observations suggest a novel mechanism where GECs induce Th17 development and proliferation, which is likely a crucial mechanism in inflammation during Hp infection.

W.114. Autoimmune Mediated Pulmonary Inflammation and the Involvement of Alveolar Type II Epithelial Cells

Andrea Autengruber, Marcus Gereke, Dunja Bruder. Helmholtz Centre, Braunschweig, Germany

To develop advanced therapies and treatments for asthma, COPD and other pulmonary diseases, it is essential to gain a better understanding of the basic molecular and cellular interplay in the cause of pulmonary inflammation. Therefore, we make use of a transgenic mouse model for T cell mediated lung disease, showing lymphocytic infiltrations, alveolar emphysema and severe progressive interstitial pneumonitis. Based on this model we have recently shown that AECII actively contribute to the induction of regulatory T cells in the inflamed lung, but their immune modulating role appears to be far more diverse. Data obtained by analyzing gene expression profiles of AECII derived from chronically inflamed lungs compared to healthy controls revealed an up-regulation of many T cell co-stimulatory as well as co-inhibitory genes suggesting a pronounced T cell-AECII crosstalk in the lung. Furthermore, chemoattractant genes were dynamically regulated in AECII upon inflammation which is well in line with the observed increase in numbers of macrophages and lymphocytes in BALF from diseased mice. These data suggest that AECII interact with T cells and other immune cells via receptor-ligand interactions and chemokines, thereby influencing the course of ongoing inflammation and contributing to the re-establishment of self-tolerance in the lung.

W.115. A Novel Spontaneous Murine Model of Pruritic Skin Disease, Eosinophilic Esophagitis and Portal Inflammation

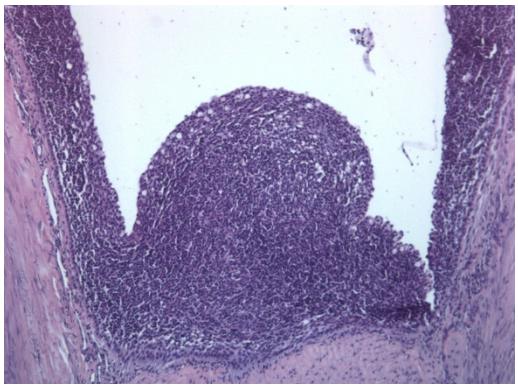
Johnthomas Kang, Alexander Khoruts. University of Minnesota, Minneapolis, MN

Despite a well-recognized rise in the incidence of eczema and eosinophilic esophagitis in the industrialized world, there is relative paucity in animal models of these important diseases that could allow mechanistic dissection of their pathogenesis. Here we describe a novel spontaneous model of pruritic inflammatory disease involving skin, esophagus, and portal areas in the liver characterized by eosinophilic infiltration in lethally irradiated mice transplanted with B7-1/B7-2-deficient bone marrow. This disease is mediated by CD4 T cells and depends on B7 expression by radio-resistant host antigen-presenting cells. Th2 cytokine signatures are dominant in the functional analysis of CD4 T cells emerging in these animals. Interestingly, B7 expression by the host cells in these radiation chimeras is insufficient to maintain the homeostasis of regulatory Foxp3+CD4 T cells (Tregs). The resultant Treg deficiency leads to unrestrained accumulation of pathogenic CD4 T cells specific for antigens in the skin and esophagus. The model may be used for mechanistic dissection of Treg function in maintenance of immunologic tolerance along the surfaces lined by the squamous epithelium, as well as other aspects of pathogenesis of eczema and eosinophilic esophagitis.

W.116. Interbrancheal Lymphoid Tissue in Salmonids - An Intraepithelial T Cell Aggregate

Erling Koppang[1], Uwe Fischer[2], Emilio Jirillo[3], Fumio Taizawa[2], Lars Austbø[1], Mohasina Syed[1], Ivar Hordvik[4]. [1]Norwegian School of Veterinary Science, Oslo, Norway; [2]Friedrich-Loeffler Institute, Greifswald, Germany; [3]University of Bari, Bari, Italy; [4]Uniersity of Bergen, Bergen, Norway

It has been assumed that lower vertebrates do not possess lymph nodes or organised mucosa-associated lymphoid tissue. Recently, we discovered a hitherto unreported lymphoid cell aggregate in the gills of salmonid fish. The aggregate is located on the caudal edge of the gill arch as a continuous structure from its dorsal to ventral attachment. Laser-assisted micro-dissection and subsequent gene expression analysis indicated presence of T cells and only very few B cells. Recombination activating gene expression was only detectable in one out of seven samples. Sequencing of T cell clones revealed a high diversity in T cell receptor constructs. To verify presence of T cells, polyclonal antisera against the CD3 epsilon chain and monoclonal antibodies against CD8alpha were generated. Morphological investigations confirmed that the tissue consisted of epithelial cells embedding predominantly T cells (among those CD8+ cells) and only very few B cells. The tissue was detached from the underlying gill arch by a solid basal membrane and with an apparently capsular-like structure of epithelial cells covering its surface. Initial investigations involving gill virus infection indicates a regulation of T cell dynamics at site. Located in the evolutionary important pharyngeal region, the identified structure represents a hitherto non-described form of lymphoid tissue. Interbranchial lymphoid tissue in a 9 kg Atalntic salmon



W.117. **Mapping CEACAM5 and CD8α Binding by Biacore**

Giulia Roda, Jian Yu Xu, Lloyd Mayer. Mount Sinai School of Medicine, New York, NY

CD8+ regulatory T (TrE) cells can be activated by intestinal epithelial cells (IECs) through the complex formed by the non-classical I molecule, CD1d and gp180 on IECs. mAb B9, an anti-gp180 Ab, inhibits this interaction and maps to the N domain of CEACAM family members. CEACAM5, but not CEACAM1 or 6, binds to CD1d at the B3 domain. Our aim was to map domains where CEACAM5 interacts with CD8. Soluble proteins for wild type CEACAM5, CEACAM5 N domain mutants (K35A, N42D, and N70,81A) and CD8α were purified. Biacore analysis was performed to evaluate CEACAM5/CD8 binding affinities. CEACAM5 WT binds CD8 with a similar affinity to that seen with MHC class I. The sugarless mutation, N70,81A, results in a loss of affinity for CD8. Mutations of the B9 epitope, K35A, demontrate low affinity compared to the WT whereas N42D is similar to the WT but with an accelerated off rate. These studies indicate that CEACAM5 interacts with CD8α at sites that are overlapping (but not exclusively) with B9 epitopes. The potential role of the glycosylation site in the N domain may relate to conformational binding to CD8.

W.118. Modulation of Gene Expression in Intestinal Epithelial Cells by Intraepithelial Lymphocyte During Homeostatic and Inflammatory Conditions

Jennifer Brasseit, Leslie Saurer, Christoph Mueller. University of Bern, Bern, Switzerland

Intestinal epithelial cells (IEC) can actively influence local immune responses. The gut is also home to various lymphoid cells like intraepithelial lymphocytes (IEL), or innate lymphoid cells (ILC), like LTi's, NKR-LTi's or NK cells. The impact of distinct lymphoid cells on IEC function is yet poorly understood. To directly determine the effects mediated by distinct lymphoid subset on IEC functions during inflammation, colitogenic T cells were transferred into either lymphopenic RAG2-/- mice; RAG2-/- mice containing a monoclonal population of CD8ααTCRαβ IEL (318xH8xRAG-/-), or lacking ILC (RAG-/-xγc-/-mice). Expression of pro-, and anti-inflammatory genes was assessed in purified IEC by qRT-PCR. Compared to control IEC, IEC from colitic mice showed enhanced expression of various pro-inflammatory genes (ICAM-1, CXCL-5, CXCL-9, CCL-20). No attenuating effects on pro-inflammatory chemokine gene expression by IEC were observed by the presence of CD8ααTCRαβ IEL during colitis. In contrast, the absence of ILC prevented the upregulation of pro-inflammatory genes in IEC. Our findings indicate that some lymphoid cell subsets indeed critically modulate the events leading to intestinal inflammation. To further investigate the specific role of ILC for the induction of colitis we now investigate the effect ILC and IEL on the differentiation of the transferred CD4 T cells.

W.119. A New Experimental System Identifies a Novel Functional Synergy Between Epithelial Cells and Intraepithelial Lymphocytes

Lucie Abeler-Dörner[1], Anna Bas[2], Mahima Swamy[3], Sarah Clarke[4], Adrian Hayday[1]. [1]King's College London, London, United Kingdom; [2]University of Gothenburg, Gothenburg, Sweden; [3]Cancer Research UK, London, United Kingdom;

Intestinal intraepithelial lymphocytes (IEL) constitute one of the largest T cell compartments, and include substantial numbers of unconventional T cells. However, because IEL rapidly undergo apoptosis *ex vivo*, their activation requirements, effector potentials and functional interactions with epithelial cells are very poorly understood, and they are commonly excluded from models of gut immune function. This study addresses this problem, describing a protocol to establish viable, resting IEL, thereby permitting their activation requirements and effector potentials to be defined; conventional and unconventional T cells to be compared; and interactions with intestinal epithelial cells (IEC) to be investigated. Among several findings, this approach has identified a hitherto unrecognized cytokine collaboration by which TNF and IL-1α produced by activated IEL provoke IEC

to increase IL-6 production. This is important for a variety of settings, elevated IL-6 levels being associated with increased epithelial cell turnover, inflammation and carcinogenesis in the gut. We go on to show that jointly, TNF, IL-1α and IL-6, derived from IEL+IEC may regulate Th17-production by gut T cells *in vitro*. Hence, this newly identified IEL:IEC cross-talk offers a novel source of factors regulating mucosal homeostasis, infection, and pathology.

W.120. Synergistic Interactions Between Intestinal Intraepithelial Lymphocytes and Epithelial Cells Determine Responses to Pathogen-associated Molecules

Mahima Swamy[1], Lucie Abeler-Dorner[1], Anna Bas[1], Adrian Hayday[2]. [1]King's College London, London, United Kingdom; [2]Cancer Research UK, London, United Kingdom

The epithelial lining of the gut is subject to frequent microbial and non-microbial challenges, suggesting that initiation and control of an immune response at the level of the epithelium is very important. In this scenario, functional interactions of intestinal epithelial (IEC) cells with intraepithelial lymphocytes (IELs) must occur, to disseminate information about the nature and level of immune response required to external insults. We find that IECs are directly capable of responding to Toll-like receptor and Nod ligands. Furthermore, they can communicate these activation events to IELs, leading to increased inflammatory cytokine production from IELs. Feedback from activated IEL can in turn lead to synergistic increase in epithelial cytokine production. This potential of epithelial cells to amplify inflammation is consistent with the contribution of epithelial dysregulation to multiple inflammatory diseases. In this context, we are also investigating the role of a family of immunoglobulin-related molecules, the butyrophilin-like (BTNL) family, in the regulation of communication between IECs and IELs. We have been able to identify an IEC-intrinsic regulatory function for Btnl1 in its response to inflammatory cues from IELs. Thus our work has provided new insight into epithelial cell-mediated immune responsiveness in the context of inflammation in the gut.

W.121. Cigarette Smoking Alters Intestinal Barrier Function and Peyer's Patch Composition

Stephanie Verschuere, Ken Bracke, Tine Demoor, Maud Plantinga, Bart Lambrecht, Guy Brusselle, Claude Cuvelier. Ghent University, Ghent, Belgium

Smokers have a two-fold increased risk to develop Crohn's disease (CD). However, little is known about the mechanisms through which smoking affects CD pathogenesis. Interestingly, the Peyer's patches in the terminal ileum are the sites where the first CD lesions develop. To investigate whether smoke exposure causes alterations in Peyer's patches, we studied C57BL/6 mice after exposure to air or cigarette smoke for 24 weeks. First, barrier function of the follicle-associated epithelium overlying Peyer's patches was evaluated. We demonstrate that chronic smoke exposure is associated with increased apoptosis in the follicle-associated epithelium. Furthermore, immune cell numbers and differentiation along with chemokine expression were determined in the ileal Peyer's patches. We observed significant increases in total dendritic cells (DC), CD4+ T cells (including regulatory T cells) and CD8+ T cells after smoke exposure compared with air-exposed animals. The CD11b+ DC subset almost doubled. Interestingly, these changes were accompanied by an up-regulated mRNA expression of the chemokines CCL9 and CCL20, which are known to attract CD11b+ DC towards the subepithelial dome of Peyer's patches. Our results demonstrate that cigarette smoke exposure induces apoptosis in follicle-associated epithelium and is associated with immune cell accumulation in Peyer's patches, changes which can predispose to the development of CD.

W.122. Enhanced Epithelial IRAK1 Expression Causes Post-ischemic Innate Immune Hypersensitivity

Cécilia Chassin[1], Cordelia Hempel[1], Silvia Stockinger[1], Aline Dupont[1], Joachim Kuebler[1], Jochen Wedemeyer[1], Alain Vandewalle[2], Mathias Hornef[1]. [1]Hannover Medical School, Hannover, Germany; [2]X Bichat, Paris, France

The host microbial homeostasis at the intestinal epithelium is critically determined by regulatory control mechanisms of innate immune signalling. The tight and highly dynamic regulation of the essential Toll-like receptor signalling mediator interleukin 1 receptor associated kinase (IRAK1) during the neonatal period prompted us to investigate whether alterations of epithelial IRAK1 protein expression might also occur in adult individuals and contribute to immune-mediated tissue damage in relevant clinical conditions. We found an increase of IRAK1 protein in an *in vitro* model of hypoxia using intestinal epithelial mICcl2 cells, in epithelial cells isolated from mice following intestinal ischemia-reperfusion, and in hypoxic human intestinal biopsies. Increased expression of IRAK1 significantly enhanced the cellular response towards LPS. In contrast, IRAK1 knock-out mice were protected from innate immune hyperresponsiveness and ischemia-mediated tissue damage. Pharmacological inhibition of enhanced epithelial IRAK1 protein expression with microRNA-146a mimic or inducer reduced the functional consequences of epithelial innate immune hyperresponsiveness and facilitated maintenance of epithelial barrier integrity after ischemia-reperfusion. Our findings present a novel strategy to prevent post-ischemic tissue damage and organ dysfunction by modulating epithelial IRAK1 protein levels.

W.123. Cholera Toxin Induces a Pro-inflammatory Response in Mouse Intestinal Epithelial Cells *in vitro*, which can be Reversed by Diclofenac

Marianne Bol-Schoenmakers[1], Emma Cook[1], Manon van Roest[1], Daniëlle Fiechter[1], Joost Smit[2], Raymond Pieters[1]. [1]Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands; [2]Utrecht Center for Food Allergy, Utrecht, Netherlands

Recently we have shown that diclofenac enhances allergic responses in a mouse model of peanut allergy. In this model cholera toxin is used as oral adjuvant to elicit sensitization. Administration of diclofenac during sensitization resulted in a more flattened appearance of intestinal epithelial cells, accompanied by a decrease in IL-6 production. The present study aimed to investigate the effects of diclofenac and cholera toxin on intestinal epithelial cells. Incubation with cholera toxin increased mitochondrial activity, COX-2 activity and IL-6 and MIP-2 production by MODE-K epithelial cells. Co-incubation with diclofenac decreased cholera toxin-induced COX-2 expression as well as production of IL6, and to lesser extent of MIP-2. Therefore, induction of food allergic responses by cholera toxin may be partly due to a direct pro-inflammatory effect on intestinal epithelial cells. The anti-inflammatory *in vitro* effects of diclofenac appear contradictory to the increased peanut allergic responses in mice after diclofenac exposure.

W.124. Distorted Ultrastructural and Molecular Tightness of the Mucosal Barrier Correlates with Mucosal MC Activation and Bowel Dysfunction in the Jejunum of Diarrhoea-predominant Irritable Bowel Syndrome Patients (IBS-D)

Cristina Martinez, Maria Vicario, Beatriz Lobo, Meritxell Guila, Laura Ramos, Carmen Alonso, Ana Maria Gonzalez-Castro, Marc Pigrau, Mar Guilarte, Maria Antolin, Ines deTorres, Fernando Azpiroz, Javier Santos. Institut Recerca Hospital Universitario Valle Hebron, Barcelona, Spain

Background & aims: Increased intestinal epithelial permeability and mast cell activation are central events in IBS pathophysiology. However, the clinical translation of abnormalities in TJ-related proteins and the myosin light chain kinase (MLCK)-dependent pathway has not been addressed. Methods: We assessed jejunal biopsies from 23 Healthy (H) and 25 diarrhea-IBS patients (IBS-D) for expression of tryptase and TJ proteins by western blot and immunofluorescence. TJ ultrastructure was evaluated by electron microscopy. Symptoms were recorded in the prior 10 days to the jejunal biopsy. Results: Tryptase expression was increased in IBS-D (1.8 fold-change). Expression of zonula occludens-1(ZO-1)was reduced in IBS-D(H:128±28; IBS-D:45±20 arbitrary units;P<0.0001), showing redistribution to the cytoplasm. ZO-2 was also reduced in IBS-D(H:423±156; IBS-D:281±149 arbitrary units;P<0.01), while ZO-3 remain unchanged. Moreover, IBS-D showed increased MLCK1 expression(1.6 fold-change), and reduced MLC phosphatase(0.7 fold-change)and, consequently, enhanced phosphorylation of MLC(pMLC)(H:140±28; IBS-D:187±31 arbitrary units;P<0.01). In addition, IBS-D patients showed a significant increase of apical intercellular distance(H:21±2nm; IBS-D:25±3nm;P<0.05)and higher proportion of TJ with peri-junctional cytoskeleton condensation(H:28±8%; IBS-D:39±5%;P<0.01). Remarkably, ZO-1 expression significantly correlated with tryptase expression(p<0.05). Altered TJ ultraestructure and level of pMLC and ZO-1 significantly correlated with clinical manifestations. Conclusion: Clinical manifestations of IBS-D may rely on distinctive mast cell-related changes of jejunal TJ integrity.

	pMLC protein	ZO-1 protein	Intercellular distance (nm)	Cytoskeleton condensation
Bowel Movements	r= 0.569; P= 0.021	r= -0.73 P<0.0001	r= 0.584; P= 0.046	r= -0.704; P= 0.011
Bristol	r= 0.509; P= 0.044	r= -0.71 P<0.0001	r= 0.618; P= 0.032	r= 0.794; P= 0.002

W.125. Induced ß-defensin Accumulates in the Intestinal Mucus Layer During Salmonella Infection

Joseph Russell[1], Pak Yu[2], Sebastian Winter[1], Robert Crawford[1], L. Adams[3], Andreas Baumler[1], Charles Bevins[1]. [1]University of California, Davis, CA; [2]Massey University, Palmerston North, New Zealand; [3]Texas A&M University, College Station, TX

Enteric beta-defensin (EBD) is an inducible antimicrobial peptide expressed by the bovine intestinal epithelium and has been implicated in innate immunity; however, like other defensins, little is known about the abundance and activity of EBD during infection *in vivo*. To gain better insight into the induced defensin response, we analyzed Salmonella Typhimurium infection using a bovine ligated ileal loop model, which allowed unique access to tissue, mucus and lumenal compartments. By 8hr after infection, quantitative western blot analysis revealed *in vivo* tissue EBD concentrations of 98.3±13.1µg/ml, exceeding the estimated MIC of 10-20µg/ml against Typhimurium *in vitro*. Further analysis of the mucus revealed an EBD concentration of 53.8±18.0µg/ml, and lumenal fluid concentrations neared 6.4±1.3µg/ml EBD. Colony forming unit (CFU) counts revealed a significant decrease in mucosal CFUs, as compared to the lumenal CFUs. These *in vivo* data quantify for the first time an accumulation of defensin in infected tissue, as well as, a partitioning of secreted defensin between distinct mucus and lumenal compartments in the intestine. This induced defensin response suggests an antimicrobial gradient that may enable the host to better manage pathogenic challenge by generating a concentration gradient of host defense molecules at the mucous interface.

W.126. Retinoic Acids Regulate Mucosal Barrier Functions

Yoh Ishiguro, Hiroto Hiraga, Hirotake Sakuraba, Keisuke Hasui, Shinsaku Fukuda. Hirosaki University School of Medicine, Hirosaki, Japan

Vitamin A and its biologically active derivatives, retinoids participate in a variety of biological processes including maintenance of normal tissues. To identify the role of vitamin A in acute intestinal inflammation, we examined the effect of vitamin A-deficiency (VAD) on dextran sulfate sodium (DSS)-induced colitis. Vitamin A-sufficient (VAS) and VAD female C57BL/6 (B6) mice were fed 4% DSS. All of VAD B6 mice died within 10 days, while all of

VAS B6 mice survived for 10 days. Similar results were observed when DSS-treated SCID mice were used. To test the functional role of vitamin A for mucosal barrier function, we examined the effect of VAD on S. typhimurium infection induced by oral gavage in SCID mice. VAD SCID mice showed significantly higher bacterial loads in the spleen, and liver compared with VAS SCID mice. To test the mucosal permeability, FITC dextran was orally administered and plasma FITC levels were evaluated. Plasma FITC levels were significantly higher in VAD mice as compared with wild type mice. We also evaluated the effects of RAR siRNA on TER of Caco-2 monolayer and the results showed that siRAR significantly suppressed TER. Mitochondrial trans-membrane potential was also lower in siRAR treatment cells. These results indicate that Retinoic acids play an important role in the regulation of mucosal barrier function to conserve the homeostasis of the gastrointestinal tract.

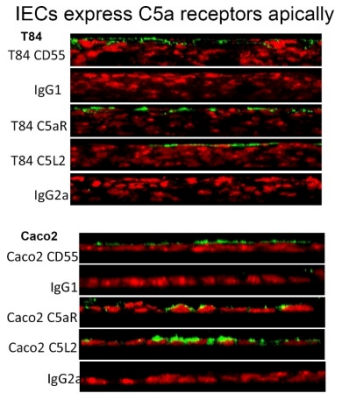
W.127. Expression of Suppressor of Cytokine Signalling 3 (SOCS3) Protein in UPEC-infected Human Bladder Epithelial Cells

Isak Demirel, Katarina Persson. Örebro University, Örebro, Sweden

Suppressor of cytokine signalling (SOCS) proteins are negative regulators of the innate immune system in immune cells. Pathogens appear to have the ability to modify the host SOCS proteins to evade the immune response. Uropathogenic escherichia coli (UPEC) are able to suppress the pro-inflammatory host response evoked by bladder epithelial cells, but the mechanisms are not fully understood. The purpose of this study was to investigate if SOCS proteins are induced in bladder epithelial cells in response to UPEC. RT-PCR studies showed an increased SOCS3 expression in isolated bladder epithelial cells (RT-4) in response to UPEC strain IA2, with a peak 5.1±2.3 fold increase after 6 hours of infection. In contrast, UPEC did not increase SOCS1 transcription. Both SOCS1 and SOCS3 transcripts were increased in RT-4 cells upon stimulation with a cytokine mixture of IL-1β, TNF-α and IFN-γ. The increase of SOCS3 transcript was confirmed at the protein level by western blotting. This study showed an increased expression of SOCS3, but not SOCS1, in bladder epithelial cells in response to UPEC infection *in vitro*. The data suggest that uroepithelial cells may use SOCS proteins to counterbalance the immune response similar to what has been established for immune cells.

W.128. Expression and Role of Anaphylatoxin Receptors on Human Colonic Epithelial Cells

Qi Cao, Andrew Stadnyk. Dalhousie University, Halifax, NS, Canada



Studies have implicated the complement system as impacting on colitis. To address the question of whether intestinal epithelial cells (IECs) can detect activated complement we investigated the expression and roles of anaphylatoxin receptors on human colonic epithelial cell lines. RT-PCR and western blotting were used to detect mRNA and proteins, respectively, in the T84, Caco-2 and HT-29 cell lines. We discovered that the C5aR, C5L2, and C3aR are expressed. Confocal microscopy further showed the expression of C5aR and C5L2 on the apical i.e. luminal surface, but not on the basal surface in polarized cell lines in Transwells. C3a or C5a treatment up-regulated mRNA for selected chemokines and activated the ERK pathway in the cells. EGF-activated ERK did not result in increased chemokine mRNA. Despite the increased chemokine mRNA there was only a limited increase in protein secretion. Pharmacologically blocking ERK inhibited the chemokine mRNA changes due to C5a, indicating ERK is necessary not sufficient for the increase in IECs. Our findings that human IECs are capable of detecting anaphylatoxins in the gut lumen introduce the possibility that the IECs amplify the inflammatory signal arising from complement-related events in the lumen.

W.129. Interferon Lambda Expression in Human Uterine Epithelial Cells

Mickey Patel, Charles Wira. Dartmouth Medical School, Lebanon, NH

The interferon (IFN) lambda (λ) family of cytokines directs a protective antiviral response. However, their expression and response to pathogen stimulation in the female reproductive tract (FRT) epithelium are unknown. Polarized cultures of primary human uterine epithelial cells were stimulated with Toll-like receptor (TLR) agonists for 3-48 hours in the presence or absence of 17-β estradiol (5x10-8M). IFN λ1 and 2 were potently upregulated by PolyIC (TLR3 agonist) with maximal upregulation between 6-12 hours. Both were consistently upregulated between 3-25 fold higher than IFNβ. PolyIC increased IFNλ receptor up to 10-fold. Agonists for TLRs 4, 7, 8 and 9 had no effect on IFNλ or IFNλ receptor expression. Exogenous application of IFNλ1 caused the rapid upregulation of the interferon-stimulated genes MxA and OAS2 within 4 hours in a dose-dependent manner. Estradiol had no effect on IFNλ or IFNλ receptor expression and did not alter the PolyIC-induced upregulation of IFNλ. IFNλ in the upper FRT is exquisitely sensitive to the presence of dsRNA and may play a greater role in mucosal defense than IFNβ. The lack of an estradiol effect suggests that the IFNλ response is independent of hormonal effects thus providing a constant level of protection across the menstrual cycle.

W.130. The Intestinal Epithelial Cells can Induce the Integrin CD103 After Interacting with Chitosan

María Magdalena Canali, Silvia Correa. CIBICI-CONICET, Córdoba, Argentina

The polysaccharide Chitosan (Ch) triggers regulatory cytokine production, modulates the function of mesenteric lymph node (MLN) T cells and promotes tolerance at mucosal inductive sites. To evaluate the contribution of intestinal epithelial cells (IECs) to the activity of Ch we studied i) the endocytosis of FITC conjugated Ch using different cell lines and IECs from normal rats; ii) the signals derived from IECs after 16 h of Ch ingestion

evaluated as CD103 expression on spleen (Sp) or MLN mononuclear cells co-cultured with them. After 30 min we found an increment in % and MIF of FITC-Ch+ cells, dose and temperature dependent (4°C vs. 37°C), suggesting the endocytosis of Ch; wich was confirmed by confocal microscopy. The contact with control group IECs induced CD103 expression in CD3+CD4+, CD3+CD4- and CD11b/c+ cells in Sp and MLN ($p<0.05$). This effect was stronger after co-cultures with Ch-treated IECs ($p<0.01$) and was inhibited using transwell inserts ($p<0.05$). It is well accepted that the subset CD103+ of MLN DCs is efficient at inducing gut-homing receptors and regulatory T cell differentiation. Therefore, the ability of IECs to induce the expression of this marker after Ch contact provides a clue to understand the mechanism of its mucosal activity.

W.131. Defects in Intestinal Barrier Function Result in CD4+ T Cell-mediated Compensatory Responses that Limit Acute Mucosal Inflammation

Manirath Khounlotham, Wooki Kim, Eric Peatman, Porfirio Nava, Caroline Addis, Asma Nusrat, Timothy Denning, Charles Parkos. Emory University, Atlanta, GA

Junctional Adhesion Molecule A (JAM-A) is a tight junction protein known to regulate intestinal epithelial permeability and leukocyte trafficking. JAM-A-/- mice have increased colonic permeability, B cell lymphoid hyperplasia and increased susceptibility to dextran sulfate (DSS) induced colitis. These mice also have elevated IgA and Treg suggesting adaptive immune compensation to enhanced intestinal permeability. To investigate the role of adaptive immunity in intestinal homeostasis in JAM-A-/- mice, we generated JAM-A-/- x RAG-/- animals. JAM-A-/- x RAG-/- mice did not develop spontaneous colitis, yet exhibited dramatically enhanced susceptibility to DSS colitis compared to JAM-A-/- or RAG-/- controls. Removal of B cells or IgA from JAM-A-/- mice had no detectable effect, however depletion of CD4+ T cells resulted in enhanced DSS colitis similar to JAM-A-/- x RAG-/- mice. Surprisingly, anti-CD25 antibody injection had no effect, suggesting Treg cells are not the critical CD4+ T cell compensatory population. Interestingly, TGFβ neutralization enhanced DSS colitis mimicking that observed in JAM-A-/- x RAG-/- mice. Collectively, these data demonstrate that in the context of an underlying barrier defect, CD4+ T cells and TGFβ limit acute intestinal inflammation . These findings provide evidence that increased colonic permeability combined with dysfunctional adaptive immunity predisposes to pathologic acute inflammation.

W.132. Defects in Autophagy Favor Adherent-invasive E. Coli Intramacrophagic Persistence and Increase Pro-inflammatory Response

Pierre Lapaquette, Marie-Agnès Bringer, Arlette Darfeuille-Michaud. Clermont Université, Université d'Auvergne, Clermont-Ferrand, France

Ileal lesions in Crohn's disease (CD) patients are abnormally colonized by pathogenic adherent-invasive Escherichia coli (AIEC). AIEC bacteria are able to replicate within macrophages and to induce secretion of high amounts of TNF-α by infected macrophages. CD-associated polymorphisms in NOD2, ATG16L1 and IRGM affect bacterial autophagy, a crucial innate immunity mechanism. We analyzed the impact of a loss of autophagy function on AIEC intramacrophagic replication and pro-inflammatory cytokine response by infected-macrophages. We show that AIEC bacteria induce the recruitment of the autophagy machinery at the site of phagocytosis, and that a subpopulation of intracellular bacteria is located within LC3-positive autophagosomes soon after infection. Functional autophagy limits the number of intramacrophagic AIEC. Impaired ATG16L1, IRGM or NOD2 expression induced an increase in the number of intramacrophagic AIEC bacteria and amplified pro-inflammatory, IL-6 and TNF-α, cytokine secretion. In contrast, forced induction of autophagy resulted in a decreased number of intramacrophagic AIEC bacteria associated with a decrease in pro-inflammatory cytokine release. On the basis of our findings, we speculate that in patients with abnormal colonization of ileal mucosa by AIEC, CD-associated polymorphisms in ATG16L1, IRGM or NOD2, which result in autophagy defects, can profoundly tip the balance toward a proinflammatory state.

W.133. Remodelling of Epithelial Barrier Function in a Rat Model of Post-inflammatory Gut Dysfunction with Persistent Mucosal Mast Cell Infiltration

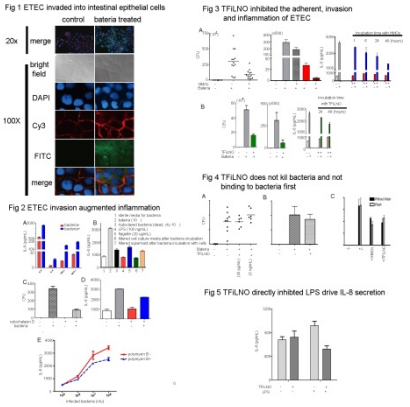
Joan Fernández-Blanco, Vicente Martinez, Patri Vergara. Universitat Autònoma de Barcelona, Bellaterra, Spain

Interactions among mucosal mast cells (MMCs), epithelial barrier function (EBF) and the enteric nervous system are the bases of inflammatory and functional gastrointestinal disorders. We characterized post-inflammatory EBF alterations in a model of MMC-dependent intestinal dysfunction in rats. SD rats were infected with Trichinella spiralis (TS). Jejunal histology and MMC (rMCP-2 immunohistochemistry) were evaluated from day 2 to 60 post-infection. Serum rMCP-2 levels (ELISA) and EBF were assessed on day 30. Basal electrophysiological parameters, FITC-dextran fluxes and responses to secretagogues and MMC degranulators were evaluated (Ussing chambers). Histological scores were significantly increased at days 6 to 14 post-infection. Thereafter, up to day 60, MMC infiltration persisted without any inflammatory infiltrate. On day 30, MMC infiltration correlated with increased serum rMCP-2 levels and altered EBF (increased secretion and permeability). MMC activation with IgE resulted in reduced secretory responses in TS-infected animals. Concanavalin A and compound 48/80 were inactive. Serotonin-induced secretion was reduced in TS-infected vs. controls, while responses to substance-P were similar. Neuronal blockade with tetrodotoxin reduced secretory responses to substance-P only in TS-infected rats. Post-inflammatory neuro-epithelial remodelling modulates EBF in a rat model of MMC-dependent gut dysfunction. Similar alterations might contribute to intestinal disorders associated with increased MMCs in humans.

W.134. A Human Milk Oligosaccharide Inhibits ETEC Invasion and Consequent Inflammation of Intestinal Epithelial Cells

YingYing He, David Newburg. Boston College, Chestnut Hill, MA

Enterotoxigenic Escherichia coli (ETEC) is a major cause of diarrhea, and a major cause of mortality in children under 5 years of age. Nursing infants have lower risk of diarrhea than those artificially fed. Stable and labile toxins of E. coli (STa & LT) are considered the major pathogenic agents of ETEC, but other mechanisms of pathogenesis have been reported, including invasion. We found that invasion of intestinal epithelial cells (T84, Caco2) by ETEC resulted in LPS-dependent increase in concentrations of cytokine inflammatory markers (i.e., IL-8, IL-6, MCP-1 and TNF-a. The upregulation was inhibited by the human milk oligosaccharide fraction when tested at equivalent concentrations to that in milk. It was also inhibited by a single oligosaccharide, TFiLNO, (trifucosyl (1,2-1,2-1,3)-lacto-N-octoase) at a concentration of 30 pg/mL (30 ppb), its estimated concentration in human milk. Milk oligosaccharides and TFiLNO also inhibited invasion of intestinal epithelial cells by ETEC, as observed by culturing cell contents after killing extracellular ETEC. Past reports indicated that some milk oligosaccharides inhibited enteric pathogen binding to host receptors. Finding other milk oligosaccharides that inhibit invasion and inflammation suggests multiple mechanisms whereby human milk glycans may act as a human milk innate immune system that protects infants. An individual oligosaccharide inhibited ETEC invasion augmented inflammation in intestinal epithelial cells.



W.135. A Clinically Relevant Model of Ischemia-reperfusion in the Rat: Possible Role of Mast Cells in Intestinal Epithelial Injury and Systemic Inflammation

Joern Karhausen[1], Amelia Gibson[2], Adam Moeser[2], Soman Abraham[1], G. Burkhard Mackensen[1]. [1]Duke University Medical Center, Durham, NC; [2]North Carolina State University, Raleigh, NC

Introduction: The basis for the severe inflammatory response associated with cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA) is currently unknown. We have developed a rat model to investigate inflammatory signals and the tissue injury associated with ischemia-reperfusion (I/R). Methods: Anesthetized and cannulated rats underwent 30min of CPB, 45min of DHCA at 16-18°C and 30min of rewarming on CPB. Sham-treated rats were cannulated without CPB/DHCA. Tissue was harvested after 2h of recovery. Serum from different time points was assayed by a multiplex cytokine-ELISA and for endotoxin levels. Transepithelial electrical resistance and FITC-Dextran Flux were assayed in Ussing chambers. For histological analysis, intestinal samples were fixed and stained with hematoxylin/eosin or tolouidine blue. Results: DHCA-associated I/R resulted in prominent macroscopical and microscopical intestinal injury and neutrophil infiltration, which translated into significant increases of FITC-Dextran Flux in all measured gut sections. In conjunction with histological gut injury, an unusual intraepithelial infiltration by mast cells was seen. Serum levels of MCP1, MIP1alpha, IL4, IL1beta, IL6, IP10, IL10 and TNFalpha as well as endotoxin were markedly increased but only after reperfusion. Conclusion: Employing our clinically relevant model of I/R we have gained first indications that mucosal mast cells play a critical role in the local and systemic inflammation associated with CPB and DHCA.

W.136. **Functions and Regulatory Targets of p38α MAPK in Intestinal Homeostasis and Inflammation**

Celia Caballero-Franco, Yasuso Sano, Jin Mo Park. Harvard Medical School/Massachusetts General Hospital, Charlestown, MA

Perturbation of mucosal homeostasis can lead to uncontrolled intestinal inflammation. The ubiquitously expressed protein kinase p38α signals to modulate tissue homeostasis and inflammatory responses, yet its function is believed to be cell-type specific. In order to investigate the physiological function of p38α in the intestinal epithelium, we have generated mutant mice in which p38α expression is specifically ablated in intestinal epithelial cells (p38αF/F-VillinCre; p38αΔIEC). This condition caused a disturbance in epithelial turnover and increased susceptibility to dextran-sodium-sulfate-induced colitis in the mutant mice. Further molecular analysis using the MODE-K intestinal cell line and primary intestinal epithelial cells from wild-type and p38αΔIEC mice revealed key regulatory targets of p38α MAPK involved in the control of cell proliferation and apoptosis via suppressing the activation of the Jun N-terminal kinase (JNK) pathway. This study reveals new insight into the role of p38α-JNK signaling circuitry in intestinal epithelial cells.

W.137. Analysis of the Genome Locus, Expression and Activity Studies of the Bovine RNases Suggests a Role in Mucosal Host Defense

Tom Wheeler, Sandeep Gupta, Paul Harris, Rita Lee. AgResearch, Hamilton, New Zealand

Members of the mammalian secretory RNaseA family are expressed in a range of mucosal epithelial tissues, including the digestive tract, urogenital tract, the male and female reproductive tracts, mammary gland, and in immune cells, including eosinophils and neutrophils. Their pattern of expression, together with the antimicrobial activity of some members of the family, has led to the suggestion they play a role in host defence at mucosal sites. Characterisation of the RNase family locus in cattle revealed the presence of eight genes apparently unique to the bovine lineage, supporting the idea that in this species the family has undergone rapid evolution through gene duplication. These bovine-specific RNases were all

found to be expressed in host-defence associated cells or tissues but each with their own unique pattern. RNase4 and RNase5 were found to be were present in both bovine milk and reproductive tract secretions. RNase4 and RNase5, purified from milk, were shown to modulate nucleic acid stimulated pro-inflammatory signalling in macrophages. Furthermore, RNase5 bound to the opportunistic mucosal pathogen, Candida albicans and suppressed its growth under certain conditions. Collectively, these data suggest that RNases contribute to host defence on mucosal epithelia.

W.138. Modulating Role of Cathelicidin-related Antimicrobial Peptide in Maintaining Mucosal Homeostasis

Sae-Hae Kim, In-Young Yang Kyung-Yeol Lee, Yong-Suk Jang. Chonbuk National University, Jeonju, Republic of Korea

Gastrointestinal mucosa is exposed to heavy load of commensal and pathogenic microorganisms and maintains the mucosal homeostasis with dendritic cells (DCs) conditioned by mucosal environment. However, the balance is lost by infection or an antigen influx. These counter events occur in mucosal inductive site, Peyer's patch (PP), since both commensal bacteria and luminal antigens transported by M cells present in the site, although the factors regulating the balance are not well understood yet. We assume that cathelicidin-related antimicrobial peptides, whose production is induced by secondary product of commensal bacteria, play a key role in maintaining mucosal homeostasis in steady-state condition through modulating $CX3CR1^{+}CD70^{+}$ DC associated with Th17 cell differentiation. The homeostatic conditioning by the peptides could be broken by pro-inflammatory environment through reduction of the peptide expression and predominant cell population in PP is changed during infection or antigen influx. To confirm this assumption, we prepared PP lymphocytes under steady-state and characterized the modulation of DCs and the degree of functional Th17 cell differentiation by addition of a cathelicidin-related antimicrobial peptide, LL-37. Collectively, we suggest that regulation of LL-37 expression by commensal bacteria is closely associated with mucosal homeostasis and immune response induction. This study was supported by the IPET.

W.139. Expression and Function of Scavenger Receptors in Activated Airway Epithelial Cells: Role in the Response to TLR3 Agonist

Philippe Gosset[1], Audrey Dieudonné[2], David Torres[3], Simon Blanchard[4], Yves Delneste[4], Pascale Jeannin[4], Muriel Pichavant[1], François Trottein[1]. [1]Institut Pasteur, Lille, France; [2]CH de Valenciennes, Valenciennes, France; [3]Université Libre de Bruxelles, Gosselies, Belgium; [4]INSERM U892, Angers, France

Background: Airway epithelium participates in host defence and inflammation. Danger signals are recognized by toll like receptors (TLR) which control defense mechanisms. Airway epithelial cells (AEC) have been described to functionally express TLR, the most powerful activator being TLR3. Since scavenger receptors (SR) and TLR cooperate to adjust the pathogen response, the expression of SR and their function was analyzed in AEC particularly in response to TLR3 ligand. Methods: We evaluated SR expression and their *in vitro* role as a TLR3 coreceptor in activated BEAS-2B cells and primary cultures of AEC. The capacity of SR ligands to control lung inflammation induced by dsRNA was determined in mice. Results: AEC constitutively express mRNA for several SR except SR-A1. TNF-α-induced increased expression of LOX-1 and CXCL16 was associated with higher binding of the SR ligand maleylated ovalbumin (mOVA). *In vitro*, mOVA inhibits dsRNA binding and TLR3-dependent cytokine production. mOVA also decreases the TLR3-dependent mobilization of IRF3 and NF-κB. *in vivo*, mOVA inhibits dsRNA-induced bronchoalveolar inflammatory infiltrate and cell mobilization in draining lymph nodes. Conclusion: SR expression in AEC is upregulated by TNF-α and controls the response to TLR3 ligand. SR mobilization inhibits dsRNA-induced immune cell recruitment and activation in airway mucosa.

W.140. Effects upon Epithelial Cells Induced by TLR2

José Miguel Cabral, Patrício Soares-da-Silva, Fernando Magro. University Porto, Porto, Portugal

Type 2 toll-like receptors (TLR2) are expressed in the cell membrane and recognize a wide range of pathogen-associated molecular patterns derived from bacteria as lipoteichoic acid (LTA). Aims: Evaluate the effect of TLR2 activation by LTA on activity of type 1 and 2 Na+/H+ exchanger (NHE), pHi and potential membrane in T84 intestinal epithelial cells. Results: Short-term TLR2 activation significantly decreased basal pH levels and inhibited NHE1/NHE2 activity in a concentration dependent manner. Long-term TLR2 activation significantly decreased basal pH levels and inhibited NHE1/NHE2 activity ; a significant increase in TLR2 protein expression was observed after long-term treatment with 10 μg/ml LTA. Inhibition of PKA, inhibition of PLC, and down regulation of PKC, prevented the short- and long-term TLR2-mediated inhibition of NHE1/NHE2 activity. LTA treatment produced a significant increase in cAMP levels. An increase in adenylyl cyclase 3 expression was observed after long-term exposure to LTA. Inhibition of NHE1/NHE2 activity was observed after treatment of T84 cells with forskolin, db-cAMP or m-3M3FBS. LTA produced a hyperpolarization of the cell membrane. Conclusions: Activation of TLR2 exerts marked inhibition of NHE1/NHE2 activity, decreases pHi and membrane hyperpolarization of epithelial cells. Transduction mechanisms set into motion during TLR2-mediated inhibition of NHE1/NHE2 activity involves PKA, PLC and PKC. However, short- and long-term TLR2 activation might use different signaling pathways.

W.141. Short and Long-term Regulation of Intestinal Na+/H+ Exchange by TLR4

José Miguel Cabral, Patrício Soares-da-Silva, Fernando Magro. University Porto, Porto, Portugal

The ability of recognizing microbiological infections is largely dependent on toll-like receptors (TLR). TLR4 is expressed in the cell membrane and is activated by bacterial lipopolysaccharide. Aims: Evaluate the effect of TLR4 activation by Monophosphoryl Lipid A (MPLA) on activity of type 1 and 2 Na+/H+ exchanger (NHE) in T84 intestinal epithelial cells. Results: Short-term TLR4 activation significantly decreased basal pH levels and inhibited

NHE1/NHE2 activity in a concentration dependent manner. Long-term TLR4 activation inhibited NHE1/NHE2 activity; a significant increase in TLR4 protein expression was observed after long-term treatment with MPLA. Long-term activation of TLR4 induced at low concentrations a decreased in NHE3 activity and at 100 µg/ml the inhibitory effect was showed only upon NHE1 activity. MPLA treatment produced a significant increase in cAMP levels. An increase in adenylyl cyclase 3 expression was observed after long-term exposure to MPLA. Inhibition of NHE1/NHE2 activity was observed after treatment with forskolin, db-cAMP or m-3M3FBS. MPLA significantly reduced membrane potential. Conclusions: Shot-term activation of TLR4 exerts marked inhibition of NHE1/NHE2 activity. Long-term stimulation of TLR4 at low concentrations preferentially inhibits NHE3 activity in T84 cells. Transduction mechanisms set into motion during short- and long-term TLR4-mediated inhibition of NHE1/NHE2 activity involves PKA, PLC and PKC.

W.142. High Levels of Soluble HLA-G in the Female Genital Tract of Beninese Commercial Sex Workers are Associated with HIV-1 Infection

Valérie Thibodeau[1], Julie Lajoie[2], Annie-Claude Labbé[1], Michel Alary[3], Johanne Poudrier[1], Michel Roger[1]. [1]Université de Montreal, Montreal, QC, Canada; [2]Université de Winnipeg, Winnipeg, MB, Canada; [3] Universitaire de Québec, Université Laval, Québec, QC, Canada

HLA-G is a powerful suppressor of the immune response, and HIV can modulate its expression. The aim of this study was to investigate whether soluble HLA-G (sHLA-G) expression in cervicovaginal lavages (CVLs) is associated with risk of HIV-1 infection among highly HIV-exposed commercial sex workers (CSWs). Levels of sHLA-G in CVLs were compared between 50 HIV-1-uninfected and 43 HIV-1-Infected CSWs as well as 67 HIV-1-uninfected non-CSW women at low risk of exposure, recruited in Cotonou, Benin. HIV-1-infected CSWs had significantly higher genital mucosa sHLA-G levels when compared to HIV-1-uninfected CSWs and non-CSW groups. The association remained significant after adjustment was made for all variables other than HIV that can influence HLA-G expression. This study demonstrates that sHLA-G expression in the female genital tract is associated with HIV-1 infection, and suggests that high levels of sHLA-G may contribute to the tolerogenic environment favouring viral immune escape and dissemination at the initial site of exposure.

W.143. Innate Lymphoid Cells Regulate Intestinal Epithelial Barrier Function

Gregory Sonnenberg, David Artis. University of Pennsylvania, Philadelphia, PA

A diverse array of beneficial microbial communities colonize barrier surfaces of the body and can influence tissue homeostasis, metabolism, immunity and inflammation. However, loss of barrier function can result in microbial translocation and systemic immune activation, hallmarks of inflammatory bowel disease (IBD) and HIV infection that have been proposed to contribute to disease progression. Innate lymphoid cells (ILCs) are a recently identified heterogeneous population of cells that have been implicated in promoting inflammation or immunity at barrier surfaces. However, whether ILCs contribute to maintenance of epithelial barrier function or prevention of microbial dissemination in the steady state has not been investigated. We demonstrate that depletion of ILCs in Rag-/- mice results in loss of intestinal barrier function and systemic dissemination of commensal bacteria. This loss of barrier function was evident by culturable bacteria in the liver and blood, systemic immune activation and hepatic inflammation. Moreover, ILCs were found to be critical for maintenance of barrier function in both lymphocyte-deficient and lymphocyte-replete hosts. Collectively, these results identify a previously unrecognized function of ILCs in maintaining barrier function and implicate this cell population as a target in treating or preventing the pathogenesis associated with chronic inflammation, microbial translocation and systemic immune activation.

W.144. Activation of Invariant Natural Killer T Lymphocytes in Response to α-galactosylceramide Encapsulated in PLGA-base Particles

Elodie Macho Fernandez[1], Jiang Chang[2], Josette Fontaine[1], Emilie Bialecki[1], Fabien Rodriguez[1], Elisabeth Werkmeister[5], Vanessa Krieger[3], Christophe Ehret[4], Béatrice Heurtault[4], Sylvie Fournel[3], Benoit Frisch[4], Didier Betbeder[2], Christelle Faveeuw[1], François Trottein[1]. [1]INSERM U1019, Lille, France; [2]University Lille Nord de Franc, Lille, France; [3]CNRS UPR 9021, Strasbourg, France; [4]CNRS UMR 7199, Illkirch, France; [5]IFR142, Lille, France

Invariant Natural Killer T (iNKT) cells are non-conventional T lymphocytes that respond rapidly to glycolipid antigens presented by the CD1d molecule. In response to α-galactosylceramide (α-GalCer), iNKT cells promptly secrete high amounts of cytokines that leads to downstream activation of numerous immune cells. Through this activation cascade, α-GalCer exerts potent anti-tumoral activity *in vivo*, rendering it a powerful candidate for anti-tumor therapy. However, no or moderate clinical responses were observed among the patients repeatedly inoculated with α-GalCer. We hypothesized that encapsulation of α-GalCer in particles would improve the efficacy of the treatment. In this study, we compare the effect of α-GalCer encapsulated in poly(lactic-co-glycolic acid) (PLGA)-based nanoparticles (NP) and microparticles (MP) on iNKT cell activation. Our data show that whatever the size of the particles, vectorized α-GalCer induced potent activation of iNKT cells *in vitro* and *in vivo*. Endocytosis of particles by DC is mediated by a clathrin-dependent mechanism and this event is important to stimulate iNKT cells. Finally, we report that α-GalCer vectorized in NP and MP exhibited different behaviours *in vivo*. Collectively, our data validate the concept that α-GalCer encapsulated in PLGA-based particles can be used as delivery systems to activate iNKT cells *in vitro* and *in vivo*.

W.145. Protease Treatment of Native or Deamidated Gluten Epitopes Prevents Recognition by a Gliadin-specific CD4+ T Cell Clone

Henrik Toft-Hansen[1], Torben Barington[1], Erwin Roggen[2], Ludvig Sollid[3], Søren Lillevang[1], Steffen Husby[1]. [1]Odense University Hospital, Odense, Denmark; [2]Novozymes, Bagsværd, Denmark; [3]University of Oslo, Oslo, Norway

Gluten-specific CD4+ T cells are pathogenic in celiac disease. We tested five proteases for their potential to prevent the proliferative response by a gluten-specific CD4+ T cell clone to protease-digested gluten epitopes using the substrates gluten, gliadin, or the highly immunogenic 33-mer from alpha2 gliadin. The protease treatment was carried out at pH 7.8 for 2 hours at 37 degrees C, either before or after deamidation of substrate with tissue transglutaminase. Proliferation of a gliadin-specific CD4+ T cell clone was measured by incorporation of radioactive thymidine using an assay with HLA-DQ2 homozygous B cells as antigen presenting cells. One of the proteases was particularly efficient at diminishing proliferation after stimulation with cleaved antigen, and blocked the response against all three antigens at a ratio of protease to substrate of 1:250 (w:w). The other four proteases showed a moderate effect. Equivalent results were obtained when cleaving both native and deamidated gluten epitopes. In conclusion, we identified proteases which could cleave both native and deamidated substrates. One protease blocked the response to protease-digested gluten epitopes by a gliadin-specific CD4+ T cell clone. We intend to further investigate the spectrum of gluten epitopes that can be cleaved by these proteases.

W.146. Airway Epithelial Cells Modify Reactivity of Dendritic Cells through Secretion of Prostaglandin E_2

Lotte Schmidt[1], Maria Belvisi[2], Konrad Bode[1], Maria-Thersia Suchy[3], Dimitrios Tsikas[3], Jutta Scheuerer[1], Felix Lasitschka[1], Alexander Dalpke[1]. [1]University Heidelberg, Heidelberg, Germany; [2]Imperial College London, London, United Kingdom; [3]Hannover Medical School, Hannover, Germany

We report that airway epithelial cells modify dendritic cells (DCs) towards a tolerogenic phenotype characterized by reduced secretion of proinflammatory cytokines, up-regulation of IL-10 and decreased T cell activation. Modification was dependent on constitutively secreted, soluble factors among which we identified prostaglandin E_2 (PGE_2) to mediate inhibition of IL-12. Blocking PGE_2 synthesis through Cox2 inhibition or inhibition of PGE_2 activity using EP4 deficient DCs reduced the inhibitory effects of airway epithelial cells. Moreover, *in vitro* cultivated epithelial cells as well as laser capture microscopy of epithelial cells from tracheal sections showed constitutive expression of Cox2. Manipulating cAMP and PKA signalling that are coupled to EP4 receptors in DCs again released the latter cells from control by epithelial cells. Genome wide expression profiling further revealed that epithelial cells modify DCs to adopt a phenotype that resembles alternatively activated myeloid cells with high expression of arginase-1, YM1 and a newly identified molecule MS4A8A. *Ex vivo* isolated primary lung DCs showed a comparable phenotype to ECM-DCs as indicated by the expression of arginase-1 and Ym1. The experiments reveal that airway epithelial cell-derived PGE_2 contributes to the modulation of DCs supporting a concept in which the local microenvironment shapes reactivity of professional immune cells.

W.147. Homeostatic Microbiota-driven Signalling at the Epithelium of the Intestinal Tract

Silvia Stockinger[1], Tamas Dolowschiak[1], Claudia Duerr[1], Johanna Pott[1], Martijn Brugman[2], Mathias Hornef[1]. [1]Hannover Medical School, Hanover, Germany; [2]Leiden University Medical Center, Leiden, Netherlands

Intestinal epithelial cells (IECs) provide the barrier between the intestinal lumen densely colonized by the enteric microbiota and the largely sterile underlying lamina propria. Similar to professional immune cells, IECs express receptors of the innate immune system. Given the enormous amount of potential proinflammatory stimuli within the gut lumen, a tight regulation of the interaction between the commensal microbiota and the mucosal immune system is required to prevent an inappropriate inflammatory reaction. On the other hand, flora-driven epithelial cell responses may contribute to a stable host-microbial interaction and facilitate maintenance of homeostasis at the mucosal interface. Here we systematically studied epithelial gene expression involved in various aspects of epithelial barrier formation such as antimicrobial host defence, cell proliferation, and homeostatic immune signalling. IECs prepared from both conventionally raised and germfree mice as well as animals deficient in various innate immune signalling mediators were examined by microarray analysis. The results were validated on the protein level by immunoblot and immunohistology. The functional relevance was elucidated using *in vivo* models of oral infection with enteric pathogens as well as transient epithelial damage. Our results indicate a critical role of epithelial innate immune signalling induced by microbial ligands to maintain the mucosal barrier and enteric host-microbial homeostasis.

W.148. Post Operative Ileus is Triggered by Mast Cell Derived Proteinase Activity and IL1beta Release

Susanne Snoek, Sjoerd van Bree, Guy Boeckxstaens, Wouter De Jonge. Amsterdam Medical College, Amsterdam, Netherlands

Post-operative ileus (POI) is a common consequence of intestinal surgery that enhances morbidity and hospitalization. Its pathogenesis involves the activation of intestinal dendritic cells (DCs) and a subsequent Th1 response, but it is unclear how these DCs are activated. We show in a mouse model for POI that IM leads to bacterial translocation and DC activation, which was dependent on MyD88 -but not TLR 2, 4, or 9-, indicating a role for IL1R activation. In conjunction, treatment with the IL1R antagonist Anakinra prevented DC activation, bacterial translocation, and POI. Furthermore, we show that mast cell- derived IL1beta is crucially important for DC-derived IL12 production and POI. In conjunction, mast cell deficient mice did not develop POI after surgery, and mast cell reconstitution recapitulates POI. Surprisingly, the IL1beta production was independent on inflammasome formation in this model, and consequently ASC1 or NALP3 deficient mice were not protected against POI. Instead mast cell

secreted proteinase activity was required to processes IL1beta to stimulate DC cytokine release. We conclude that POI is triggered by mast cell activation, proteinase activity and IL1beta release. This study implies that targeting mast cells, or IL1beta, is a bona fide strategy to treat POI.

W.149. Role of Secretory Leukocyte Protease Inhibitor (SLPI) in Intestinal Inflammation: Results from Spontaneous and Induced Mouse Models of Colitis

Rajaraman Eri[1], Hui Tong[2], Timothy Florin[2], Michael McGuckin[2]. [1]University of Tasmania, Launceston, Australia; [2]Mater Medical Research Institute, Brisbane, QLD, Australia

Secretory leukocyte protease inhibitor (SLPI), a 11.7 kDa protease inhibitor found in mucosal surfaces and fluids, has been described as possessing anti-inflammatory, wound healing, anti-microbial and immune modulatory activities. While SLPI polymorphisms have been associated with IBD risk, its exact role in intestinal inflammation is unknown. We recently demonstrated the pivotal role of ER stress in intestinal goblet cells in the spontaneous colitis Winnie mouse1,2. Winnie colons exhibit a 20-fold increase in SLPI mRNA and the stressed goblet cells produce large quantities of SLPI (13-fold greater than wild-type). Age and sex matched wild-type and SLPI-/- mice were subjected to 3% DSS for 7 days, all the clinical and histological parameters were analysed. There was no difference in non-DSS treated wild-type and SLPI-/- mice. In DSS treated group, SLPI-/- had reduced weight loss, significantly reduced colon weights and increased colon lengths. Histological colitis scores showed a significantly reduced overall colonic inflammation but regional analysis revealed protection, especially in the distal recto-colon region. Our results demonstrate a hitherto unassigned role for SLPI in colonic inflammation especially affecting the distal rectocolon. Heazelwood CK, Cook MC, Eri RD, et al., PLoS Medicine (2008).Eri RD et al., Mucosal Immunology (2010).

W.150. Blocking Integrins in a Human Cervical Mucosa Tissue Model Inhibits HIV-1 Infection

Veronica Tjomsland[1], Rada Ellegard[1], Karlhans Che[1], Preben Kjölhede[1], Jorma Hinkula[1], Jeffrey Lifson[2], Marie Larsson[1]. [1]Clinical and Experimental Medicine, Linköping, Sweden; [2]National Cancer Institute at Frederick, Frederick, MD

Slightly more than half of the HIV-1 infected individuals in the world are women and almost all acquire the infection through sexual intercourse. The initial interaction between HIV-1 and the host occurs at the mucosa and the most common mode to access the submucosa is through dendritic cells (DCs). In the cervical mucosa, HIV-1 exist both as free and opsonized virions and this might influence infection. We used a cervical tissue explant model and both free and opsonized HIV-1 to study HIV-1 transmission and how it can be prevented. We found that complement opsonization significantly enhanced HIV-1 infection of DCs compared to free HIV-1 but this increase in infection was not seen for CD4+ T cells. Blocking of α4, beta β7, and β1 integrins demonstrated significant inhibition of infection of both DCs and CD4+ T cells emigrating from mucosa independent on the use of free or complement opsonized HIV-1. Blocking CD1a, a receptor expressed on antigen presenting cells, almost abolished the infection of mucosal DCs but did not affect the infection of CD4+ T cells. Our findings should be taken into consideration when developing future microbicides, which are currently one of the best strategies to prevent HIV infection and transmission.

W.151. The Cholinergic Anti-inflammatory Pathway: Role of Acetylcholine in Regulating Epithelial Integrity During Inflammation in the Gut

Shobhit Dhawan[1], Ida Hiemstra[2], Linette Willemsen[3], Joke den Haan[2], Wouter De Jonge[1]. [1]Academic Medical Center, Amsterdam, Netherlands; [2]Vrije Universiteit, Amsterdam, Netherlands; [3]Utrecht University, Utrecht, Netherlands

We have shown previously that vagus nerve derived acetylcholine ameliorates intestinal inflammation via activation of cholinergic receptors on innate immune cells. Here we aim to illustrate the protective effect of acetylcholine on enterocytes and intestinal barrier function under healthy and inflammatory conditions. We measured the effect of cholinergic receptor activation on barrier function in human and mouse epithelial cell lines by electrical impedance (ECIS) across the monolayer, as well as in transwell experiments. IL1β induced dissociation of tight junction proteins (occludin & ZO1) was observed by immunostaining, which was restored upon pre-treatment with mAChR agonists. In addition, IL1β led to NFkB activation and IL8 production in Caco2 and CMT93 cell lines which was antagonised by acetylcholine, muscarine and neostigmine. Treatment with IL1β enhanced permeability (transwell) and reduced impedance (ECIS) across the monolayer, counteracted by preincubation with acetylcholine, bethanechol and neostigmine. The protective effects of acetylcholine were antagonised by atropine. In epithelial cells isolated from IBD patient resection material a significantly reduced choline acetyltransferase and acetylcholine esterase expression was observed compared to unaffected control tissue. In conclusion, acetylcholine restores epithelial barrier function under inflammatory conditions. Reduced epithelial acetylcholine metabolic enzyme expression in an inflammatory IBD setting may contribute to barrier dysfunction.

W.152. Engineering of a Multi-cellular Organotypic Model of the Human Intestinal Mucosa

Rosangela Mezghanni, Alessio Fasano, Marcelo Sztein. University of Maryland Baltimore, Baltimore, MD

The engineering of multicellular organotypic models of the human intestinal mucosa has wide-ranging potential as a tool for discovery in both health and disease, including interaction with pathogens, antigen trafficking, inflammatory and physiological processes. However, current models remain relatively simple composed of few cell types. Here we describe an organotypic model of the human intestinal mucosa comprised of multiple cell types including fibroblasts, lymphocytes epithelial and endothelial cells. Methods: In our model fibroblasts and endothelial cells were embedded in a collagen-rich extracellular matrix and cultured with epithelial cells and lymphocytes under microgravity conditions provided by a rotating wall vessel

bioreactor. Results: We observed that the epithelial cells (i) displayed a monolayer organization, (ii) had the appropriate polarity, tight junctions, desmosomes and microvilli, (iii) produced cytokines upon antigenic stimulation, (iv) transported nutrients and (v) differentiated into multi-lineage progeny (i.e., absorptive enterocyte, goblet and M cells). We also observed well preserved fibroblasts, lymphocytes and endothelial cells dispersed throughout the extra-cellular matrix. Conclusions: We report the development of an organotypic model resembling structurally and functionally the human intestinal mucosa. Finally, to our knowledge, previous attempts to integrate multiple cell types in constructs grown under microgravity have been unsuccessful.

W.153. HIV-induced Innate Immune Responses in Uterine Epithelial Cells

Mickey Patel, Mimi Ghosh, John Fahey, Charles Wira. Dartmouth Medical School, Lebanon, NH

The mucosal epithelium of female reproductive tract is the primary site of HIV transmission during heterosexual intercourse with R5-tropic strains accounting for the majority of transmission events. However, the early innate immune response of epithelial cells to HIV has not been adequately studied. Primary uterine epithelial cells were cultured on transwell inserts and stimulated apically with varying concentrations of BaL (R5) and 3B (X4) HIV for up to 48 hours. Both viruses upregulated TNFα mRNA expression. Interferon (IFN) beta was induced at very low levels by both viruses. Intriguingly, BaL down-regulated the production of IFN lambda while 3B had no effect. The interferon-stimulated genes (ISG) MxA, OAS2 and PKR were only induced by 3B and not BaL. This finding extended to mRNA levels of the anti-HIV molecules CCL20 and Elafin, which were induced by 3B in a dose-dependent manner. The potent innate immune response elicited by 3B (X4) in uterine epithelial cells may partially account for the increased transmissibility of R5 viruses. How BaL avoids inducing an ISG and anti-HIV response is unknown. Furthermore, the upregulation of interferon-stimulated genes in the absence of a robust IFN beta or lambda response hints at a novel response mechanism elicited by 3B.

W.154. HIV Exposed Seronegative Women Express Lower Level of IFN-g Inductible Chemokine in their Cervico-vaginal Lavages

Julie Lajoie, Adam Burgerner, Joshua Kimani, Blake Ball, Keith Fowke. University of Manitoba, Winnipeg, MB, Canada

Over three-quarters of HIV/AIDS cases occur through heterosexual transmission. However, little is know about the factors influencing the susceptibility to HIV infection and the immune response in the female genital tract (FGT). Studies, including those with female sex worker (FSW), have reported natural resistance to HIV-1 infection. Reduced levels of immune activation, termed immune quiescence, has been associated with this resistant. The aim of this study is to analyse immune mucosal factors that could be implicated in the susceptibility to HIV infection. Methods: 213 CVL from FSW from the Majengo clinic in Nairobi, Kenya (57 new negatives (NN); 68 HIV+ and 55 highly exposed non infected (HESN)) and 33 HIV uninfected low risk women were analysed for the presence of cytokines and chemokines. Our results show a significant difference between the three groups for the chemokines CXCL-9 (MIG) and CXCL-10 (IP_10). MIG and IP-10 were decreased in the CVL of the HESN compared to the other groups (anova $p=0.0002$ and $p<0.0001$ respectively).Conclusion: These results suggest a decreased of the immune activation in the FGT of the HESN consistent with the immune quiescence hypothesis. This study will allow us to furthering our understanding of the mechanisms involved in the host immune response versus HIV. Funded by CIHR.

W.155. Role of Toll-like Receptors in Development of Postpartum Endometritis

Olga Lebedeva[1], Natalya Samborskaya[1], Sergei Pakhomov[1], Mikhail Churnosov[1], Pavel Kalutsky[2], Robert Qirko[3], Petr Karpov[1], Pavel Dovgiy[1]. [1]Belgorod State University, Belgorod, Russian Federation; [2]Kursk State Medical University, Kursk, Russian Federation; [3]Tirana University, Tirana, Albania

Toll-like receptors (TLRs) play a crucial role in defense against pathogens invading the female reproductive tract. Binding of specific ligands with TLRs promotes pro-inflammatory cytokine production and development of inflammation. The aim of the study was to establish the association between expression of TLR1 (ligands - triacyl lipopeptides), TLR2 (ligands - lipopeptides, lipoteichoic acid, zymosan), and TLR 5 (ligand - flagellin) of cervical epithelial cells *in vivo* and development of postpartum endometritis in women with high infection risk. Materials and methods: Expression of TLR1, TLR2 and TLR5 mRNA in epithelial cells of cervix utery was detected using qRealTime PCR on third day after delivery per vias naturalis. Samples were taken from 9 women with clinical endometritis and 17 women with normal postpartum period. mRNA was extracted using Trizol (Invitrogen). First-strand cDNA synthesis was performed using oligo dT primers and Mint reverse transcriptase kit (Eurogen). Quantitative real-time PCR was performed using qPCRmix-HS SYBR kit (Eurogen). Results were analyzed using an iCycler (Bio-rad laboratories). The threshold cycle values were normalized against the threshold value of human beta-actin and analyzed using Statistics 6.0. Results. It was shown that mean relative expression of TLR5 is significantly lower in women with postpartum endometritis compared with expression during normal postpartum period. Levels of TLR1 and TLR2 has not significant difference in both groups. Level of TLR5 was strongly correlated with data of histological results of placental tissue. Conclusions. It was shown, that decrease of TLR5 expression plays significant role in postpartum endometritis development. Research supported by grant of President of Russian Federation MK-1564.2010.7.

W.156. Antibodies and the Neonatal Fc Receptor (FcRn): Enhancement Versus Neutralization of Chlamydia Infection

Charles Armitage[1], Timothy Kuo[2], Peter Timms[1], Richard Blumberg[2], Kenneth Beagley[1]. [1]Queensland University of Technology, Brisbane, QLD, Australia; [2]Brigham & Women's Hospital and Harvard Medical School, Boston, MA

Until recently, the protective effect of antibodies was thought to be limited to the extracellular space. However, recent studies have shown that antibody neutralization is not limited to extracellular pathogens. We have used polyclonal IgG antibodies directed against 3 chlamydial antigens, MOMP (expressed by both the extracellular EB and the intracellular RB), and IncA and NrdB (both expressed exclusively by the intracellular replicating RB) to determine the effects on chlamydial infection in pH-polarized epithelial cells. Epithelial cells were able to internalize antibodies directed against the chlamydial intracellular antigens IncA and NrdB, which resulted in the reduction of chlamydial infection levels demonstrating that they not only entered the cells, but were able to interfere with normal chlamydial growth. The neutralization was not observed when FcRn was silenced by siRNA. Conversely, IgG anti-MOMP enhanced chlamydial infection of epithelial cells but this was abrogated by FcRn knockdown. Both the male and female reproductive tracts readily express FcRn and have an acidic environment, which favors binding and uptake of IgG, the dominant immunoglobulin in both tracts. Thus, it is important to consider the potential for FcRn and IgG-mediated enhancement or neutralization of infection when designing vaccines to target sexually transmitted infections.

W.157. Matrix Metalloproteinases in Serum and Follicular Fluid of Women Treated by *in vitro* Fertilization

Petra Horká, Karin Malickova, Radka Jarosova, Ivana Janatkova, Tomas Zima, Marta Kalousova. University and General University Hospital, Prague, Czech Republic

Aim: Analyze the relation of fertility and pregnancy of women of childbearing age of two matrix metalloproteinases: MMP-2 and MMP-9. Materials and Methods: Fifty-eight women evaluated for infertility were divided into two cohorts based on the success of IVF treatment. In the first cohort were 29 women, who became pregnant. The second cohort consisted of 29 women, whose treatment was unsuccessful. Concentrations of MMP-2 and MMP-9 were measured by means of commercially available ELISA-kits. Results: Women who become pregnant after IVF had a higher follicular fluid concentrations of MMP-9 than women for whom IVF was unsuccessful ($p = 0.003$). The production of MMP-9 in serum ($p = 0.127$) between the two groups of women was found to have no significant difference. The concentration of MMP-2 in follicular fluid and also in serum across the two groups did not differ. This study shows that women who had high concentrations of MMP-9 in follicular fluid had a higher probability of pregnancy during IVF. Conclusion: It seems that a high concentration of MMP-9 in follicular fluid is important for successful IVF, while the concentration of MMP-2 and concentration of MMP-9 (serum) do not play a key role in the initial stage of pregnancy.

W.158. Menstrual Blood as a Potential Source of Endometrial Derived Lymphocytes

Steffanie Sabbaj, Zdenek Hel, Houng Vu, Zina Moldoveanu, Holly Richter, Jiri Mestecky, Paul Goepfert. University of Alabama at Birmingham, Birmingham, AL

Studies of cell-mediated immunity in the female genital tract (FGT) have been problematic due to difficulties associated with the collection of mucosal samples. Most studies rely on biopsies from the lower FGT or tissue from hysterectomies. Samples from healthy women are limited as most studies are carried out in women with underlying pathologies. Menstruation is the cyclical sloughing off of endometrial tissue, and should be a source of endometrial cells without the need for a biopsy. We isolated and compared mononuclear cells from menstrual (MB) and peripheral blood and from endometrial biopsy-derived tissue from healthy women to determine the cellular composition and function of this compartment. We demonstrate that T cells isolated from MB are a heterogeneous population of cells with markers reminiscent of blood and mucosal cells as well as unique phenotypes not represented in either compartment. T cells isolated from MB expressed increased levels of HLA-DR, αEβ7 and CXCR4 and reduced levels of CD62L relative to PBMC. MB T cells were enriched for cells expressing CCR7 and CD45RA (naïve) T cells and were functional as determined by antigen-specific intracellular cytokine production. These data may open new avenues of investigation involving the FRT without the need for biopsies.

W.159. Omega-3 Fatty Acids (FA) Contribute to the Prevention of Pre-term Delivery in Mice Model Through Downregulation of Cervical Local Inflammation

Aki Yamashita, Kei Kawana, Kensuke Tomio, Satoko Kojima, Seisuke Sayama, Takeshi Nagamatsu, Tomoyuki Fujii. University of Tokyo, Tokyo, Japan

Omega-3 fatty acids (FA) are reported epidemiologically to have preventable effect on preterm delivery. But the mechanism remains unclear. We addressed effect of omega-3 FA on cervical inflammation and preterm delivery using Fat-1 transgenic mice which have a Fat-1 enzyme converting omega-6 to omega-3 FA. For preterm delivery mice model, pregnant mice of wild type (WT) or Fat-1 mice received intracervical injections of either vehicles or Lipopolysaccharides (LPSs). We defined preterm birth as at least one pup delivery within 48 hours after the injection. Rates of reterm birth were compared between WT and Fat-1 mice. Local immune cells, including peritoneal washing cells, were also analyzed by flow cytometry comparing each group. Local cytokine secretion was measured by ELISA. LPS-induced preterm birth rates were 45 % in WT and 20% in Fat-1 mice ($p=0.001$) while all vehicle-injected mice were term-delivered. Peritoneal neutrophils, IL6 and IL-1b secretion in Fat-1 mice were significantly less than in WT mice. Omega-3 FA may effect on preventing preterm delivery through downregulation of inflammatory reaction of genital mucosal

immune cells, especially neutrophils.

W.160. Characterization of Gut-derived Intraepithelial Lymphocyte (IEL) Residing in Human Papillomavirus (HPV)-infected Intraepithelial Neoplastic Lesions

Satoko Kojima, Kei Kawana, Kensuke Tomio, Aki Yamashita, Seisuke Sayama, Takeshi Nagamatsu, Tomoyuki Fujii. University of Tokyo, Tokyo, Japan

Human papillomavirus (HPV) infected to cervical mucosal epithelium. Most HPV infections resolve spontaneously, but the remainders persist and may progress to cervical cancer in some women. Since precursor lesion of cervical cancer, cervical intraepithelial neoplasia (CIN), develops in the mucosal epithelium, mucosal CD3+ T cell, especially intraepithelial lymphocyte (IEL), should be a direct effector in the host anti-HPV adaptive immunity for regression of CIN lesions. We characterized population of cervical T cells collected from cervical mucosa of HPV-positive CIN patients. About 25 % of cervical T cells were integrin β7 and CCR9 positive, indicating that gut-derived mucosal T cells are homing to cervical mucosa. Integrin β7+ cells we collected here expressed αE but not α4 subunit, meaning these cells were IELs but not lamina propria lymphocytes (LPLs). Interestingly, the population of integrin αEβ7+ T cells in CIN regression cases was significantly higher than that of non-regression cases (p=0.001). About half of the integrin αEβ7+ T cells were CD45RA-negative memory T cells. Taken together, CIN may regress spontaneously through anti-HPV adaptive immune response when gut-derived memory effector IEL retain abundantly in the epithelial lesions. This study first demonstrated association of cervical IEL with HPV-infected CIN course.

W.161. Bladder Conditioning upon Repeated BCG Instillations is Required for a Sustained Local Th1-immune Response

Claire Biot[1], Cyrill Rentsch[2], Joel Gsponer[2], Hélène Saklani[1], Fabrice Lemaitre[1], Charlotte Auriau[1], Caroline Demangel[1], Lucie Peduto[1], Matthew Albert[1]. [1]Institut Pasteur, Paris, France; [2]University of Basel, Basel, Switzerland

Intravesical BCG therapy as a treatment for bladder cancer is one of the most effective immunotherapies, with response rates ranging from 50-70%. Success relies on repeated instillations of live BCG, shortly after tumor resection, and correlates with a strong Th1-immune response. Using an experimental mouse model, we show that repeated instillations of live BCG into a naïve animal are required for T cell infiltration in the bladder, yet a single instillation is sufficient to achieve BCG dissemination to draining lymph nodes and T cell priming. Both live and heat-killed bacteria trigger acute inflammation of the bladder, but only repeated live BCG achieves sustained activation of bladder dendritic cells, which correlates with recruitment of CXCR3+ T cells (consistent with findings of IP-10 production in bladder CD11c+ cells). Interestingly, T cell recruitment is independent of TLR activation or IFNα/β signaling, but requires IL1R signaling - current investigations are assessing upstream sensors and if NLR activation accounts for the requirement for live bacilli. Finally, we show that prior systemic vaccination does not influence local recruitment of T cells, which supports our interpretation that multiple instillations of BCG influence “tissue conditioning” thus accounting for efficient trafficking of lymphocytes to the bladder mucosa.

W.162. Induction of Immunity in Human Genital Tract: Effect of Intranasal Immunization

Zina Moldoveanu, Gordon Bates, Wen-Qiang Huang, Jiri Mestecky. University of Alabama at Birmingham, Birmingham, AL

To validate in humans the effectiveness of intranasal immunization in the induction of immune responses in the genital tract reported for experimental animals, female and male volunteers were immunized either intranasally with live-attenuated influenza viruses trivalent vaccine (FluMist) or, as control, intramuscularly with the correspondent inactivated vaccine. Levels of vaccine-specific antibodies were determined in blood and secretions collected before, and at 3-4 weeks post immunization. Total and vaccine-specific antibody-secreting cells (ASC) were evaluated at one week after immunization in the peripheral blood and in cells sorted according to the α4β1 homing receptor. All immunized volunteers displayed in the circulation influenza virus-specific ASC, the majority being present in the α4+β1+ cell population, particularly in intranasally immunized volunteers. IgG was the predominant isotype, however most individuals receiving FluMist had a higher proportion of IgA ASC. The increase in influenza-specific antibodies, detected in sera of all immunized volunteers, was lower in subjects receiving the FluMist. The two immunization protocols induced influenza virus-specific IgA and in IgG in cervico-vaginal lavages and in semen, at slightly higher levels in the intranasally vaccinated volunteers. These studies suggest that intranasal immunization can induce responses in human female and male genital tracts.

W.163. Molecular Markers Associated with Inflammation and Repair Precede Decreased Allograft and Isograft Function Following Rat Renal Transplantation

Lea Novak, John Thompson. University of Alabama at Birmingham, Birmingham, AL

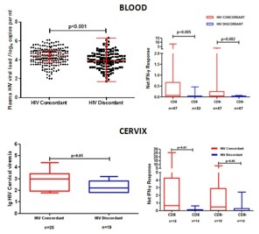
Molecular changes in the transitional epithelium (TE) and underlying submucosa (SM) of the renal pelvis and ureter of rat transplanted kidneys were correlated with renal function. Rat donor kidneys were transplanted orthotopically into syngeneic (n=11) or allogeneic (n=27) recipients. Grafts harvested post-transplant at 8, 12, 26, 32 weeks were examined for molecular markers associated with inflammation and repair: proliferation (PCNA), acidic fibroblast growth factor (FGF-1), nitric oxide synthases (iNOS, eNOS), nitrotyrosine (Y-NO2), and osteopontin (OPN). Prior to graft harvest, 24-hour urine was analyzed for albumin. Normal (n=6) and sham-operated animals (n=6) served as controls. Compared to controls, allografts demonstrated an early onset (8 weeks) of TE lesions and SM inflammation that preceded changes in isografts (12 weeks). Allografts

showed significantly more cellular proliferation at 8 weeks compared to isografts and controls. Compared to controls, immunostains for FGF-1, iNOS, eNOS, OPN, and Y-NO2 were increased in both transplant groups. The immonoappearance of these markers in allografts (8 weeks) preceded that observed in isografts (12 weeks). Urinary albumin levels were increasing after 16 weeks in both transplant groups, with higher levels in allografts. Both allografts and isografts exhibited post-transplant ischemic/reperfusion as well as immunological injuries that preceded decreased renal function.

W.164. Impact of Partner Human Immunodeficiency Virus Status on Human Immunodeficiency Virus Specific T Cell Responses in Blood and Cervix of Individuals in Concordant and Discordant Relationship

Shameem Jaumdally[1], Pamela Gumbi[1], Anna-Lise Williamson[1], David Coetzee[2], Jo-Ann Passmore[1]. [1]Institute of Infectious Disease & Molecular Medicine, University of Cape Town, Cape Town, South Africa; [2]University of Cape Town, Cape Town, South Africa

Mucosal immunity in the female genital tract is likely to be important in protection against heterosexually acquired HIV infection. Several studies have shown that biological and behavioural differences exist between infected individuals in HIV concordant and discordant relationships. Our study evaluated the impact of HIV status on HIV specific T cell responses in blood and cervix of individuals in HIV concordant and discordant relationship, and the effect of other biological factors on HIV-1 pathogenesis. We found that individuals in HIV concordant couples were significantly more likely than individuals in HIV discordant couples to have an HIV plasma load of above 10 000 copies/ml. Men in HIV concordant relationships were more likely to have concomitant HPV infection. We found that CD8+ and CD4+ T cell IFN-γ responses to Gag were detectable in blood and at the cervix of HIV infected individuals. HIV concordant participants showed a significantly higher net IFN-γ response than HIV discordant participants in both blood and the female genital compartment. Our results suggest that increased viral exposure in HIV infected participants leads to a higher degree of priming of HIV specific T cells in blood, and this translates to the genital tract.



W.165. The Effect of Delayed Processing on the Stability of Cervical Cytobrush-derived Mononuclear Cells from the Female Genital Tract

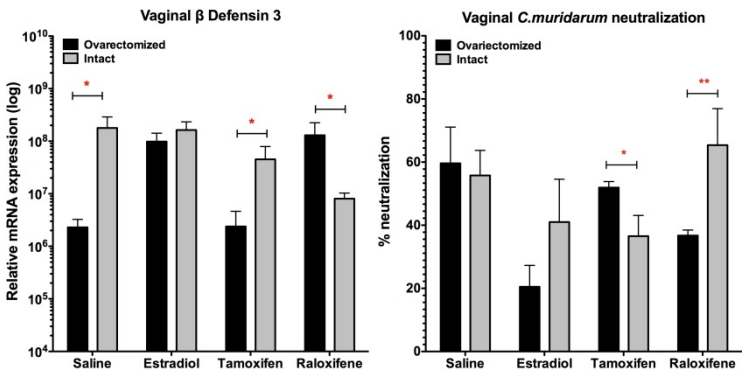
Lenine Liebenberg[1], Hoyam Gamieldien[1], Nonhlanhla Mkhize[1], Shameem Jaumdally[1], Pamela Gumbi[1], Lynette Denny[2], Jo-Ann Passmore[1]. [1]Institute of Infectious Disease & Molecular Medicine, University of Cape Town, Cape Town, South Africa; [2]University of Cape Town, Cape Town, South Africa

Sampling from the female genital tract yields few cells for immune studies. Therefore, standardisation of methods for collection, processing, and immune analysis of cells derived from this compartment is important. The aim of this study was to assess the effect of transport conditions on the viability, recovery and antigenic responsiveness of cervical T cells. Cervical cytobrushes were collected from 185 HIV+ women. Cytobrushes were either processed immediately, after cryopreservation, or after 24hrs at 37, 4 or ~22 degrees Celsius. T cell numbers were enumerated and viability was assessed. IFN-γ responses to PMA, PHA and CEF peptides were examined in cytobrush-derived T cells *ex vivo* and after delayed processing. Thawed cervical lymphocytes were expanded polyclonally *in vitro* before assay. T cell yield and viability was similar in cytobrushes processed immediately or after 24hrs at each condition. Half of the T cell yield was recovered after cryopreservation and 50% of this expanded. IFN-γ production following mitogenic stimulation was similar *ex vivo* and after 24hrs. Maintaining cytobrushes at 37°C prior to processing improved CEF-specific T cell detection compared to *ex vivo*. Cervical cytobrush-derived T cells are robust and can preserve their viability, phenotype and function over 24 hours of mock transport.

W.166. Estradiol, Tamoxifen & Raloxifene Treatment of Intact & Ovariectomized Mice Differently Alters Female Reproductive Tract (FRT) Antimicrobial Expression & Protection Against Chlamydia Muridarum

Danica Hickey, Charles Wira. Dartmouth Medical School, Lebanon, NH

This study investigated the ability of estradiol & the selective estrogen receptor modulators (SERMs), tamoxifen & raloxifene to mediate innate immunity in the FRT. Differences in vaginal β-defensins 1-4 expression of intact cycling & ovariectomized mice were characterized & we determined the capacity of vaginal secretions to neutralize Chlamydia infectivity. Female BALB/c intact or ovariectomized mice were treated with saline, 0.5μg 17β-estradiol, 10μg tamoxifen or 10μg raloxifene (3 daily subcutaneous injections). Message RNA was isolated from whole vaginal tissue & the expression of β-defensins 1-4 was determined by real-time PCR. Vaginal washes were collected for



Chlamydia muridarum neutralization *in vitro*. No significant differences in both the Chlamydia neutralizing capacity & β-defensin expression were observed between intact & ovariectomized mice treated with 17β-estradiol. However, vaginal washes collected from cycling animals treated with tamoxifen had a greater ability to neutralize Chlamydia in culture, neutralization correlated with β-defensin 3 mRNA. In other studies, raloxifene increased β-defensin 2 & 3 in ovariectomized but not cycling animals and enhanced neutralization of vaginal lavages in ovariectomized mice. Tamoxifen & raloxifene bind to estrogen receptors and are used clinically to treat breast cancer & osteoporosis. Our studies demonstrate that SERMs regulate the levels of innate antimicrobial peptides in the FRT & suggest that women on selected SERMs may have enhanced protection a gainst sexually acquired infections. This work was supported by NIH grant AI-013541. Figure: Vaginal mouse β-defensin 3 (left) mRNA expression and corresponding *in vitro* Chlamydia muridarum neutralization (right) observed by Estradiol or SERM treated intact cycling and ovariectomized mice.

W.167. The Isolation of Viable T Cells from Vaginal Mucosa

Elizabeth McGee, Pam Gigliotti, Hendrikus van Dessel. Virginia Commonwealth University, Richmond, VA

In order to determine the populations of immune cells in vaginal mucosa in normal and disease states, we developed a technique to isolate T cells from intact vaginal mucosa biopsies. Vaginal mucosal was carefully dissected from young adult female mice, post mortum. Tissue was dissected free of fat and connective tissue under a dissecting microscope and rinsed with sterile PBS then sterile DMEM/F12 medium. Mucosa was cut with sterile scissors into 1mm pieces and ground with glass homogenizers. The resulting product was pressed through a #10 stainless mesh and filtered through graded meshes from 450 to 25 microns. A variety of enzymes including collagenase and trypsin were tested in this protocol but did not add to cell recovery but did increase cell mortality. Isolated cells are suspended in DMEM/F12 supplemented with 5% FBS. For Fc receptor blocking, the suspended cells are immersed in mouse FcγII/III (Mouse BD FcBlockTM) for 10 minutes on ice to reduce non-specific staining. In shielded light, the cells are incubated at 4 degrees for 30 min with mouse APC labeled anti CD4-antibody or negative control. Cells were washed with PBS, resuspended with FACS buffer and samples run on a Beckman Coulter Elite XL-MCL using standard techniques and analysis software. After gating for P1; 239,832 events were noted. For Q1 gating 692 APC positive cells were counted reflecting 0.3 % of cells identified as T cells. This is compared to a total of 5 cells that were in Q1 for the cells incubated with negative control antibody. Thus demonstrating that a reasonable population of living immune cells can be isolated from a mucosal dispersate from the vagina for additional studies. Further studies are also under way to further charaterize vaginal mucosal T cells and to identify other populations of immune cells present in vaginal mucosa.

W.168. Zonulin as the Key Determinant for Gut Epithelial Barrier Permeability

Dina Damlund[1], Stine Metzdorff[1], Matilde Kristensen[2], Hanne Frøkiær[1]. [1]University of Copenhagen, Frederiksberg, Denmark; [2]Technical University of Denmark, Søborg, Denmark

The integrity and permeability of the intestine epithelium are regulated by tight junctions. Multiple tight junction integral proteins are involved and identified in controlling permeability of the epithelial cell layer, including Zona occludens 1-3 and Zonulin. Increased intestinal permeability is associated with Type 1 diabetes and these patient's relatives (Sapone et al., 2006). In a newly commenced study, where we aim at characterizing the intestinal response to gluten and microorganism, Caco2 cells are used as an *in vivo* model to mimic the epithelial cells in the gut. Caco2 cells are cocultured with dendritic cells in order to investigate the mechanisms involved in gut permeability and immune response (Zeuthen, Fink, & Frokiaer, 2008). In this study different bacteria toxins, LPS and gliadin are added to Caco2 cells and the influence on the expression of tight junction genes is analyzed. Probiotics are suggested to play a role in the prevention of increased intestinal permeability (Montalto et al., 2004). To investigate the role of probiotics in the expression of tight junction genes, different strains of lactic acid bacteria and bifidobacteria are tested alone or in combination with gliadin for the effect on the permeability of the Caco2 monolayer.

W.169. Determinants of Antigen-specific Effector CD8 T Cell Mediated Killing of Keratinocytes

Purnima Bhat, Michelle Yong, Veronica Martin, Ian Frazer. University of Queensland Diamantina Institute, Woolloongabba, NSW, Australia

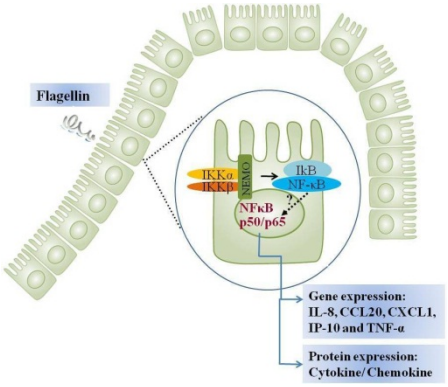
Keratinocytes are often the first cells in contact with the external environment of the host, in skin, or the mucous membranes of the mouth or reproductive tract. As such, their specific role in maintaining the immune regulation of the host is unique, striking a balance between tolerance and inflammation. We investigated the basic mechanisms that CD8 effector T cells utilize in inducing apoptosis in these cells when they express non-self antigen. We cultured primary keratinocytes expressing ova together with effector CD8 T cells and imaged these using time-lapse fluorescent and confocal microscopy. Movies over 30 hours long showed that keratinocytes, but not ova-expressing fibroblasts or T cells, apoptosed after prolonged, sustained contact (> 10 hours) with effector T cells, and this was abrogated by inhibition of caspase-3. The amount of antigen, and CD44/CD62L expression by the effector T cells both directly increased killing. CD8 T cells require perforin to kill keratinocytes, but not interferon-γ, which, when added externally, was inhibitory on T cell activity. We are examining these conditions for keratinocytes expressing tumour antigens, such as the E7 protein of human papillomavirus, to determine whether the determinants of keratinocyte killing are altered by oncoprotein expression.

Poster Session: Thursday, July 7
Authors Present: 13:00-14:30

T.1. Dynamic Response of Intestinal Epithelial Cells to Purified PAMPS, Commensal and Pathogenic Bacteria using an Anaerobic Cell Culture System

Vanessa Ferraria[1], Annaig Lan[2], Denise Kelly[1]. [1]Rowett Institute of Nutrition and Health, Aberdeen, United Kingdom; [2]AgroParisTech, Paris, France

The healthy human gut is the organ with largest communities of commensal bacteria belonging to the Firmicutes, Bacteroidetes and Actinobacteria phyla and co-existing without inducing overt inflammation. Nevertheless, in inflammatory bowel disease (IBD), microbial diversity is altered and has been associated with the presence of invasive pathogenic bacteria, including the enteroinvasive Escherischia coli. The recognition of gut bacteria is mediated, in part, by toll-like receptors (TLRs) expressed on intestinal epithelial cells. Both commensal and pathogenic bacteria activate these receptors the downstream, nuclear factor kappa B (NF-κB) and mapkinases signalling pathways triggering distinct patterns of proinflammatory gene and protein expression. This study has revealed differential mechanisms of immune regulation by commensal and pathogenic bacteria which determine the net inflammatory responses and the expression of negative regulators of NF-κB. The significance of these findings in relation to immune homeostasis and inflammation will be presented.



T.2. ElrA Operon of Enterococcus Faecalis Contributes to Macrophage Evasion

Naima Cortes-Perez, Romain Dumoulin, Pascale Serror. INRA, Jouy en Josas, France

Enterococcus faecalis is a commensal bacterium of the human microbiota. Under normal conditions E. faecalis is non pathogenic, however it is also a major opportunistic bacteria responsible for nosocomial infections. Enterococcal leucine-rich protein A (ElrA) is the first protein encoded by the elrA operon (opelrA) which is induced *in vivo* conditions. We have previously characterized the opelrA and we have demonstrated that inactivation of elrA gene significantly reduces virulence in a mouse peritonitis model. In the present study, we determined the effect of opelrA in the interaction with either epithelial cells (Caco-2) or macrophages (Raw 264.7 cell line). To circumvent the lack of opelrA expression in the *in vitro* conditions, we constructed a genetically modified strain harboring a constitutive promoter upstream opelrA (opelrA+). Our results show that expression of opelrA has no effect on bacterial adhesion to CaCo-2 cells. On the other hand, the expression of opelrA gives to E. faecalis the ability to escape phagocytosis. Interestingly, expression of opelrA abolishes IL-6 and IL-10 production by macrophages. Altogether, these results suggest that opelrA is involved in E. faecalis immune evasion.

T.4. Stress- and Antibiotic-induced Dysbiosis of Gut Commensal Microbiota (GCM) Disrupts the Mucus Layer and Promots Bacterial Wall Adherence in Mice

Mònica Aguilera, Javier Estevez, Patri Vergara, Vicente Martinez. Universidad Autonoma de Barcelona, Bellaterra, Spain

We assessed how antibiotics and stress, individually or combined, affect GCM and bacterial wall adherence. C57BL/6 female mice, maintained in standard conditions, were treated orally with non-absorbable, broad spectrum antibiotics [bacitracin and neomycin (0.4 mg/mouse/day for each)]. Stress groups were subjected to psychological stress (water avoidance, 1 h/day) for 7 days. Animals were euthanized after last stress session. Colonic luminal and wall-adhered microbiota were characterized using FISH. At necropsy, enlargement of the cecum was observed in antibiotic-treated groups, with multifocal-to-diffuse inflammatory infiltrate and submucosal edema upon histopathological analysis. Stress diminished by 60 % the colonic mucus layer ($P<0.05$), while antibiotics had a minor effect (20% reduction). Antibiotics induced a specific perturbation of luminal GCM with a reduction in Clostridia and increases in Lactobacillus/Enterococcus and Enterobacteria, without changes in total bacterial counts. Antibiotics favoured Clostridium spp. and Enterobacteria adherence (incidence: 83% and 67%, respectively); however addition of stress restricted adherence to Clostridia (incidence: 83%). In vehicle-treated mice stress increased the counts and adherence (incidence: 67%) of Clostridia. No bacterial adherence was observed in vehicle-non-stressed animals. Antibiotic treatment and/or stress alter the intestinal mucous layer and induce a specific dysbiosis of the GCM. These changes favour bacterial wall adherence, disrupting gastrointestinal homeostasis.

T.6. Anti-inflammatory Properties of Bifidobacterium Spp. Strains Isolated from Healthy Infant Feces

Andrei Shkoporov, Ekaterina Khokhlova. Russian State Medical University, Moscow, Russian Federation

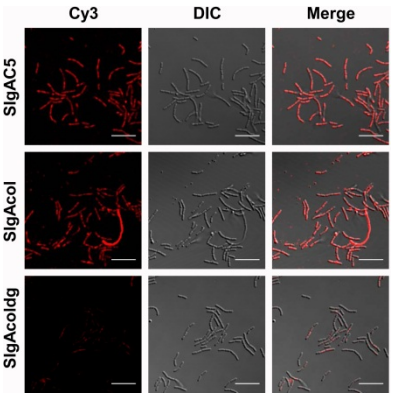
Several groups have reported recently, that certain probiotic Bifidobacterium strains are able to inhibit inflammatory response in intestinal epithelial cells *in vitro* and *in vivo*. However neither chemical nature nor precise mechanism of action of putative anti-inflammatory agents have been determined. In addition it is unclear whether such immunomodulating activity is a property of some specific Bifidobacterium strains or is common for the whole genus. Here we report on partial characterization of similar anti-inflammatory properties in several Bifidobacterium strains, isolated from healthy infant feces. Conditioned media (CM) of all tested strains was capable to attenuate, albeit to varying extent, TNFα- and LPS-induced

inflammatory response in HT-29 cell line. In contrast, neither killed bifidobacterial cells, nor cell extracts possess such activities. The active substance present in bifidobacterial CM was resistant to protease and nuclease treatment, heat-labile, non-lipophilic, and had a molecular weight of less than 3 kDa. The anti-inflammatory effect of bifidobacterial culture supernatants was dose- and time-dependent and was accompanied by inhibition of IκB phosphorylation and NF-κB-dependent promoter activation. Bifidobacterium CM didn't influence gene expression pattern in the absence of pro-inflammatory stimuli. However, combined treatment of cells with CM and LPS or TNFα resulted in upregulation of TGFβ1, IκBζ, and p21CIP mRNAs.

T.7. Impact of Diet in Fecal Diversity in Patients with Crohn's Disease

J. Antonio Quiros[1], Sumathi Sankaran[2], Thomas Prindiville[2], Sathya Dandekar[2]. [1]California Pacific Medical Center, San Francisco, CA; [2]University of California Davis, Davis, CA

Loss of microbial diversity has been reported as a feature of Crohn's disease. The objective of this study is to compare the effects of low residue diet(LRD) and the Specific Carbohydrate Diet(SCD) on the microbiota in Crohn's disease. Methods: This was a pilot randomized, single-blind crossover study. We evaluated fecal micobiome in 6 Crohn's patients, in remission, following LRD and SCD. Controls where collected from healthy people in same geographical area. The microbiome was assayed using DNA-microarray technology. Results: The overall abundance and diversity of bacterial families was lower in Crohn's as compared with controls. Bacteria belonging to the class Clostridia accounted for 50% of bacteria while those belonging to Bacteriodetes accounted for 21% of bacteria in Crohn's. LRD diet was associated with a shrinking microbiome with 11 bacteria belonging to 3 familes dissapearing. SCD increased diversity to include 376 bacteria belonging to 32 different classes. Discussion: In conclusion, diet changes in patients with Crohn's appear to result in dramatic changes in the large intestinal microbiome and will need to be investigated further. Small number of subjects was a limiting factor in this study. Also, multiple technical issues regarding stability of different standard extraction methods when using this technology was noted.



T.8. Activation of G-protein Coupled Receptor 43 by Short Chain Fatty Acids Regulate Inflammation on Acute Gout: Role of Diet and Commensal Microbiota

Angelica Vieira[2], Ellen De Leon[1], Doris Shim[1], Kendle Maslowski[1], Heidi Shilter[1], Flavio Amaral[2], Mauro Teixeira[2], Charles Mackay[3]. [1]Garvan Medical Research, Sydney, NSW, Australia; [2]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; [3]Monash University, Melborne, VIC, Australia

Short-chain fatty acids (SCFAs) are produced by bacterial fermentation of dietary fiber and bind to GPR43 modulating inflammatory responses. Gout is an inflammatory disease characterized by release of uric acid crystals into the joint cavity and neutrophil infiltration that leads to tissue damage. Our aims are understand the mechanism of SCFAs as well as, the role of GPR43 in gout. Treatment with SCFA protected mice from injury by intra-articular injection of Monossodium Uric Acid crystal showing reduction of pro-inflammatory cytokines and increasing anti-inflammatory mediators, such as, IL-10 and TGFbeta inducing neutrophils's apoptosis. Interestingly, we observed that GPR43ko mice presented reduction in inflammatory parameters after gout induction due deficiency on recruitment of cells, when compared to MSU-injected WT mice. Germ-free mice, which no express SCFAs, showed similar "hiporresponsive" and inhibited recruitment of cells. However if we treated GF with acetate before challenged with MSU, this "phenotype" was reversed. Thus, microbiota-induced SCFA and GPR43 activation are necessary for proper neutrophil recruitment. However, SCFA treatment during an established inflammatory response promoted resolution of the inflammation. We suggest that endogenous microbiota shapes the host's ability to respond to inflammatory stimuli. The presence of SCFAs provides a link between diet, gastrointestinal bacterial metabolism and inflammation.

T.9. Nonspecific Recognition of Intestinal Bacteria by Hybridoma-derived and Colostral Secretory IgA is Mediated by Carbohydrates

Amandine Mathias, Blaise Corthésy. Lausanne University Hospital, Lausanne, Switzerland

The molecular bases pertaining to the interaction between secretory IgA (SIgA) and bacteria residing in the intestine are not known. Previous studies have demonstrated that commensals are naturally coated by SIgA in the gut lumen. Thus, understanding how natural SIgA interacts with commensal bacteria can provide new clues on its multiple functions at mucosal surfaces. Using fluorescently labeled, non-specific SIgA or secretory component (SC), we visualized by confocal microscopy the interaction with various commensal bacteria including Lactobacillus, Bifidobacteria or Escherichia coli strains. These experiments revealed that the interaction between SIgA and commensal bacteria involves Fab and Fc-independent structural motifs, featuring SC as a crucial partner. Removal of glycans present on free SC or bound in SIgA resulted in a drastic drop in the interaction with Gram positive bacteria, indicating the essential role of carbohydrates in the process. The interaction with Gram-negative bacteria was preserved whatever the protein used, suggesting different mechanisms involved. Purified SIgA and SC from either mouse hybridoma cells or human colostrum exhibited identical patterns of recognition for Gram-positive bacteria, emphasizing conserved plasticity between species. Thus, nonspecific sugar-mediated binding of commensals by SIgA identifies a novel feature of the antibody in contributing to mucosal symbiosis.Colocalization (seen as red

dots) of Lactobacillus bacteria (visualized by differential interference contrast (DIC)) with nonspecific hybridoma-derived SIgAC5 and native or deglycosylated SIgA purified from human colostrum (respectively SIgAcol and SIgAcoldg). One representative field obtained from 10 different observations following analysis of 5 different slides is depicted. Scale bars: 10 μm.

T.10. Investigating the Mechanism by which Early-life Environment Predisposes to Inflammatory, Autoimmune and Allergic Disease

Marie Lewis[1], Charlotte Innan[2], Chelsea Hicks[1], Denise Kelly[3], Christopher Stokes[1], Mick Bailey[1]. [1]University of Bristol, Bristol, United Kingdom; [2]University of Birmingham, Birmingham, United Kingdom; [3]University of Aberdeen, Aberdeen, United Kingdom

There is a strong, but complex link between early-life environment, acquisition of the gut microbiota and development of immune disease in later life, although the exact mechanisms remain unknown. Recent genetic and microbiota analyses suggests that pigs share more similarities with humans than do mice, indicating that pigs are valuable intermediates between rodent models and human clinical trials. In our experimental model, piglet full-siblings can be reared from 24h old, either in low-hygiene farm environments suckling the mother, or in a high-hygiene SPF facility fed milk formula. We have used fluorescence immunohistology to determine the effects of these different environments on the developing mucosal immune system. Our results demonstrate early differences in recruitment of dendritic cell subsets followed later by differences in CD4+ T cells. Farm piglets developed fewer CD4+ T cells overall ($p=0.005$), and a higher proportion of these were FoxP3+ ($p=0.01$). Thus, the ratio of effector (Th1, Th2, Th17) to regulatory CD4+ T cells was higher in the isolator than the farm piglets. In humans, early exposure to farm environments has been correlated with protection against the later development of allergy and studies in piglets may lead to mechanistic understanding of how early-life environment predisposes to immune disease.

T.11. Early Life Treatment with Vancomycin Reduces Diabetes Incidence in NOD Mice

Camilla Hansen, Dennis Sandris Nielsen, Finn Kvist Vogensen, Axel Kornerup Hansen. University of Copenhagen, Frederiksberg, Denmark

Type 1 diabetes (T1D) results from an uncontrolled T cell mediated destruction of the insulin-producing beta-cells in the pancreas. Causal factors include a combination of genetics, early life incidents and the food we eat. The involved adaptive immune response can be down regulated by a regulatory immune response and a fine-tuned balance between these immunological components is crucial for characteristics of the disease, such as severity, onset time and recovery. The balance between the regulatory and the adaptive immune response is heavily influenced by early life bacterial stimulation. An interplay that is likely to represent a critical environmental component to diabetes induction. In a period after birth alterations of the early microbial colonization of the gut therefore can be expected to have an immense impact on diabetes progression later in life. In this study neonate NOD mice were treated with the antibiotic vancomycin in four weeks from birth. Diabetes incidence and onset time were compared with a control group and we found that neonate vancomycin treatment attenuates T1D. By changing the gut flora composition in the beginning of life we also demonstrated a disruption of the mechanisms regulating intestinal immune homeostasis toward a proinflammatory mucosal environment.

T.12. Specificity of Intestinal Adaptive B Cell Responses to the Microbiota

Emelyne Lécuyer[2], Sabine Rakotobe[2], Hélène Lengliné[1], Nadine Cerf-Bensussan[1], Valérie Gaboriau-Routhiau[2]. [1]INSERM, Paris, France; [2]INRA, Jouy-en-Josas, France

Our recent work using gnotobiotic mice showed that Segmented Filamentous Bacterium (SFB) induced strong and coordinated intestinal T and IgA responses while other members of the microbiota, such as the commensal Escherichia coli MG1655 strain could stimulate intestinal IgA in the absence of detectable T cell responses (Immunity 2009, 31(4):677). In order to define whether Immunoglobulin (Ig) and notably IgA responses elicited upon mono-colonisation by these two strains differ by their kinetics, specificity and site(s) of induction, antibody-secreting cells were analyzed by ELISPOT in lamina propria, mesenteric lymph nodes, Peyer's patches and spleens of mono-associated mice. Both E. coli and SFB stimulated strong total IgA responses in lamina propria. Unexpectedly, the IgA response to E. coli showed a significantly higher specificity than that to SFB (~25% vs ~4% in lamina propria, respectively). Studies in progress aim to determine whether the sites of induction of Ig responses differ between the two species and to identify the bacterial impact on peripheral Ig responses.

T.13. IgA and IgG Antibodies Against Bifidobacteria Spp. in One-year Old Children with Differences in their Prognosis for Pancreatic Beta-cell Autoimmunity

Raivo Uibo[1], Ija Talja[1], Epp Sepp[1], Marika Mikelsaar[1], Riitta Veijola[2], Mikael Knip[3], Olli Simell[1], Jorma Ilonen[1], Meeme Utt[1]. [1]University of Tartu, Tartu, Estonia; [2]University of Oulu, Oulu, Finland; [3]University of Helsinki, Helsinki, Finland

Exposure to microorganisms in early childhood is a significant modulating factor in immune system maturation and might be involved in immune-mediated disease development later in life. Although the impact of external microbes has been confirmed in type 1 diabetes (T1D) development the role of commensal microflora is still open. In the present study we analyzed the occurrence of serum IgA and IgG antibodies against proteins of three Bifidobacterium strains lysates by immunoblot assay in two groups of 12-month old children having different propensity for T1D development as assessed by the presence of two or more types of pancreatic islet cell autoantibodies later in their life. Our results showed serum antibodies reactivity up to 44 antigenic proteins in three strains. Among tested variabilities IgA antibodies against B. adolescentis DSM 20083 proteins were more frequently detected in children who later developed islet cell autoantibodies compared to children who did not develope islet cell autoimmunity

(P=0.003). The results suggest that the B. adolescentis strain DSM 20083 or microorganism(s) with similar antigenic structure might be involved in the modulation of the intestinal immune milieu prone for development of pancreatic beta-cell autoimmunity.

T.15. Immune Imprinting by Commensal Microbiota on the Generation of Immunity versus Tolerance Towards HBV

Miao-Tzu Huang, Bor-Luen Chiang, Ding-Shinn Chen, Pei-Jer Chen. National Taiwan University Hospital, Taipei, Taiwan

Unlike HBV infection of the adult, ninety percent of the infection in neonates and childhood leads to chronic HBV persistence. The fact that liver is constantly exposed to antigens derived from food and intestinal microorganisms suggest a role of the microbiota in modulating the balance between immune tolerance and immunity towards HBV in the liver. By using hydrodynamic injection of replication-competent HBV DNA, we established a mouse model of *in vivo* HBV transfection in which HBV persistence was generated in young C57BL/6 mice of 4-6 w/o, but not in mice older than 10 w/o under specific pathogen-free (SPF) condition. In contrast, adult germ-free mice cleared HBV much slower than their SPF counterparts. Instead, they cleared HBV as inefficient as the young. In livers of SPF C57BL/6 mice, IFN-beta expression was significantly induced in the young mice after HBV transfection but not in the adult. Under germ-free condition, however, adult mice restored IFN-beta production in response to HBV. These data suggest that IFN-beta executes active suppression towards generation of HBV immunity and the microbiota negatively regulate IFN-beta secretion of the adult mice in response to HBV. Our preliminary data support that commensal microbiota promote effective liver immunity towards HBV through mechanisms involving IFN-beta.

T.16. Gut Microbiota Drive the Immune System Abnormalities Present in Nod2-Deficient Mice

Tomas Hrncir[1], Lucia Hrncirova[1], Helena Tlaskalova-Hogenova[1], Koichi Kobayashi[2]. [1]Academy of Sciences of the Czech Republic, Novy Hradek, Czech Republic; [2]Dana-Farber Cancer Institute, Boston, MA

Mutations in Nod2 gene have been associated with Crohn's disease, Blau syndrome and graft-versus-host disease. However, the mechanisms by which these mutations predispose to the development of inflammatory pathology remain unclear. We show that gut microbiota play an essential role in the development of immune system abnormalities seen in Nod2-deficient mice. To assess the effect of gut microbiota on the immune system in Nod2-deficient mice we transferred mice into germ-free conditions and analyzed lymphocyte subpopulations and cytokine profiles of cells isolated from spleen, mesenteric lymph nodes, terminal ileum and colon. We show that immune system deviations found in Nod2-deficient mice bred under specific-pathogen free conditions do not develop under germ-free conditions. In other words, in the absence of gut microbiota Nod2-deficient mice have almost identical immunophenotype as wild-type mice. Thus, we conclude that the immune system alterations seen in Nod2-deficient mice are driven by gut microbiota.

T.17. IL-1 Receptor-associated Kinase-M-deficient Mice Show Hyperactivated Phenotype with Increased Production of Pro-inflammatory and Anti-inflammatory Cytokines

Lucia Hrncirova[1], Tomas Hrncir[1], Helena Tlaskalova-Hogenova[1], Koichi Kobayashi[2]. [1]Academy of Sciences of the Czech Republic, Novy Hradek, Czech Republic; [2]Dana-Farber Cancer Institute, Boston, MA

Abnormal recognition of enteric bacterial products in inflammatory bowel disease appears to depend at least in some Crohn's disease patients on genetic mutations of specific bacterial sensing gene products. IL-1R-associated kinase (IRAK)-M is a cytoplasmic protein that blocks toll-like receptor action in macrophages and possibly other cell types by stabilizing the signaling complex and thus downregulates and limits excessive TLR signaling leading to tissue damage and autoimmune disease. We compared cytokine production and leukocyte subsets distribution in spleen, mesenteric lymph nodes, Peyer's patches and colonic lamina propria in C57BL/6 and IRAK-M knockout mice bred in specific pathogen free condition. We have found an increased numbers of CD4+Foxp3+ and CD4+IFNg+ T cells and increased levels of IL-1, IL-5, IL-6, IL-13, IFNγ and IL-10 cytokines in Peyer's patches, mesenteric lymph nodes and spleen of IRAK-M-deficient mice. We conclude that IRAK-M-deficient mice have an increased activation of immune responses in spleen and colon.

T.18. Regulatory T Cells in the Helicobacter Pylori-infected Human Gastric Mucosa are Primarily Helios+ Natural Tregs

Khiyam Hussain, Darren Letley, John Atherton, Karen Robinson. University of Nottingham, Nottingham, United Kingdom

H. pylori (Hp), the leading cause of peptic ulcer disease (PUD), stimulates a strong regulatory T cell (Treg) response. Recent studies demonstrated that 70% of natural Tregs, which recognise self-antigens, express the transcription factor Helios. In contrast, induced Tregs are Helios-. We hypothesised that Hp stimulates an induced Treg response, lowering the risk of PUD, and we aimed to characterise gastric mucosal Tregs in patients. Gastric antral biopsy Tregs from 43 Hp+ and 28 Hp- patients, were isolated, stained for CD4, CD25, FOXP3, IL-10, CTLA-4 and Helios, and analysed by flow cytometry. Hp+ biopsies contained increased levels of CD4+CD25hiFOXP3+ (p=0.03), CD4+CD25hiIL10+ (p=0.001) and CD4+CD25hiCTLA-4+ (p=0.014) cells compared with the Hp- biopsies. Increased frequencies of Helios+ (p=0.002) and Helios- (p=0.007) Tregs were observed in the Hp+ gastric mucosa; however, the majority (74%) were FOXP3+Helios+ natural Tregs. Amongst Hp+ patients, those with PUD had lower levels of FOXP3+ (p=0.026) and IL10+ (p=0.02) Tregs. IL-10+ Tregs in the Hp-infected gastric mucosa could be exerting bystander suppression on PUD. The majority of Hp associated Tregs were FOXP3+Helios+ and this was unexpected. Helios+ natural Tregs may arise because

Hp expresses antigens of high homology with human proteins, and could explain protective associations between Hp and autoimmunity.

T.19. **Crucial Involvement of β7 In**tegrin in the Regulation of DSS-induced Colitis

Angela Schippers[1], Moritz Muschaweck[1], Thomas Clahsen[1], Amelie Dralle[1], Nikolaus Gassler[1], Werner Müller[2], Norbert Wagner[1]. [1]RWTH Aachen Unviversity, Aachen, Germany; [2]Manchester University, Manchester, United Kingdom

The lymphocyte adhesion molecule β7 integrin directs the migration of leukocytes into the gut associated lymphoid tissues (GALT) mainly via interaction with its endothelial ligand MAdCAM-1. By analyzing β7-/- and β7-/-Rag2-/- double deficient mice in the DSS induced colitis model we wanted to get more insight into the impact of these molecules for inflammatory processes of the gut. β7-/- mice exhibited attenuation in acute and chronic DSS-induced colitis signs as exemplified by decreased body weight loss and shortening of the colon, reduced tissue destruction and attenuated inflammatory cell infiltration in comparison to wildtype mice. Moreover the migration of CD11b F4/80 Ly6C+ inflammatory macrophages into the lamina propria of β7-/- mice was decreased. To investigate the role of β7 integrin for the migration of myeloid cells we induced DSS colitis in Rag2-/- and β7-/-Rag2-/- double deficient mice. Interestingly severity of colitis expression was much less in β7-/-Rag2-/- mice compared to Rag2-/- mice accompanied by decreased migration of inflammatory macrophages. This finding indicates a role for β7 integrin on innate immune cells i.e. dendritic cells, granulocytes and most probably inflammatory macrophages during the progression of DSS colitis.

T.20. The Muc1 Mucin Regulates Helicobacter Pylori-induced Gastritis via Interactions with the Inflammasome

Philip Sutton[1], Garrett Ng[1], Alison Every[1], Michael McGuckin[2]. [1]University of Melbourne, Melbourne, VIC, Australia; [2]Mater Medical Research Institute, Brisbane, QLD, Australia

We previously showed that the Muc1 mucin limits the severity of Helicobacter pylori associated gastritis in mice. Polymorphisms in this mucin are associated with gastric cancer in humans. Aim: To identify the mechanism by which Muc1 regulates H. pylori-induced inflammation in the gastric mucosa and thereby protects against cancer progression. Results: Muc1 is expressed by mucosal epithelial and immune cells. Using bone marrow chimaeras, we showed that it is Muc1 expression by haematopoietic cells, and not epithelial cells that regulates Helicobacter-induced gastritis severity. This increased severity of inflammation in the gastric mucosa of Muc1-/- mice was associated with increased levels of IL-1β, but not other inflammatory cytokines. H. pylori stimulation of macrophages and dendritic cells induced IL-1β secretion that was inhibited by the NLRP3 inflammasome inhibitor, glyburide. Muc1-/- macrophages secreted increased levels of IL-1β when stimulated with cytosolic MDP (NLRP3 inflammasome activator) but not cytosolic DNA (AIM2 inflammasome activator), compared to wild-type control macrophages. Conclusions: Muc1 expression by haematopoietic-derived immune cells regulates Helicobacter-induced gastritis. We show for the first time that H. pylori activates and Muc1 negatively regulates the NLRP3 inflammasome. This provides an immune mechanism by which Muc1 can protect against the pathological consequences of H. pylori infection.

T.21. Persistence of Gastric Mucosal Th17 Cells in Humans with Evidence of Past Helicobacter Pylori Infection is Associated with Elevated Levels of Gastric IL-1beta

Victoria Serelli-Lee[1], Lai Han Yeong[1], Cassandra Ho[1], Gek Keow Lim[2], Khoon Lin Ling[2], Justin Wong[1]. [1]National University of Singapore, Singapore; [2]Singapore General Hospital, Singapore

Chronic gastritis induced by Helicobacter pylori (HP) plays a significant role in gastric cancer pathogenesis. Despite bacterial eradication, gastric inflammation and cancer risk persists in patients with previous HP infection. To characterize this persistent gastric lymphocytic infiltrate, we compared T cell responses in patients who had past HP infection (P), with responses in actively infected patients (A), and patients naïve to HP (N). In both peripheral blood and gastric biopsy samples, we observed elevated IL-17A expression and increased numbers of Th17 cells in group P and group A patients compared to group N. Using whole HP lysate, we detected HP-specific gastric mucosal Th17 cells in individuals from groups P and A. We performed gene expression profiling and found elevated levels of IL-1β mRNA in gastric samples from group P patients; however, IL-23, IL-6 and TGF-β levels were similar to those found in group N. We also demonstrated the presence of mature IL-1β protein in gastric samples from group P and A patients but not from group N. In conclusion, we have found an association between elevated levels of gastric IL-1β and the persistence of HP-specific gastric mucosal Th17 cells in patients with a previous history of HP infection.

T.22. IRAK-M Expression Mediated by H. Pylori Antigens May Induce Regulatory Activity in Dendritic Cells

Jessica Shiu, Thomas Blanchard. University of Maryland School of Medicine, Baltimore, MD

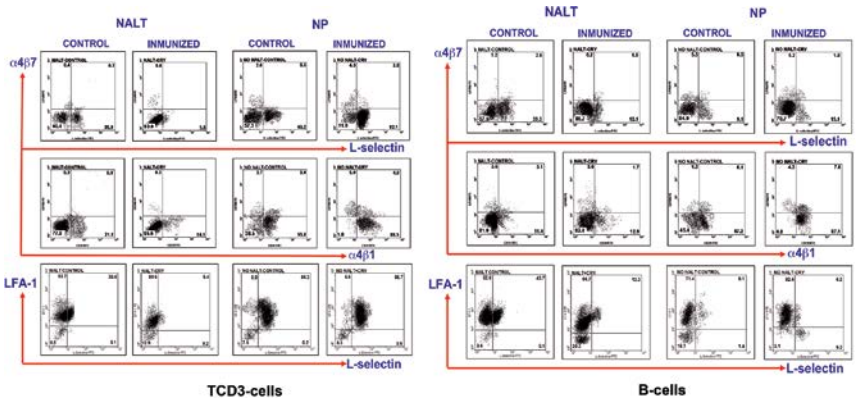
Background: Helicobacter pylori (Hp) infection of the stomach lasts for life if left untreated. Recent studies suggest that Hp induces regulatory dendritic cells (DCs) and Treg cells but the mechanisms of induction remain unknown. Aim: To determine molecular pathways involved in Hp-mediated immunoregulation. Methods: Bone marrow-derived DC were stimulated with Hp or E. coli (Ec) antigens and evaluated for gene expression and the ability to induce Treg cells in co-culture assays. Results: Hp antigen, but not Ec antigen consistently induced T cell FoxP3 expression (3.3 - 5.5%). Whereas Ec antigen changed the expression of over 2000 genes in DCs, only 10 genes were affected by Hp antigen including IκBζ, Vanin3 and IRAK-M. IRAK-M induction by Hp and Ec antigens was confirmed by RT-PCR at multiple time points with the greatest differential observed at 24 hours ($P < 0.003$). IRAK-M expression was also compared between wild type, TLR2-/- and TLR4-/- DCs following activation. A marked reduction in

IRAK-M expression was observed in both knockout models. Conclusions: Hp antigens induce changes in DC that favor Treg induction. The antigens may act through DC TLR2 and/or TLR4 signaling pathways to induce IRAK-M which may contribute to the tolerogenic phenotype observed in Hp infections.

T.23. Differential and Temporal **Immunomodulation of α4** Integrin Receptors on Memory T Cells by Bordetella Pertussis and Bordetella Parapertussis Infection in Mice

Tzvia Abramson[1], Tuan Andrew Nguyen[1], Dipti Ravindra[1], Sana Waheed[1], Brian Kwong[1], LinhPhuong Nguyen[2], Eugene Butcher[2]. [1]San Jose State University, San Jose, CA; [2]Stanford University School of Medicine, Stanford, CA

Despite widespread vaccination, whooping cough disease caused by Bordetella pertussis (Bp) has made a fierce comeback across the world. Mechanisms of selective trafficking of lymphocytes to respiratory tissues are likely to be critical in the immune response to airborne pathogens. We compare mouse infection with pertussis toxin (PTX)-producing Bp and Bordetella parapertussis (Bpp), a pathogen that lacks PTX, to study the trafficking receptors (TR) required for homing to the airways as well as the imprinting of TR programs by dendritic cells (DC). Hematoxylin and Eosin (H&E) lung sections reflect impaired leukocyte recruitment at 5 days post infection (p.i.) with Bp but not with Bpp, which is restored at day 25 p.i. Integrin receptors α4β1, α4β7 on blood effector/memory T lymphocytes (CD4+, CD44++, and CD45RBlow) are highly up-regulated in mice infected with Bpp 5 days p.i. While their comparative levels in Bp infection are significantly reduced at day 5 p.i, yet restored at day 25 p.i., indicating delayed TR imprinting. No such differences are observed in the lung-memory T cells. Lung DCs from both Bp and Bpp infected mice express high levels of maturation markers 5 days p.i. While those drop to uninfected levels in Bpp mice at 25 days p.i., they strongly persist in the Bp model, even when bacteria are no longer recovered from the lungs. Finally, 4 days of co-culturing isolated lung DC with allogeneic isolated T cells (1:5) suggest a compromised imprinting of α4β1, α4β7 by Bp-lung DC but not by the respective Bpp or Bp-PTX mutant lung DC. We suggest that α4 integrins (in combination with β7 and β1) may play an important role in memory T cell migration during infection with Bp and that this outcome may be addressed in the design of new vaccines.



T.24. A Double Mutant Heat Labile Toxin From E. Coli as an Adjuvant in a Vaccine Against Helicobacter Pylori Infection

Louise Sjökvist Ottsjö[1], Carl-Fredrik Flach[1], John Clements[2], Jan Holmgren[1], Sukanya Raghavan[1]. [1]Institute of Microbiology and Immunology, Gothenburg, Sweden; [2]Tulane University Health Science Center, New Orleans, LA

The prevalence of H. pylori infections is high in developing countries and so far no vaccines are available to contain the spread of the infection and its associated disease. The aim of this study was to evaluate the efficacy of a mucosal non toxic adjuvant, double mutant (dm) heat-labile toxin (LT) produced by Enterotoxigenic E. coli bacteria in inducing immune responses and protection against H. pylori infection. Mice were sublingually (SL) immunized with H. pylori lysate antigens together with dmLT followed by intragastric challenge with live H. pylori bacteria. SL immunization with lysate antigens together with dmLT as an adjuvant resulted in significant ($p<0.01$) protection against H. pylori infection with a corresponding increase in serum antibody titers to H. pylori antigens. Enhanced *in vitro* proliferation to H. pylori antigens of spleen and mesenteric lymph nodes (MLN) cells was associated with an increase in secretion of both IL-17 and IFNγ in SL immunized mice. RT-PCR analysis showed an up-regulation of IFNγ, IL-17, TNF, IL-12p40 gene expression in the stomach of immunized protected mice, compared to the unimmunized infection controls. Our results suggest that the mechanism of adjuvant function of dmLT is via expansion of Th1 and Th17 cells to H. pylori antigens.

T.25. Expression of Homing Integrins of B and T Cells from Superficial Lamina Propria of Proximal and Distal Small Gut

Ivonne Maciel Arciniega-Martinez[1], Rafael Campos-Rodriguez[1], Judith Pacheco-Yepez[2], Luvia Sánchez-Torres[3], Gabriela Alicia Hernández-Echaurregui[1], Aldo Arturo Resendiz-Albor[1]. [1]Escuela Superior de Medicina-Instituto Politécnico Nacional, México, Mexico; [2]Universidad La Salle, Mexico, Mexico; [3]Escuela Nacional de Ciencias Biologicas-Instituto Politécnico Nacional, Mexico, Mexico

Recently there was an interintestinal compartment of specialized B cells, described as the superficial lamina propria (SLP). Nevertheless, few reports exist on the phenotype and function of this compartment. In the current study an examination was made of the differences in expression of five adhesion molecules on B and T cells from the SLP of proximal and distal small gut. The adhesion molecules evaluated were the homing integrins α4β7, αEβ7 and α4β1 as well CCR9 and L-selectin, and their expression was analyzed on three populations B220+/CD19+ , CD4+ T cells and CD8+ T cells, all by flow cytometry. From the SLP of proximal and distal small gut, differences were detected in the expression of: CCR9, α4β1 and L-selectin on Lc B, αEβ7, α4β7 and α4β1 on TCD4+, and integrins αEβ7, α4β7, α4β1 and L-selectin on Lc TCD8+. Additionally, in Peyers patches

differences were found in the expression of: L-selectin on LcB, CCR9, α4β7, and α4β1 on T CD4+, and CCR9, αEβ7, α4β7 and L-selectin on T CD8+. These results show striking differences in the cell surface phenotypes of B and T cells of the SLP between proximal and distal small intestine from normal Balb/c mice. This work was supported by SIP and COFAA IPN.

T.26. Inhibition of Colitis by IL-25 Associates with Induction of Alternatively Activated Macrophages

Angelamaria Rizzo, Ivan Monteleone, Daniele Fina, Carmine Stolfi, Roberta Caruso, Massimo Fantini, Eleonora Franzè, Francesco Pallone, Giovanni Monteleone. University Tor Vergata, Rome, Italy

Interleukin (IL)-25, a Th2-related factor, inhibits macrophage-mediated inflammatory responses in the gut, but the mechanism underlying the counter-regulatory effect of IL-25 remains unknown. Since Th2-cytokines abrogate inflammation by inducing alternatively activated macrophages (AAMs), we evaluated whether AAMs are involved in the IL-25-mediated anti-colitic effect. *in vivo* in mice, IL-25 administration enhanced the expression of AAM gens in F4/80+ cells infiltrating the peritoneum and colon of wild-type and colitic mice. To prove that IL-25-induced AAMs exert anti-inflammatory effects in the gut, peritoneal F4/80+ cells isolated from IL-25-treated mice were injected to recipient mice with TNBS-colitis. Such a cell transfer reduced the severity of TNBS-colitis. Since IL-25 did not directly promote AAM differentiation *in vitro* and, *in vivo* in mice, IL-25 administration enhanced the synthesis of IL-4, IL-13 and TGF-β1, which are known to favour AAM polarization, we assessed whether such cytokines are involved in the IL-25-driven AAM induction. Blockade of IL-4, IL-13 and TGF-β1 with neutralising antibodies in mice did not inhibit the stimulatory effect of IL-25 on AAM gene expression. Our data indicate that the IL-25-mediated anti-colitic effect is associated with induction of AAMs.

T.27. Characterization of Homing Receptors Profile and Adhesion Molecules in NALT and NP After Inmnunization with pCry1Ac

Marco Aurelio Rodriguez-Monroy[1], Lucio Rivera-Santiago[3], Oscar de Jesus Nieto-Yañez[3], Maria Concepción Peña-Juarez[3], Maria Margarita Canales-Martinez[2], Saul Rojas-Hernandez[3], Arturo Contis-Montes de Oca[3], Xochitl Olivares Lopez[3], Maricela Carrasco-Yepez[3], Ricardo Ortíz-Ortega[2], Leticia Moreno-Fierros[2]. [1]Universidad Nacional Autonoma de Mexico, Tlalnepantla, Mexico; [2]Unam Fes Iztacala, Tlalnepantla, Mexico; [3]ESM-IPN, Mexico, Mexico

The nasal mucosal is the first site of contact with inhaled antigens. However, the nature of local immune responses and the role of homing receptors and adhesión molecules of NALT in those responses have not fully studied. In this study we characterized the expression of integrins and adhesion molecules in different cells of both NALT and nasal-passages (NP) after intranasal immunization with 50 micrograms of pCry1Ac. The percentage of different cell populations and the detection of homing receptors on their surface molecule expression activation of NALT and NP lymphocytes was performed by flow-cytometry while detecting the expression of molecules the NALT HEV adhesion was performed by immunohistochemical procedures. pCry1Ac modifies the expression of homing receptors on T and B lymphocytes in the NALT and NP, and may cause changes in the pattern of expression of adhesion molecules in NALT. Homing receptors showed that major changes were L-selectin, alfa4beta1, LFA-1. Adresinas profile expressed in the NALT is considerably different PP. pCry1Ac modifies the expression of adhesion molecules in NALT significantly reducing the network of PNAd and increasing the expression of ICAM-1 and VCAM-1 on HEV.

T.28. Multicolor Flow Cytometry of Peripheral Blood Lymphocytes in Pediatric Patients with Inflammatory Bowel Disease

Tzvia Abramson[1], Nicole Tarlton[1], Caroline Green[1], Oren Abramson[2], Anthony Wong[2], Nicole Lazarus[3], Lusijah Rott[4], Chulie Ulloa[3], Eugene Butcher[3]. [1]San Jose State University, San Jose, CA; [2]Kaiser Permanente, San Jose, CA; [3]Stanford University, Stanford, CA; [4]Veteran's Affairs Palo Alto Health Care System, Palo Alto, CA

While the etiology and mechanism of Inflammatory Bowel Disease (IBD) is not fully understood, immunologic abnormalities play a role in these diseases. Monitoring of disease progression and response to therapeutics is typically invasive and lacking. Increased recruitment of lymphocytes to the gut has been demonstrated in IBD, leading to inflammation and ultimately to gut tissue damage. Lymphocyte circulation is tightly controlled by trafficking receptors (TRs) that act in combination to govern migration to the target organ. Plasmablasts (PBs) are immature circulating B cells that may reflect tissue localized plasma cells. We hypothesize that the PB blood levels and trafficking patterns can inform on the location of tissue damage and the clinical condition of the patient. Using multicolor flow cytometry we examined PB (IgA+/CD38high) and memory B cell (IgA+/CD19+) blood levels, and TRs expressed on these cells. We show that an increase in IgA+ PB blood levels reflect an aggravated clinical state, and a significantly greater amount of these cells in Ulcerative colitis patients express colon associated TRs (CCR10+) as opposed to small intestine associated TRs (CCR9+). Additionally, these trafficking patterns are more evident on IgA+ PBs rather than memory B cells. This work in progress suggests that the analyses of the TR phenotype of circulating immature and mature B lymphocytes may provide valuable information for non-invasive monitoring of disease activity and progression.

T.29. Immunization with Cry1Ac Increase Differentially the Expression of Homing Receptors in Small and Large Intestine

Aldo Arturo Resendiz-Albor[1], Leticia Moreno-Fierros[2]. [1]Escuela Superior de Medicina-Instituto Politécnico Nacional, México, Mexico; [2]UBIMED-FES Iztacala, México, Mexico

Cry1Ac protoxin from Bacillus thuringiensis is attractive for use in vaccination due to its innocuity, low production cost, stability, and mucosal immunogenicity and adjuvanticity. To further characterize its immunological effects we evaluated if intraperitoneal immunization of mice with Cry1Ac

affected the intestinal intraepithelial and lamina propria lymphocyte populations of the large and the small intestine. We found striking differences between of the large and small intestine on the phenotype of lamina propria lymphocytes. In the large intestine (LI) most lymphocytes were B cells, and T cells were predominantly TCR-αβ+ CD8+. In contrast in the small intestine (SI) most lymphocytes were T cells CD4+, and most T cells expressed TCR-αβ but a higher percent expressed TCR-γδ. Slight differences were found between control and immunized mice respect to the proportion of T and B cells. We observed that the expression of homing receptors α4β7, α4β1,αEβ7 and L-selectin on T cells was also different between the small and large intestine. Interestingly intraperitoneal immunization of mice with protoxin Cry1Ac increases the percent of intraepithelial and lamina propria T lymphocytes expressing α4β7 in the small intestine and αEβ7 in the large intestine. The differential increase on expression of α4β7 and αEβ7 observed on intestinal T lymphocytes from immunized mice suggest that different addressin-homing receptor interactions may exist among the small and large intestine. This work was supported by SIP and COFAA IPN.

T.30. Alterations in Dendritic and T Cell Homing Profiles in Crohn's Disease Cutaneous Wounds

Najib Daulatzai[1], Rebecca Slater[2], David Bernardo[1], Hafid Al-Hassi[1], Simon Peake[1], Goher Rahbour[1], Cheng Tee[1], Johnathan Landy[1], Ailsa Hart[1], Robin Phillips[2], Elizabeth Mann[1], Stella Knight[1]. [1]Imperial College London, London, United Kingdom; [2]Northwest London Hospitals NHS Trust, London, United Kingdom

Background: Crohn's disease (CD) patients have increased predisposition to wound failure following surgery. Dendritic cells (DC) initiate T cell responses and play a central role in CD pathogenesis. We hypothesised that changes in immune cells within CD wounds contribute to wound failure. Hence, we aimed to determine the differences in homing profile of DC and T cells from CD and control (non-CD) wounds. Methods: Peripheral blood mononuclear cells (PBMC) were obtained by centrifugation over Ficoll-gradient. Wound tissue cells were obtained by an overnight cell "walkout" assay. Expression of gut-homing marker (β7) and skin-homing marker (CLA) was determined on DC and T cells by flow cytometry. Results: A lower proportion of DC and T cells from CD wounds expressed CLA and β7 compared with control wounds. However, there was no difference in leucocyte homing profiles of blood DC/T cells. A greater proportion of T cells in CD wounds were of memory phenotype compared with control wounds. Discussion: Reduced expression of gut- and skin-homing molecules on local wound tissue DC and T cells in CD, reported here for the first time, is likely to reflect changed functions contributing to aberrant wound healing. Higher proportions of memory T cells suggest alterations in effector function within CD wounds.

T.31. Kinetic Analysis of the Development of Effector- and Memory-type CD4$^+$ T Cells in the Intestinal Mucosa

Ryoki Kobayashi[1], Shinichi Sekine[1], Keiko Fujihashi[1], Rebekah Gilbert[1], Yoshiko Fukuyama[1], Masafumi Yamamoto[2], Kohtaro Fujihashi[1]. [1]University of Alabama at Birmingham, Birmingham, AL; [2]Nihon University School of Dentistry at Matsudo, Matsudo, Japan

CD4$^+$ memory T cells (T_M) play key roles in the induction of antigen-specific protective immunity against infectious diseases; however, the development of mucosal CD4$^+$ T_M cells still remains to be elucidated. C57BL/6 mice were orally immunized for 3 consecutive weeks with OVA plus native cholera toxin (CT) as mucosal adjuvant. Sixteen, 28 and 42 days after the initial immunization, mononuclear cells from Peyer's patches (PPs), mesenteric lymph nodes (MLNs), intestinal lamina propria (iLP) and spleen were stained for FACS analysis in order to determine effector and memory phenotypes. PPs, MLNs and spleen contained increased numbers of CD62L^{Low} CD44$^+$ CD4$^+$ T cells at days 16 and 28. In contrast, iLP revealed increasing CD62L^{High} CD44$^+$ CD4$^+$ T cells from day 16 through to day 42. Interestingly, IL-7 receptor α (IL-7Rα) expression by CD62L^{Low} (PPs, MLNs, and spleen) and CD62L^{High} (iLP) populations were significantly down-regulated at day 16 but return to normal or higher level by day 28. These results indicate that CD4$^+$ effector T (T_E) cells are developed by day 16 and begin to differentiate into CD4$^+$ T_M cells over the next 12 days. We are currently testing mucosal homing receptor expression by CD62L^{Low} and CD62L^{High} CD4$^+$ T cells in these tissues.

T.32. TLR2 Protects Against Commensal-**dependent Pancolitis Exacerbation in MDR1α Deficiency**

Birgit Ey[1], Joachim Göthert[1], Guido Gerken[1], Daniel Podolsky[2], Elke Cario[1]. [1]University Duisburg-Essen, Essen, Germany; [2]UT Southwestern Medical Center, Dallas, TX

Mice deficient in MDR1α develop spontaneous chronic colitis, but the underlying innate immune mechanisms have not been delineated yet. The aim of our study was to define the functional role of TLR2 in the pathogenesis of colitis associated with MDR1α deficiency. We have generated TLR2-/-MDR1α-/- [FVB/N;F8] mice (controls: TLR2+/+MDR1α-/-) and colitis activity was examined in age-matched male mice (n>10 per group; SPF: helicobacter-/MNV-free). We found that deletion of TLR2 significantly worsened colonic inflammation in MDR1α-/- mice. In the absence of TLR2, fulminant pancolitis developed early with many CD11b+ monocytes in bone marrow and peripheral blood of MDR1α-/- mice. Abundant CD11b+ monocytes and CD4+ T cells infiltrated the colonic lamina propria of TLR2-/-MDR1α-/-, but not TLR2+/+MDR1α-/-, already at 5 weeks of age. Accelerated recruitment was paralleled by a rapid shift towards Th1-immune responses (IL-12p40, IL-1β, IFNγ), while Th2 (IL-4) and TH17 (IL-17, IL-21, IL-27) were not induced. Broad-spectrum antibiotic treatment ameliorated colitis in TLR2-/-MDR1α-/- mice, implying that commensal bacteria play an important role in triggering monocyte/Th1-dependent disease. We conclude that the presence of TLR2 prevents pancolitis exacerbation in the context of MDR1α deficiency. Ulcerative Colitis patients with combined genetic defects in TLR2 and MDR1A may exhibit a more severe disease phenotype.

T.33. IL-**1β Promotes Susceptibility of TLR5KO Mice to Colitis**

Frederic Carvalho, Matam Vijay-Kumar, Jesse Aitken, Andrew Gewirtz. Emory University, Atlanta, GA

Background: The extent to which numerous strains of genetically engineered mice, including mice lacking toll-like receptor 5 (T5KO), display colitis that is environment-dependent with the gut microbiota underlying much of the variance in phenotype. Accordingly, embryonic rederivation of T5KO mice ameliorated their spontaneous colitis despite only partially correcting elevations in pro-inflammatory gene expression. We postulated that absence of overt inflammation in these mice required activation of endogenous anti-inflammatory pathways. Consequently, we hypothesized that neutralization of the anti-inflammatory cytokine IL-10 might induce uniform colitis in T5KO mice and thus provide a practical means to study mechanisms underlying their inflammation. Methods: Two distinct strains of non-colitic T5KO mice, mice lacking MyD88, TLR4 and IL-1R as well as various double-KO were treated weekly for 4 weeks with 1 mg/mouse of IL-10 receptor neutralizing antibody (IL-10R mAb) and colitis assayed 1 week after the final injection. Results: Anti-IL-10R mAb treatment led to severe uniform intestinal inflammation in both strains of T5KO mice. Such neutralization of IL-10 signaling did not cause colitis in WT littermate mice nor mice lacking TLR4, Myd88 or IL-1R. The susceptibility of T5KO mice to this colitis model was not rescued by absence of TLR4 in that TLR4/T5-DKO mice displayed severe colitis in response to anti-IL-10R mAb treatment. Finally, we observed that ablation of IL-1β signaling was crucial for this colitis model as IL-1R/TLR5-DKO were completely protected from colitis in response to IL-10R mAb treatment.Conclusion: Regardless of whether they harbor a “colitiogenic microbiota,” loss of T5 predisposes mice to colitis triggered by immune dysregulation via an IL-1β-dependant pathway.

T.34. The Circadian Gene Period 2 Regulates DSS-induced Colitis in Mice

Atsuhito Nakao, Kyoko Oh-oka. University of Yamanashi, Chuo/Yamanashi, Japan

Circadian rhythms are daily oscillations of multiple biological processes with a roughly 24-hour cycle such as the sleep-wake cycle, blood pressure, and behavioral patterns. Previous studies reveal that the circadian rhythms are driven by a set of clock genes including Period2 (Per2) that form transcriptional feedback loops inside the cells. In mammals, the suprachasmatric nucleus in the brain is a master pacemaker regulating the circadian rhythms while peripheral organs including the intestine also have a set of clock genes showing circadian oscillation. Recent evidence suggests that the circadian clock system is also involved in the regulation of many pathological conditions including inflammation. This study investigated whether the circadian clock system affects inflammatory bowel disease (IBD)-related experimental colitis. We compared the development of dextran sodium sulfate (DSS)-induced colitis between wild-type mice and mice with a “loss of function” mutation of Per2 (mPer2m/m). mPer2m/m mice were more resistant to DSS-induced colitis than wild-type mice. Bone marrow chimera experiments showed that the Per2 mutation in non-hematopoietic cells protected mice against DSS-induced colitis. These results suggest that Per2 in non-hematopoietic cells plays a critical role in the suppression of IBD-like colitis. How Per2 in non-hematopoietic cells mediates protection against DSS-induced colitis are under investigations.

T.35. Luminal CD4$^+$ T Cells Penetrate Gut Epithelial Monolayers and Egress from Lamina Propria to Blood Circulation

Yasuhiro Nemoto[1], Takanori Kanai[2], Tamako Shinohara[1], Tetsuya Nakamura[1], Ryuichi Okamoto[1], Kiichiro Tsuchiya[1], Mamoru Watanabe[1]. [1]Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan; [2]Keio University, Tokyo, Japan

Although accumulated evidence has revealed how effector/memory T cells migrate to peripheral tissues, there are still many enigmas about how they egress from peripheral tissues to blood circulation. Recent reports proved that the egress of memory T cells from peripheral tissues such as lung and skin into the draining lymph nodes requires the expression of CCR7 on these cells. In the intestine, however, the unique phenotype of the resident memory T cells in the lamina propria (LP), which lack CCR7 suggests that they are tissue-bound and don't exit the intestine as if it is ‘graveyard’ of lymphocytes. To challenge this dogma, we developed novel and unique cell transfer system using intearectal administration of lymphocyte to mice. SCID mice administered intrarectally with splenic CD4$^+$ T cells obtained from normal mice developed colitis with their expansion not only in the LP, but also in mesenteric lymph node (MLN), peripheral blood and spleen. Intrarectally-administered GFP$^+$CD4$^+$ T cells resided in the LP, but were not found in MLN and spleen 6h after administration. They egressed to MLN until 24h after and finally reached spleen until 168h after. Immunohistochemical and electron microscopic analysis revealed that CD4$^+$ T cells were detected in intraepithelial space just 3h after intrarectal administration. CCR7 deficiency did not impair egress of CD4$^+$ T cells from LP to systemic circulation. We here demonstrates for the first time that CD4$^+$ T cells can not only penetrate from the intestinal luminal side to the LP, but also actively egress from the LP to the blood circulation. This method may be a useful tool to investigate cell trafficking in intestinal mucosa, and it suggest a new concept of cell therapy for intestinal diseases by enema administration.

T.37. Cyclosporine Ameliorates Apoptosis-mediated Epithelial Damage via Transforming Growth Factor-**β Related Pathway**

Hirotake Sakuraba, Yoh Ishiguro, Satoh Yuki, Hiroto Hiraga, Shogo Kawaguchi, Keisuke Hasui, Shinsaku Fukuda, Akio Nakane. Hirosaki University School of Medicine, Hirosaki, Japan

Introduction: Cyclosporine showed a rapid improvement in the treatment of ulcerative colitis, but the precise mechanism is still obscure. We hypothesized that cyclosporine would affect the TGF-β expression of intestinal mucosa and TGF-β-related signaling of epithelial cells. Methods: Colitis was induced by feeding of 4% DSS in C57BL/6, C.B17 SCID, and CD4+CD25+cells-transferred SCID mice. DSS colitis was induced in vehicle- or cyclosporine-treated mice with or without anti-TGF-βmAb. The body weight change, histological damage score and epithelial apoptosis

was assessed. Colonic TGF-β-level was evaluated using ELISA. Expression of cFLIP, and caspase-activities in purified IECs were analyzed by western blot and colorimetric protease assay. Result: Treatment of cyclosporine ameliorated mucosal destruction through reduction of epithelial apoptosis. Cyclosporine up-regulated the TGF-β in the colon and smad2 phosphorylation in IECs. Increase of cFLIP expression and decrease of caspase-8 activity were observed in IECs from cyclosporine-treated mice. TGF- β expression was significantly suppressed and treatment of cyclosporine failed to up-regulate the expression of TGF-β in SCID mice. On the other hand, treatment of cyclosporine up-regulated TGF-β expression in CD4+CD25+cells- transferred SCID mice. Conclusion: These results demonstrate that treatment of cyclosporine can ameliorate epithelial damage through TGF-β-related pathway and CD4+CD25+T cells would mediate the protective effect of cyclosporine.

T.38. Acute Enteritis Alters Toll-like Receptors (TLR) Expression, Gut Commensal Microbiota (GCM) and Bacterial Wall Adherence in Rats

Mònica Aguilera, Evangelina Terán-Ventura, Patri Vergara, Vicente Martinez. Universidad Autonoma de Barcelona, Bellaterra, Spain

We assessed spontaneous changes of GCM, bacterial wall adherence and expression of TLR 2 and 4 during acute enteritis in rats. Enteritis was induced in SD rats by systemic indomethacin (7.5 mg/kg, sc, 2 doses/48 h). Animals were euthanized at day 4, corresponding to the acute phase of inflammation, as assessed through disease activity parameters. Ileal and cecal luminal and wall-adhered microbiota were characterized using FISH. Expression of TRL-2/4 was assessed by RT-PCR. Disease activity parameters were increased in indomethacin-treated animals, indicating active enteritis. Cecal expression of TLR-2/4 increased by 3-fold during inflammation. Similar changes were observed in the ileum. During inflammation, Bacteroides spp., Enterobacteria and Clostridium cluster XIV were increased in the ileum. In control conditions, only Lactobacillus/Enterococcus were found attached to the ileal mucosa. During inflammation, adherence of enterobacteria (incidence: 86%), clostridia (incidence: 83%) and bifidobacteria (incidence: 67%) was observed. Cecal microbiota showed similar qualitative changes. Acute inflammation implies qualitative and quantitative changes in both luminal and wall-adhered microbiota as well as in the expression of microbiota-recognition systems (TRL-2 and 4). This suggests that alterations of the GCM and its interaction with the host might be important pathogenic factors in the development and maintenance of intestinal inflammation.

T.39. HSP70 has an Anti-inflammatory Activity via MIF Suppression in Human and Murine Colitis

Keisuke Hasui, Yoh Ishiguro, Hitoshi Fujita, Hirotake Sakuraba, Shinsaku Fukuda. Hirosaki University Graduate School of Medicine, Hirosaki, Japan

Heat shock proteins (HSPs) are categorized into HSP90, HSP70, HSP60, HSP20, and HSP8.5 on the basis of their molecular weight and highly conserved molecules present in both prokaryotic and eukaryotic cells. HSPs play an important role in cellular function and stress conditions and HSP70 proteins constitute the central part of the chaperone system. We reported that MIF had an important role in the glucocorticoids (GC) resistant inflammatory response in ulcerative colitis (UC), and that HSP70 might be a negative regulator of MIF activity. In this study, we assessed the effects of Geranylgeranylacetone (GGA) on IL-10-/- transfer colitis model and the results showed that GGA treatment ameliorate the colitis with MIF and IL-17, TNF-α suppression. To elucidate the roles of HSP70 in inflammatory response in human UC, colonic samples were obtained from GC responsive cases, GC refractory cases, and controls, and qPCR, western blot and organ culture were performed. HSP70 was significantly reduced at mRNA levels and protein levels in refractory cases rather than responsive cases with UC and controls. In organ culture system, TNF-α production by mucosal specimens from refractory cases was significantly reduced by rHSP70. In addition, concentration of MIF in supernatant was also down-regulated by rHSP70. These results suggest that HSP70 might have an anti-inflammatory activity via MIF suppression in colitis.

T.40. Jagged1 is Upregulated in Human Colonic Tissue of Inflammatory Bowel Diseases in Active Phase, and its Downregulation is Not Good for Mucosal Healing

Hirotsugu Imaeda, Akira Andoh, Shigaki Bamba, Osamu Inatomi, Tomoyuki Tsujikawa, Yoshihide Fujiyama. Shiga University of Medical Science, Otsu, Japan

Background: In healing mucosa of ulcerative colitis (UC), decreasing of goblet cells and increasing ectopic Paneth cells are observed. Expression of Notch ligands in intestine is unknown. Methods: 2%(wt/wt) dextran sulfate sodium (DSS) in drinking water administrated C57BL/6J mice were observed with colon. Separated epithelium with subepithelial tissue by EDTA, relative expressions of Jagged1 (JAG1), Delta-like protein 1 (DLL1) and Delta-4 mRNA were observed by real-time PCR. 2% DSS colitis model mice were intraperitoneally injected with 1μg/body IL-33 in every other day, and observed disease activity index (DAI), colon length, colon weight and investigated expression of JAG1, DLL1, Delta-4, Math-1 mRNA. Isolated mouse colonic subepithelial myofibroblasts (SEMFs) were incubated with IL-1β, TNF-α and IL-33, and investigated expression of JAG1 mRNA. Biopsies on H/C (n=6), Crohn's disease (CD) (n=19) and UC (n=40) were investigated expression of JAG1 and DLL1 mRNA. Results: JAG1, DLL1 and Delta-4 were significantly upregulated in DSS induced colitis mice, and JAG1 expression increased in epithelium more than subepithelial tissue. JAG1 and Math-1 expression were downregulated in IL-33 injected mice. Conclusions: JAG1 could activate Notch signals in intestinal epithelial cells and would be one of the targets to treat mucosal healing of IBD patients.

T.41. Influence of Escherichia Coli Nissle 1917 on the Development of Acute Intestinal Inflammation-induced Dextran Sulfate Sodium

Tomas Hudcovic, Renata Stepankova, Hana Kozakova, Tomas Hrncir, Martin Schwarzer, Dagmar Srutkova, Helena Tlaskalova-Hogenova. Institute of Microbiology ASCR, Novy Hradek, Czech Republic

The participation of E. coli strains, in the development of the acute intestinal inflammation (model of ulcerative colitis) was studied in gnotobiotic mice. Experimental colitis was evoked by an administration of 2.5 % dextran sulfate sodium (DSS) in drinking water (7 days). Germ-free mice monoassociated by E. coli Nissle 1917 were associated by E. coli O6K13 after weaning and than DSS was administrated. Colon morphology and mucin production were evaluated. The level of cytokine was determined in supernatant of cultivated intestinal pieces of colon descendens. Mice monoassociated with E. coli O6K13 developed intestinal inflammation in colon whereas colonization with E. coli Nissle 1917 strain protected mice against inflammation. In this group, the level of pro-inflammatory cytokine TNF-alpha and IL-6 was reduced markedly in colon compared with controls. Mice monoassociated with E. coli Nissle 1917 strain and reassociated by E. coli O6K13 strain developed intestinal inflammation in colon in 33% of experimental mice only. We conclude that E. coli Nissle 1917 colonization protects mice against intestinal inflammation induced by DSS treatment. Supported by grants 303/08/0367 of the Czech Science Foundation and grants 2B06053 and 2B06155 of the Ministry of Education, Youth and Sports of the Czech Republic.

T.42. The Recurrent TNBS-induced Colitis Model: A Time-course Study of Acute and Chronic Disease Development

Bas Kremer[1], Rob Mariman[2], Sabine Mourits[1], Marjan van Erk[3], Lex Nagelkerken[1]. [1]TNO, Leiden, Netherlands; [2]Leiden University Medical Center, Leiden, Netherlands; [3]TNO, Zeist, Netherlands

The 28-day recurrent TNBS-induced colitis model enables evaluation of anti-inflammatory or immunomodulatory effects of prolonged administration of pharmaceuticals and nutrition. Demonstrator studies using corticosteroid treatment and probiotic treatment have shown protective effects of these interventions against deleterious effects of TNBS challenges. This study presents an in-depth characterization of the effects of the TNBS challenges in time. Each TNBS challenge induced an acute response characterized by body weight loss, shortened colons and severely damaged mucosal tissue. These acute responses were associated with a significant upregulation of expression of a large panel of genes encoding acute phase proteins, such as TNF-α, haptoglobin, SAA and calgranulin. At end-point, colitis was associated with significantly increased colon weight, and inflammatory cell infiltrates of mucosa and submucosa, consisting of T cells, antigen presenting cells and mast cells. The developing inflammatory response coincided with increased gene expression of mast cell-associated proteins, such as mast cell proteases, and Paneth cell-associated peptides, such as α-defensins. Systemically, colitis development was reflected by a balanced Th1/2/17 cytokine profile. In summary, this recurrent TNBS colitis model combining acute and chronic phases of inflammation is a suitable model for mechanistic and preclinical efficacy studies with pharmaceuticals and nutrition.

T.43. Liver Tolerance is Maintained by Tolerogenic Immature CCR9^{+} pDCs and their Breakdown is Caused by Activated Macrophages in IBD Model Mice

Yohei Mikami, Takanori Kanai, Tomohisa Sujino, Shinta Mizuno, Atsushi Hayashi, Yuichi Ono, Tomomitsu Doi, Katsuyoshi Matsuoka, Tadakazu Hisamatsu, Hirotoshi Ebinuma, Nobuhiro Nakamoto, Toshifumi Hibi. Keio University, Tokyo, Japan

Patients with inflammatory bowel disease often present liver disorders. Consistently, microbe-associated molecular patterns (MAMPs) are uptaken in the gut lumen and transported continuously to the liver via portal-venous blood. This may imply mucosal immune system plays important roles in the induction of liver dysfunction, but it is still unclear how immune dysregulation in the liver is caused during the development of colitis. We here show that liver of normal mice contains especially greater amount of CD11b^{-}CD11c^{+}PDCA-1^{+}CCR9^{+} immature plasmacytoid dendritic cells (pDCs) than other organs, and these pDCs support the development of Foxp3^{+} regulatory T cells from naïve T cells. Interestingly, CCR9^{+} pDCs are decreased and activated CD11b^{+}F4/80^{+}CD80^{high} macrophages are conversely increased in colitic CD4^{+}CD45RB^{high} T cell-adoptively-transferred RAG-2$^{-/-}$ mice mice (CD45RB^{high}RAG-2$^{-/-}$ mice) and IL-10$^{-/-}$ mice. To exclude the effect of infiltrating CD4^{+} in the liver, we adoptively re-transferred colonic lamina propria CD4^{+} T cells from colitic CD45RB^{high}RAG-2$^{-/-}$ mice into new RAG-2$^{-/-}$ mice. In the liver of colitic re-transferred mice, only a few CD4^{+} T cells detected, CCR9^{+} pDCs were decreased, and activated macrophages were increased like original CD45RB^{high}RAG-2$^{-/-}$ mice. All the data suggest that breakdown of gut barrier in colitic conditions induce the circulations of APCs or bacterial antigens/MAMPs, which induce colitis-associated liver dysfunction.

T.44. Analyzing the Role of Mucosal Mast Cells Inducing Colitis-associated Colorectal Cancer (CRC)

Benno Weigmann[1], Michael Stassen[2], Christian Taube[2], Markus Neurath[1]. [1]University Clinic, Erlangen, Germany; [2]Johannes Gutenberg University, Mainz, Germany

Colorectal cancer is one of the most malignancies. However, the molecular pathogenesis of colorectal cancer is poorly understood. In order to investigate the functional role of mast cells, which play a more prominent role in immunological processes, we used a previously established murine colon carcinoma model (DSS/Azoxymethan) with mast cell deficient mice. Accordingly, mice were treated with AOM followed by three consecutive cycles of orally administrated dextran sulfate sodium (DSS) over a period of 7 days. To monitor tumorigenesis in mice *in vivo*, we used our mini-endoscopic system. By using this system together with methylene blue staining, we were able to detect aberrant crypt foci in DSS plus AOM-treated wild-type mice at early time point before macroscopically visible lesions were seen. First visible lesions associated with inflammation appeared in wildtype mice around day 45, which were followed by the development of more and growing tumors until day 90. In contrast, mast cell deficient mice are protected against tumor development and although they showed colitis-similar symptoms. The possibility, that mast cells play a tumorpromoting role in the development of colon tumors led us to perform a screen¬ing of the expression of involved cytokines in co¬lons and tumors of treated mice

vs untreated mice. Even in long term study, a marginal increase of the tumorprevalence concerning mast cell deficient mice could be observed. Our data contribute extensively the understanding of mast cells in colitis-associated colon cancer and encourage of rethinking the role of mast cells in colitis-associated colorectal cancer.

T.45. Role of the Metallothionein in the Murine Experimental Colitis

Toshifumi Tsuji[1], Yuji Naito[1], Tomohisa Takagi[1], Kazuhiko Uchiyama[1], Hiroyuki Yoriki[1], Munehiro Kugai[1], Ryusuke Horie[1], Katsura Mizushima[1], Yasuko Hirai[1], Kazuhiro Katada[1], Takeshi Ishikawa[1], Osamu Handa[1], Nobuaki Yagi[1], Satoshi Kokura[1], Hiroshi Ichikawa[1], Junko Suzuki[2], Rie Yanagisawa[2], Hirohisa Takano[2], Masahiko Satoh[3], Toshikazu Yoshikawa[1]. [1]Kyoto Prefectural University of Medicine, Kyoto, Japan; [2]National Institute for Environmental Studies, Tsukuba, Japan; [3]Aichi Gakuin University, Aichi, Japan

Background and Aims: Metallothionein (MT) has been shown to suppress inflammatory disorders through mechanisms not yet defined. The aim of this study was to investigate the role of MT in the intestinal inflammation. Materials and Methods: Male MT-/- mice and C57BL/6 mice were used. To induce colitis, the mice were fed with 2% Dextran sulfate sodium (DSS). A disease activity index (DAI) was determined on every two days. Histology, length of colon, myeloperoxidase (MPO) activity and mRNA expression of inflammatory cytokines and transcriptional factors were evaluated. MT expression was determined using immunohistochemical examination. The colonic expression of MT mRNA was determined in normal colon and inflamed mucosa from patients with ulcerative colitis. Results: DAI of MT-/- mice were significantly higher. MT-/- mice showed remarkable body weight loss and shortening of colon. The MPO levels were increased in MT-/- mice. Inflammatory cytokine levels were also significantly higher. Real time PCR analysis confirmed the up-regulated expression of MT in colonic mucosa in patients with ulcerative colitis. Conclusion: Our results demonstrated that the lack of MT aggravated colonic mucosal damage and enhanced inflammation in DSS colitis model, indicating that endogenous MT may play an important role for the protection of intestinal mucosa.

T.46. Gradual Disappearance of Intestinal CD103+ Dendritic Cells in Intestinal Mucosa of CCR9-/- Mice in an Experimental Chronic DSS-mediated Colitis

Marc-Andre Wurbel[1], Maria McIntire[2], Michael Pardo[1], Edda Fiebiger[1]. [1]Children's Hospital Boston and Harvard Medical School, Boston, MA; [2]Brigham and Women's Hospital and Harvard Medical School, Boston, MA

Background: CCL25/CCR9 is a chemokine/receptor pair controlling gut-specific leukocyte migration. We have previously demonstrated that CCL25/CCR9 interactions regulate murine colonic inflammation in acute colitis mediated by Dextran Sulfate Sodium (DSS) administration. We investigated here whether such interactions also play a role in chronic colitis. Methods: Chronic inflammation in wild-type (WT) and CCR9-/- mice was induced by one or several DSS cycles, each consisting of 7-day 2% DSS followed by a 10-day water oral administration. IBD scores were assessed by histological analysis. Distribution and phenotypic characterization of dendritic cell (DC) subsets were assessed by flow cytometry. Results: Our data indicate that one single DSS cycle can induce chronic inflammation in CCR9-/- mice. More profound chronic inflammation can be established in CCR9-/- mice after 2 DSS cycles. This is accompanied by an altered DC distribution with a progressive disappearance of colonic CD103+ DCs and an altered colonic plasmacytoid DC immunophenotype. Conclusions: Our results demonstrate that CCL25/CCR9 interactions regulate inflammatory immune responses in the large intestinal mucosa by balancing different DC subsets. These findings have important implications for the use of CCR9-inhibitors in therapy of human IBD as they indicate a potential risk for patients with large intestinal inflammation.

T.47. Oral Treatment with Lysate of Probiotic Lactobacillus Casei DN-114 001 Ameliorates Experimental Colitis in Mice

Zuzana Zakostelska[1], Miloslav Kverka[1], Klara Klimesova[1], Pavel Rossmann[1], Jakub Mrazek[2], Jan Kopecny[2], Tomas Hudcovic[1], Helena Tlaskalova-Hogenova[1]. [1]Institute of Microbiology ASCR, Prague, Czech Republic; [2]Institute of Animal Physiology and Genetics ASCR, Prague, Czech Republic

Microbiota composition plays an important role in pathogenesis of inflammatory bowel diseases. The aim of this study was to investigate if lysate of probiotic bacterium L. casei DN-114 001 (Lc) and its fractions could influence the development of acute dextran sodium sulfate (DSS) colitis. BALB/c or severe combined immunodeficient mice (SCID) mice received lysate orally or by parenteral administration, in four weekly doses. Seven days after last dose, the acute colitis was induced by 3% DSS dissolved in drinking water for one week. Numbers of CD4+CD25+FoxP3+ regulatory T cells (Tregs) were measured by FACS analysis and cytokine production in various parts of the gut was estimated by tissue fragment culture with subsequent ELISA. Intestinal permeability for macromolecules was determined *in vivo* using a FITC-labeled dextran method and changes in microbiota composition in the stool was assessed by 16S rDNA denaturing gradient gel electrophoresis. Oral treatment but not parenteral administration with Lc and its membraneous fraction significantly reduced the severity of acute DSS colitis. When colitis was induced in SCID mice pretreatment with Lc failed to improve acute colitis in all tested parameters.We found statistically significant increase of Treg number in MLN of DSS/Lc-treated mice as compared to DSS/PBS-treated BALB/c mice and also decrease of intestinal permeability for macromolecules. Oral treatment with Lc lysate decrease production of TNF-α, IFN-γ, and IL-10 in Peyer's patches and large intestine. Changes of the gut microbiota composition were observed. Our study provide evidence that even lifeless components of probiotic bacterium can protect from development of intestinal inflammation, thus confering a health benefit for the host.

T.48. T Cell-specific Ablation of Protein Phosphatase 4 (PP4) Induces Colitis and Prolapse: An Immune-sufficient Model for Inflammatory Bowel Disease

Ching-Yu Huang, Fang-Hsuean Liao, Yu-Jun Lin, Yu-Te Chang, Wan-I Hsou, En-Wei Hsing, Tse-Hua Tan. National Health Research Institutes, Zhunan, Miaoli County, Taiwan

Protein phosphatase 4 (PP4) is a member of the PP2A/PP4/PP6 family serine/threonine phosphatase. We have previously shown that Lck-cre-mediated ablation of PP4 in early developing thymocytes blocked T cell differentiation. Yet CD4-cre-mediated deletion of PP4 (CD4-PP4) resulted in normal number of thymocytes and peripheral CD4 and CD8 T cells. However, by six month old ~50% male and ~30% female CD4-PP4 mice developed autonomous prolapse, while the floxed (PP4f/f) mice housed in the same condition remained healthy. Histological analyses of mice with prolapse showed classical symptoms of colitis such as weight loss, shortened colon, disrupted villi structure, and lymphocyte infiltration. When Peyer's patch (PP), intra-epithelial lymphocytes (IEL), and lamina propria (LP) lymphocytes were isolated from symptom-free CD4-PP4 mice, we found a decrease of B220+ cells in LP and αβ T cells in IEL, but with a surprising increase of CD4+CD25+ T cell percentage in LP. Furthermore, dextran sulfate sodium induced acute colitis in wild type and CD4-PP4 mice with similar efficacy. Our results thus suggest that the development or function of mucosal T cells is altered in CD4-PP4 mice. We are currently investigating the molecular mechanisms for the altered T cell development or function.

T.49. The Role of Macrophages in the Suppression of TNBS-induced Colonic Mucosal Injury in Bach1 Deficient Mice

Hiroyuki Yoriki, Yuji Naito, Tomohisa Takagi, Akihito Harusato, Kazuhiko Uchiyama, Katsura Mizushima, Yasuko Hirai, Toshifumi Tsuji, Munehiro Kugai, Ryusuke Horie, Osamu Handa, Satoshi Kokura, Hiroshi Ichikawa, Akihiko Muto, Kazuhiko Igarashi, Toshikazu Yoshikawa. Kyoto Prefectural University of Medicine, Kyoto, Japan

Introduction: BTB and CNC homolog 1 (Bach1) is a transcriptional repressor of heme oxygenase-1 (HO-1). In our previous study, we revealed HO-1 expression in the intestine was increased in the macrophages in Bach1 deficient mice. The aim of this study was to investigate the role of HO-1 macrophages in the development of the intestinal inflammation in murine 2,4,6-trinitrobenzine sulfonic acid (TNBS)-induced colitis. Methods: 8-week-old female C57BL/6 (wild-type) and homozygous Bach1-/- C57BL/6 mice were used. Mucosal injuries were evaluated macroscopically, histologically and biochemically. HO-1 expression and localization were investigated using western blotting and immunofluorescent staining. We also isolated the intraperitoneal macrophages and analyzed the function of these cells. Results: Colitis was markedly inhibited in Bach1-/- mice. Immunofluorescent staining showed HO-1 expression was mainly localized in F4/80 positive macrophages. The production of pro-inflammatory cytokines after TNF-α stimulation in the peritoneal macrophages from Bach1-/- mice significantly inhibited compared to those from wild type mice. In addition, the expression of the markers of M2 macrophages, such as arginase-1 and Fizz-1, increased in the macrophages isolated from Bach1-/- mice. Conclusion: Disruption of Bach1 ameliorated TNBS-induced colitis. These results suggest that the function of M2 macrophages plays an important role in intestinal mucosal immunity in Bach1-/- mice.

T.50. Neonatal Monocolonization by Bifidobacterium Longum Ssp. Longum RB25P Strain Modulates Immune Responses and Ameliorates Experimental Colitis in Mice

Dagmar Srutkova, Martin Schwarzer, Tomas Hudcovic, Hana Kozakova. Academy of Sciences of the Czech Republic, Novy Hradek, Czech Republic

Critical role in the etiology of inflammatory bowel diseases is played by intestinal microflora. In conventionally reared mice treatment with dextransulfate sodium (DSS) induces severe intestinal inflammation similar to human ulcerative colitis. We observed that DSS administration induced mild intestinal inflammation even in germ-free (GF) mice. The aim of our study was to evaluate how neonatal monocolonization with Bifidobacterium longum ssp. longum RB25P strain affects development of immune responses and whether these mice are protected against DSS-induced inflammation. Compared to GF controls, splenocytes of bifidobacteria-monocolonized mice showed increased production of regulatory cytokines TGF-beta and IL-10, reduced production of IL-5 and IL-4 (Th2 response) and no changes in IFN-gamma (Th1 response). Bifidobacteria-monocolonized mice had elevated level of total IgA in blood sera and gut lavages. Two-month-old bifidobacteria-neonatally monocolonized mice and age-matched GF controls were one week treated by 2.5% DSS in drinking water to develop acute colitis. Monoassociation of mice with bifidobacteria led to amelioration of intestinal inflammation as well as reduced level of IFN-gamma in both spleen and mesenteric lymph node cell supernatants compared to GF DSS-treated controls. We hypothesise that regulatory cytokines induced by bifidobacteria monocolonization play the protective role in DSS-induced inflammation. Supported by grants 303/09/0449 and CZ.3.22/2.1.00/09.01574.

T.51. Regulation of Gut Macrophage Function by CX3CR1

Antonio Bravo-Blas, Calum Bain, Allan Mowat. University of Glasgow, Glasgow, Scotland

Inflammatory bowel diseases (IBD) are increasing in incidence throughout the industrialized world and are generally believed to reflect abnormal host responses to the commensal microbiota. Macrophages (mφ) in the intestinal lamina propria (LP) are one of the first point of contacts between the host and local bacteria, but are normally are hyporesponsive to inflammatory stimuli. Fractalkine (FKN - CX3CL1) is produced by intestinal epithelial cells and a remarkable feature of resident intestinal mφ is that they express very high levels of CX3CR1. Conversely the pro-inflammatory

mφ that appear in experimental colitis express lower levels of CX3CR1, suggesting that CX3CR1 may play an important role in controlling mφ function in the intestine. We show here that HEK cells expressing soluble FKN can modify the activity of BM mφ *in vitro*. Furthermore, although resting CX3CR1 KO mice have normal numbers and subsets of colonic mφ and recruit inflammatory class II MHChi CX3CR1int Ly6Chi mφ during DSS colitis, they are resistant to pathology. Thus CX3CR1 does not alter mφ recruitment in response to DSS, but may influence their activity once in the mucosa. CX3CR1-FKN interactions may play an important and complex role in intestinal inflammation and could form the basis of targeted therapy.

T.52. The Role of Interleukin-21 in the Pathogenesis of Inflammatory Bowel Disease

Catherine Poholek, Laurie Harrington. University of Alabama at Birmingham, Birmingham, AL

Upon encounter with antigen, CD4 T cells differentiate into subsets that each secrete a specific cocktail of cytokines and are each responsible for various aspects of the adaptive immune response. Th17 cells, while necessary for protecting the host against extracellular pathogens, have also been cast as pathogenic in the context of several autoimmune diseases, including Inflammatory Bowel Disease (IBD). Genome wide association studies (GWAS) have implicated interleukin (IL)-23 signaling, which is a key component of Th17 differentiation and expansion, as a potential candidate for the cause of IBD in humans, and many studies in murine models of colitis have shown Th17 cells to be partially responsible for inflammation and mucosal damage. Th17 cells produce several inflammatory cytokines, including IL-17, IL-21, IL-22, and IL-26. Although many of these cytokines may act in concert to induce inflammation in colitis, IL-21 is a very strong candidate for further scrutiny. IL-21 expression is increased in biopsies from patients with ulcerative colitis compared to healthy controls, and recent GWAS have shown an association between the locus containing IL-2/IL-21 and IBD. Using the CD45Rb^{hi} transfer model of colitis, we have shown that IL-21 is necessary for the induction of disease. Our data show that the lack of disease seen in the absence of IL-21 is not due to an overall decrease in Th17 cells, nor to an increase in Foxp3+ T regulatory cells. These data highlight a previously unrecognized role for IL-21 in the pathogenesis of IBD.

T.53. The Pro-inflammatory Role of IL-15 in Toxoplasma Gondii-induced Ileitis is Mediated by a Local Recruitment of Inflammatory Monocytes

Julie Schuthess[1], Emma Ramiro-Puig[1], Bertrand Meresse[1], Sylvie Darche[2], Christophe Combadière[1], Dominique Buzoni-Gatel[3], Nadine Cerf-Bensussan[1]. [1]INSERM, Paris, France; [2]Institut Pasteur, Paris, France; [3]INRA, Nouzilly, France

Background: IL-15 contribution to intestinal inflammation was investigated in the model of ileitis induced by Toxoplasma gondii (TG) in C57BL/6 (B6) or IL-15-/- mice. Methods: the severity of Ileitis was defined by weight loss, death rate and histology. The phenotype of immune cells and production of IFNg, granzyme, IL-1b, IL-6 and TNFa were compared in mesenteric lymph nodes (MLN) and intestine of WT and IL-15-/- mice. Results: Ileitis was markedly attenuated in IL-15-/- mice but the parasite load was not modified. The pro-inflammatory role of IL-15 was independent of cytotoxic CD8 T cells and did not result from enhanced Th1 CD4+ response as this response evaluated by RT-PCR, intracellular staining and ELISA after or not stimulation with TG was comparable in MLN and lamina propria cells in WT and IL-15-/- mice on day 7. In contrast, a significant difference was observed in the secretion of IL-1b, IL-6 and TNFa by lamina propria cells and could be ascribed to the defective recruitment of proinflammatory monocytes in the intestine of infected IL-15-/- compared to WT mice. Conclusion: IL-15 exacerbates inflammation in TG-induced ileitis by inducing the recruitment of inflammatory monocytes. Mechanisms underlying IL-15 dependent recruitment of monocytes are under study.

T.54. Th1 and Th17 Cell Differentiation in *in vivo* Models of Inflammatory Bowel Disease

Azucena Salas, Marisol Veny, Rut Mora, Miriam Esteller, Elisabeth Calderón-Gómez, Josep Maria Piqué, Julian Panés. IDIBAPS, Ciberehd, Barcelona, Spain

Th17 lymphocytes are known to play a role in autoimmunity and inflammatory bowel disease (IBD). *In vitro* studies have identified the mechanisms driving Th17 differentiation, much less is known about the generation of these effector cells in the context of IBD. We used a model of spontaneous colitis in IL-10-/- mice and a model of DSS-induced colitis to determine the *in vivo* location of Th17 (and Th1) cell differentiation, and to identify potential subsets of antigen presenting cells (APCs) that may drive their differentiation. APCs were identified by the expression of CD11b, Ly6C and CD11c. We found a marked recruitment of CD11b+Ly6C+CD11c-/low cells to the colon of colitic IL-10-/- mice as well as of DSS-colitic mice. In contrast, the percentage of Th17 and Th1 cells in colonic lamina propria was only significantly increased in colitic IL-10-/- mice, while in mice receiving DSS with comparable disease activity, it remained unchanged compared to water-treated littermates. Our results so far suggest that effector Th cell differentiation *in vivo* predominantly takes place in the intestinal lamina propria in the context of spontaneous IBD. Based on our results in DSS-colitic mice, however, recruitment of CD11b+Ly6C+CD11c-/low cells may not be related to Th cell differentiation.

T.55. Nod2 Deficiency is Associated with Increased Mucosal Regulatory Response to Commensal Microorganisms

Alessia Butera, Antonello Amendola, Monica Boirivant. Istituto Superiore di Sanita, Rome, Italy

It has been reported that Peyer's patches (PPs) of Nod2-/- show, as a consequence of the presence of ileal microbiota, an increased tissue content of IFN-g and TNF-a that are responsible for an increased permeability of the epithelium covering PP (Gut 2010;59:207). We observed, after the induction of a transient increase of intestinal permeability, a mucosal regulatory response characterized by the expansion of T regulatory cells

expressing surface TGF-b latency-associated- protein(LAP+ T cells)(Gastroenterology 2008;135:1612). In the present study we found that Nod2-/- mice, when compared with WT mice, showed an increased intestinal permeability evaluated by serum quantification of fluorescent particles after intrarectal administration of FITC-dextran. This feature was associated with a significant increase of IL12-p70 and TGF-b colonic tissue content. In addition, we observed an increased % of CD4+LAP+ T cells in lamina propria mononuclear cells isolated from colons of Nod2-/- mice, when compared with WT mice. The induction of a transient increase of intestinal permeability by intrarectal ethanol administration was associated with a stronger increase of TGF-b tissue content and CD4+LAP+ T cells in Nod2-/- mice when compared with WT . IL-12-p70 tissue content was increased in WT mice, and was decreased in Nod2-/- mice when compared to their respective baseline levels. The data suggest that Nod2 deficiency is associated with increased regulatory response to commensal microorganisms.

T.56. Mouse Whole Genome Profile in Gut of Healthy TLR2-defient Mice

Elisabet Pedrosa Tapias[1], Violeta Lorén[1], Cristina Sánchez[1], Patricia Romero[1], Eduard Cabré[2], Eugeni Domènech[2], Juanjo Lozano[3], Ildefonso Cases[4], Josep Mañé[1]. [1]Institut d'Investigació Germans Trias i Pujol, Badalona, Spain; [2]Hospital Universitari Germans Trias i Pujol, Badalona, Spain; [3]CIBERehd, Barcelona, Spain; [4]Institute for Personalize and Predictive Medicine of Cancer, Badalona, Spain

Introduction: Toll-like receptor 2 is a bacterial pattern recognition receptor implicated in immune cell polarization and intestinal barrier function maintenance. Objectives: To describe genetic expression profile in colon of healthy TLR2-deficient mice. Methods: Mouse whole genome microarray (CodelinkTM) was used to identify transcriptomic changes between wild-type and TLR2-deficient C57BL/6J mice. Statistic analysis from microarray data was performed by LIMA-R Package, GeneCodis, gene set enrichment analysis (GSEA) and informatics database consultation. Results: Although GSEA analysis showed in TLR2 deficient mice only two gene ontology enrichment, histone deacetylase complex and cell cortex, this animals presented 24 up-regulated and 1141 down-regulated genes (FDR < 0.01 and FC≥2). GeneCodis analysis showed up-regulated genes involved in retinoic acid receptor signaling pathway, phagocitosis, positive regulation of immune response (CD4Tαβ lymphocytes, IL-2 and IL-10) and negative regulation of IL-4. Down-regulated genes were implicated in apoptosis, epigenetic regulation, T cell receptor signaling pathway, and adherens junctions. Conclusions: TLR2 deficiency induces changes in regulation of mucosal immunity and para-cellular adhesive activity.

T.57. **Immunohistochemical Expression of TNFα, TGFβ and TLR4 in the Mucosa of Mice with Hapten**-induced Experimental Colitis

Sanja Plesko[1], Marko Banic[2], Branimir Anic[1], Tomislav Brkic[1], Vanda Plecko[1], Marija Urek[2], Lidija Petričušić[2], Dusko Kardum[2], Zeljko Ferencic[2], Milan Kujundzic[2]. [1]Clinical Hospital Centre, Zagreb, Croatia; [2]University Hospital Dubrava, Zagreb, Croatia

Aim: To evaluate immunohistochemical expression of TNFα, TGFβ and TLR4 in colonic mucosa of mice with hapten-induced experimental colitis. Methods: Experimental colitis was induced in animals (chall) by receiving the challenge enema of 0.025 mL 0.2% DNFB solution in acetone and olive oil. Animals in control group (control) were treated with phosphate buffered saline (PBS). On day 5, experimental colitis was analyzed with clinical disease score (0-5). Immunohystochemical analysis (0-3) was performed for TNFα, TGFβ and TLR-4 in the mucosa of all animals. Results were considered significant at P < 0.05. Results: The clinical disease score was higher in chall group (2.7±0.3), than in control group (0.0±0.0) (P < 0.05). TNFα was most expressed in in the mucosa of chall group, when compared to mucosa without lesions in control group (P = 0.021). TGFβ was most expressed in control group, when compared to mucosa of chall group (P = 0.028). TLR4 was most expressed in mucosa of animals in chall group, when compared to mucosa of control group (P = 0.0001). Conclusions: Immunohistochemical expression of TNFα, TGFβ and TLR4 was consistent to clinical disease score. This particular model of colitis could be used for further study of various components of inflammatory mucosal injury.

T.58. Anti-CD25 Antibody Treatment Increases the Severity of Intestinal Inflammation in SAMP1Yit/Fc Mice: Evidence for a Key Role of Natural Tregs in Experimental IBD

Dai Ishikawa[2], Daniele Corridoni[1], Li-guo Jia[1], Wei Xin[1], Fabio Cominelli[1]. [1]Case Western Reserve University, Cleveland, OH; [2]Juntendo University, Tokyo, Japan

Increasing evidence suggests that regulatory T cells (Treg) may play an important role in regulating gut homeostasis and intestinal inflammation. We used the SAMP1YitFc (SAMP) mice, a model of Crohn's disease-like ileitis to precisely define the role of Tregs in chronic intestinal inflammation. SAMP mice were administered anti-CD25 Abs, which deplete Treg cell populations, or isolate control antibodies. In separate experiments, MLN cell subpopulations from anti-CD25 treated SAMP mice were adoptively transferred into SCID recipient mice to induce colitis study the *in vivo* function of these cells. Treg depletion by anti-CD25 Abs increased the severity of ileitis and adoptively transferred colitis in SAMP mice compared to AKR controls. Differently from AKR control mice, SAMP CD4+CD25+ cells failed to ameliorate adoptively transferred colitis, suggesting a functional defect of Tregs in this model. Anti-CD25 Ab treatment induced proliferation of CD25-Foxp3+ Treg cells in SAMP mice, but not in AKR control mice. However, these CD25- Foxp3+cells were functionally defective and appear to promote a proinflammatory Th1/Th2 profile. This study suggests that natural Tregs play a critical role in the regulation of chronic intestinal inflammation.

T.59. Oral Tacrolimus Therapy is Useful for Patients with Intractable Ulcerative Colitis: A Result of Post-marketing Analysis

Nagamu Inoue, Riko Ichikawa, Jun Miyoshi, Katsuyoshi Matsuoka, Tadakazu Hisamatsu, Susumu Okamoto, Haruhiko Ogata, Yasushi Iwao, Takanori Kanai, Toshifumi Hibi. Keio University School of Medicine, Tokyo, Japan

Purpose: Intravenous cyclosporine A is an alternative treatment option to total proctocolectomy for patients with severely-active ulcerative colitis (UC), while benefits of oral tacrolimus therapy are not well defined. Therefore, we evaluate the efficacy and safety of tacrolimus in patients with intractable UC. Methods: Twenty-eight patients with intractable UC were orally administered tacrolimus to induce remission with high trough levels for two week and then maintained with low trough levels. Evaluation of the clinical response was based on a modified Truelove-Witts clinical activity index (CAI) and endoscopic response was also assessed. Results: CAI scores significantly decreased at week 2. Remission was achieved in 43%, improvement was achieved in 39% of patients. Endoscopic activities decreased at three months in 16 of 23 patients examined. Corticosteroids were successfully tapered or discontinued in all of 10 patients treated with corticosteroids. A life-table analysis revealed that the overall percentages of patients who had not required colectomy was 70.0% at 12 months. There were no lethal adverse reactions to tacrolimus in our series. Conclusion: These findings suggested that short-term oral administration of tacrolimus produced good clinical response comparable to cyclosporine A as well as steroid tapering effect and endoscopic improvement in patients with intractable UC.

T.60. Mucosal Intraepithelial Lymphogram by Flow Cytometry as a Diagnostic Tool in Patients with IBD

Enrique Montalvillo Álvarez[1], José Garrote[2], Beatriz Martínez Abad[1], Sara Vallejo-Díez[1], Celia Escudero-Hernández[1], Luis Fernández-Salazar[2], Benito Velayos[2], David Bernardo Ordiz[3], Eduardo Arranz[1]. [1]University of Valladolid, Valladolid, Spain; [2]Hospital Clínico Universitario, Valladolid, Spain; [3]Imperial College London, St. Mark's and Northwick Park Hospital, Valladolid, United Kingdom

We assess the use of the mucosal intraepithelial lymphogram as a helping tool in determining the involvement of colon or ileum in Cronh`s Disease (CD), and to differentiate between CD, Ulcerative Colitis (UC), and other intestinal inflammatory disorders. Biopsies were collected from active UC (affected colon n=15), active CD (affected colon n=11; unaffected colon n=9; affected ileum n=9; unaffected ileum n=8), non-IBD patients (affected colon, n=8; affected ileum, n=6), and controls (HC, colon n=8). Intraepithelial mononuclear cells were isolated and 3 populations analyzed by cytometry: total leukocytes (CD45+), T cells (CD45+CD3+), and non-T cells (NK-like, CD45+CD3-). We used the ratio between percentages of T / non-T cells for discrimination. Mucosal inflammation due to CD was defined by selecting a cut-off ratio <1.057, by a ROC curve, with the highest sensibility and specificity. We observed a specific T / non-T cell ratio in the affected colon and ileum from CD (colon=0.638, ileum=0.791). This ratio is inverted in unaffected samples from CD (colon=1.81, ileum=2.45), controls (colon=2.68, ileum=3.62), affected colon from UC (1.75) and ileum from non-IBD (1.97). Therefore, a simple T / non-T lymphocyte ratio may classify inflamed colon and/or ileum as due to CD, and this may result in a helpful tool for IBD diagnosis.

T.61. Conjugated Linoleic Acid Ameliorates Colitis

Thaís Moreira, Ana Cristina Gomes-Santos, Archimedes Castro Junior, Bernardo Horta, Ana Cecília Ribeiro, Denise Cara, Joana Amaral, Ana Faria. Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Introduction: Inflammatory bowel diseases (IBD) include ulcerative colitis and Crohn's disease, and they are characterized by an intense inflammatory response in the gastrointestinal tract. There is strong evidence that IBD result, in part, from an imbalance between inflammatory and regulatory immune responses to the commensal bacterial microbiota. Among the proposed therapeutic and preventive treatments for IBD, is conjugated linoleic acid (CLA), which consists of a mixture of short-chain fatty acid isomers. Several actions linked to human health have been described as associated with CLA administration. Studies with *in vitro* cultured lymphoid cells and with animal models have shown that CLA can modulate immune function and inflammatory responses. Objetive: This study aims to test the effect of 1% CLA (50:50 isomers) administration as dietary supplementation for 4 weeks in mice with ulcerative colitis induced by a 7-day treatment with 1.5% dextran sodium sulphate (DSS). Results: Supplementation of CLA in the diet before the induction of colitis decreased both mucosal damage at colonic mucosa and weight loss. Inflammatory alterations such as increased levels of IL-4 and decreased levels of IL-10 in colonic mucosa of DSS-treated mice were prevented by CLA supplementation. CLA-fed animals had also lower levels of IFN-γ in mesenteric lymph nodes, and of IL-17 and MCP-1 in colonic mucosa. Serum and secretory IgA levels that were reduced by colitis were restored by CLA treatment. Conclusion: CLA mediated protection against experimental colitis and might represent a novel therapeutic tool for IBD.

T.62. Compartment-specific Expression of ALCAM (CD166) in Inflammatory Bowel Diseases

Felix Lasitschka, Kristina Schroeder, Thomas Giese, Stefan Meuer, Jutta Schroeder-Braunstein. University Hospital Heidelberg, Heidelberg, Germany

Activated leukocyte cell adhesion molecule (ALCAM) is a member of the immunoglobulin superfamily of proteins. It is expressed on a wide variety of cells, particularly on activated lymphocytes, dendritic cells and monocytes, and on various epithelial cell types. Functionally, ALCAM is involved in regulating immunological processes such as inflammation, participating in cell migration/clustering, and co-stimulation of T lymphocytes. In this study, we investigated the intestinal compartment-specific expression of ALCAM in ulcerative colitis and Crohn's disease employing laser capture microdissection, followed by subsequent RNA isolation and PCR analysis. Expression of ALCAM was further assessed by immunohistochemistry and immunofluorescence as well as in a human intestinal organ culture model via ELISA, flow cytometry, and quantitative PCR analysis. Compartment-specific expression analysis reveals an up-regulation of ALCAM in mononuclear cells in the lamina propria and a down-regulation in epithelial cells in IBD compared to healthy controls. Up-regulation of ALCAM expression in lamina propria cells under inflammatory conditions occurs mostly in cells of the monocyte/macrophage and T cell lineage. It can be induced by tissue damage as revealed by a human organ culture model of

intestinal inflammation. Gene expression of a soluble isoform of ALCAM in the epithelial compartment and detection of high levels of soluble ALCAM in the supernatant of cultured healthy whole wall tissue specimen point to a constitutive release of this molecule by intestinal epithelial cells.

T.63. An IFNG SNP-associated with UC and Severity is Functionally Associated with Altered IFNG Methylation and IFN-γ Protein Secretion

Rivkah Gonsky, Richard Deem, Dermot McGovern, Talin Haritunians, Kent Taylor, Stephan Targan. Cedar Sinai Medical Center, Los Angeles, CA

Background: DNA methylation affects transcriptional activation. IBD patients display distinct IFNG methylation, correlated with enhanced IFN-γ secretion and seroreactivity to microbial antigens. GWAS identified UC-risk/severity regions linked to SNPs flanking IFNG. Many disease-linked SNPs target CpG sites, which are rare in the genome and serve as methylation sites. Allele specific methylation preferentially occurs adjacent to SNPs that alter CpG. The CpG conserved SNP, rs1861494(C/T), in IFNG third intron (2109 bp), is in the same LD-block implicated with UC disease severity. Two adjacent CpGs are 2051 and 2009 bp. It seems likely that SNPs that alter CpGs, alter methylation and may lead to unequal allelic expression, which has been reported for IFNG. Aim: In our study we examined allele specific methylation levels of rs1861494 and asked whether a functional correlation exists with gene expression. Methods: 73 UC patients were genotyped for rs1861494. Strand-specific methylation for SNP2109, 2051 and 2009 CpG sites were measured by pyrosequencing, nucleo-protein binding by EMSA and IFN-γ secretion and CBir reactivity by ELISA. Results: The 2109 T allele is unmethylated while the C allele displays 55% methylation. Allele-specific decreased methylation of C vs. T allele ($p<0.001$) was seen at 2051, but not 2009 bp. The C SNP functionally associates with lower IFN-γ secretion and immune response to CBir. Patients with C vs. T SNP exhibit delayed need for surgical intervention. Nucleo-protein binding was lower in C vs T SNP. However, methylation of the C allele markedly enhanced binding and an additional nucleo-protein complex. Conclusion: These results link the same conserved SNP with strand-specific DNA methylation and trans-factor binding, suggesting a functional role for rs1861494 SNP in regulating IFNG expression.

T.64. OX40 is Implicated in Inflammatory Bowel Disease and T Cell Expansion

Zili Zhang[1], Wenwei Zhong[1], Andrew Weinberg[2], James Rosenbaum[1]. [1]Oregon Health & Science University, Portland, OR; [2]Providence Portland Medical Center, Portland, OR

T cells play an important role in inflammatory bowel disease (IBD). Although co-stimulatory molecule OX40 promotes T cell effector function and proliferation, it is unclear if OX40 is implicated in the pathogenesis of IBD. In this study, we found that infiltrating lymphocytes in the intestinal mucosa of IBD patients exhibited a strong OX40 expression. Next we sought to characterize the role of OX40 in a murine colitis model. The treatment of OX40 specific activating antibody accelerated dextran sulfate sodium (DSS)-induced colitis. In addition, activation of OX40 substantially increased the lymphocyte population in the lamina propria. To further explore the mechanism of OX40-mediated T cell expansion, we demonstrated that the OX40 activating antibody significantly enhanced the production of IL-21 in CD4+ cells from DO11.10 mice. In addition, after antigen activation, proliferating CD4+ cells expressed more OX40 and IL-21R than quiescent lymphocytes. This result suggests that OX40+ cells are in a proliferating state and readily respond to IL-21, a common γ cytokine critical for the proliferation of multiple T cell subsets. Thus, these data reveal a pathogenic role of OX40 in IBD. Furthermore, OX40 exaggerates intestinal inflammation in part by the expansion of activated lymphocytes.

T.65. Epithelial Cells Modulate Colonic Subepithelial Myofribroblast Functions *in vitro*

George Kolios[1], Ioannis Drygiannakis[2], Vassilis Valatas[2], Elias Kouroumalis[2]. [1]Democritus University of Thrace, Alexandroupolis, Greece; [2]Medical School, University of Crete, Heraklion, Greece

There are accumulating data suggesting that colonic subepithelial myofibroblasts (SEMFs) are key cells in the process of tissue injury and repair in the gut. We investigated *in vitro* the possible cross-talk between epithelial cells and SEMFs that may be involved in fibrosis occurring in inflammatory bowel diseases. We studied the effects of epithelial cell conditioned media (ECCM) from two human colonic epithelial cells lines (HT-29, CaCO-2) on cultures of freshly isolated human SEMFs and the colonic myofibroblast cell line 18CO. Pro-inflammatory cytokines (IL-1α, TNF-α and IFN-γ) increased profibrotic mediator production by epithelial cells such as TGFβ and TIMP-1 but had no effect on SEMF cultures. ECCM from epithelial cells pre-treated with pro-inflammatory cytokines increased MMP-9 production by SEMFs in an endothelin-A receptor dependent manner. ECCM from epithelial cells alone increased total collagen production by SEMFs and the effect was enhanced if epithelial cells were pre-treated with pro-inflammatory cytokines. The process was found to be TGFβ, CTGF, TF and endothelin independent. However, ECCM from epithelial cells pre-treated with pro-inflammatory cytokines significantly delayed wound healing of SEMF cultures. These data indicate that epithelial cells may cooperate with adjacent SEMFs for the regulation of extracellular matrix especially in the context of intestinal inflammation.

T.66. Expression of the Transmembrane Glycoprotein CD98 is Upregulated in Intestinal Inflammation

Hang Nguyen, Guillaume Dalmasso, Yutao Yan, Hamed Laroui, Shanthi Sitaraman, Didier Merlin. Emory University, Atlanta, GA

By generating a transgenic (Tg) mouse model with CD98 over-expression specifically in intestinal epithelial cells (IECs), we explore the mechanistic insights into the role of CD98 in intestinal inflammation. Tg mice exhibited barrier dysfunction and perturbed inflammatory responses, both factors known to contribute to the pathophysiology of IBD. Examination of IEC ultrastructure by electron microscopy showed abnormal morphology and decreased size of microvilli at the apical membranes of enterocytes in Tg compared to wild-type littermates. IEC-specific CD98 over-expression affected integrin signaling, leading to changes in cell proliferation and survival, which may result in abnormal basal intestinal phenotype and

contribute to increased intestinal permeability. Importantly, IEC-specific CD98 over-expression increased susceptibility of mice to DSS-induced colitis as shown by increases in body weight loss, clinical and histological score, activity of myeloperoxidase-a marker for neutrophil infiltration, and production of proinflammatory cytokines and chemokines. Altogether, our study shows that IEC-specific CD98 over-expression induces gut homeostatic defects and aggravates inflammatory responses to DSS-induced colitis. Maintaining a low level of CD98 expression in IECs during inflammation could have beneficial effects on preventing mucosal barrier disruption and tissue damage.

T.67. Impaired Deoxyribonuclease I Activity in Patients with Inflammatory Bowel Diseases

Karin Malickova[1], Dana Duricova[2], Martin Bortlik[2], Terezie Fucikova[1], Milan Lukas[2]. [1]Charles University in Prague and General University Hospital, Prague, Czech Republic; [2]Iscare IVF and Charles University, Prague, Czech Republic

Aim: To investigate serum DNase I activity in patients with inflammatory bowel diseases (IBD). Patients and Methods: A cohort of 110 IBD patients was evaluated. Fifty SLE patients and 50 healthy blood donors were examined as control age-matched groups. Serum DNase I activity was determined by ELISA. Results: DNase I activity in IBD patients was significantly lower than in healthy blood donors, but higher than in SLE patients ($p<0.0001$). Patients with ulcerative colitis showed higher DNase I activity than Crohn's disease patients, $p=0.021$. DNase I activity did not oscillate during anti-inflammatory biological treatment of IBD and did not correlate with serum CRP during the treatment ($r=0.128$, $p=0.654$).DNase I activity has shown a strong negative correlation with the serum concentration of anti-nuclesomal antibodies in the autoimmune (SLE+IBD) cohort, as well as in the separate IBD cohort. However, concentrations of anti-nucleosomal autoantibodies in IBD patients did not reach as high values as SLE patients ($p=0.001$).Conclusions: Reduced serum DNase I activity probably has pathogenetic consequences in IBD; however, the significance of this enzyme is different from SLE. Induction of autoantibodies, in particular towards nucleosomes, could be a reflection of impaired DNase I activity.
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T.68. FUT2 Scretor Status is Positively Associated with Colic Type Crohn's Disease

Jun Miyoshi, Tomoharu Yajima, Susumu Okamoto, Katsuyoshi Matsuoka, Nagamu Inoue, Tadakazu Hisamatsu, Katsuyoshi Shimamura, Atsushi Nakazawa, Takanori Kanai, Haruhiko Ogata, Yasushi Iwao, Toshifumi Hibi. Keio University, Tokyo, Japan

Background: The host-intestinal microbiota interaction plays an important role in the pathogenesis of inflammatory bowel diseases (IBDs). Intestinal epithelium works as a receptor for certain bacteria and regulates intestinal flora. One known receptor is mucosal blood type antigens, which are regulated by the FUT2 gene, and individuals who express them in GI tract are called secretors. A recent research revealed that the FUT2 gene is associated with Crohn's disease (CD) in western populations. Methods: We examined the incidence of five SNPs previously reported on the FUT2 gene in Japanese patients. We also examined the expression of the antigens performing immunohistochemistry on mucosal specimens. Results: Genetic analysis revealed that all of the CD colic type patients (100%) were secretors while the incidence of secretors was 80%, 80%, 67%, and 80%, respectively for control group, CD ileocolic type, CD ileal type and ulcerative colitis ($p = 0.036$). The abnormal expression of blood type antigens was only observed in CD colic type. Conclusions: The FUT2 secretor status was associated with colic type CD. Taken together with the data from immunohistochemistry, the abnormal expression of blood type antigens in colon may be a unique and essential factor to colic type CD.

T.69. Protective Effects of TIMP3 on Gut Inflammation

Ivan Monteleone[1], Massimiliano Sarra[1], Viviana Casagrande[1], Francesca Zorzi[1], Michele Cavalera[1], Angelamaria Rizzo[1], Thomas MacDoanld[2], Francesco Pallone[1], Massimo Federici[1], Giovanni Monteleone[1]. [1]University of Tor Vergata, Rome, Italy; [2]Barts and the London School of Medicine and Dentistry, London, United Kingdom

Background: TNF-alpha is central to the orchestration of inflammation in the gut. TNF-alpha is expressed as a transmembrane-bound-molecule, and is released from the cell surface by proteolytic cleavage, primarily involving the TNF-alpha-converting-enzyme (TACE). The activity of TACE near the cell surface is strictly regulated by tissue inhibitor of matrix-metalloproteinase (TIMP)3. AIM: To evaluate if TIMP3 can dictate the induction and progression of intestinal inflammation. Methods: TIMP3 expression was evaluated in intestinal biopsies and LPMC from CD patients. Inflammatory cytokine was evaluated in CD samples cultured with rh-TIMP3. To evaluate if TIMP3 expression in IBD is dependent on Smad7, an inhibitor of TGF-beta-activity, TIMP3 was evaluated in CD biopsies cultured with a Smad7 antisense. Finally, we evaluated the susceptibility of wild-type(WT), TIMP3-knockout(KO) and TIMP3 over-expressing-transgenic(Tg) mice to experimental colitis. Results: TIMP3 expression was reduced in CD compared to normal gut and ulcerative colitis. Treatment of CD tissue and LPMC with rh-TIMP3 resulted in a reduced expression of inflammatory cytokines. TIMP3 expression was also enhanced in CD biopsies by knock-down of Smad7. TIMP3 KO mice were more susceptible to TNBS colitis while TIMP-3 over-expressing mice were resistant. Conclusions: These data suggest that defective TIMP3 expression contributes to excessive inflammation in CD.

T.70. The Transition of Peripheral Immune-characteristics from Pre-, Post- to Trans-operation in Ulcerative Colitis

Yoko Yokoyama, Ken Fukunaga, Shinichirou Kashiwamura, Koji Kamikozuru, Risa Kikuyama, Kyoichi Kato, Naohisa Takeda, Takayuki Matsumoto. Hyogo College of Medicine, Nishinomiya, Japan

We have investigated the circulating level of CD25HighCD4+ regulatory T cells (Treg), which are intimately associated with the mucosal immune

function in patients with ulcerative colitis (UC). In the present study, we were interested in the peripheral %Treg and the Th1, Th2, and Th17 cytokines in patients with UC, in active (aUC), quiescent phase (qUC) and after colectomy (cUC). The %Treg was significantly higher in the qUC vs the aUC. Similarly, in the cUC a significantly higher %Treg was found as compared with the level during active phase. Further, the rise of Th1, Th2 and Treg cytokines (TGF-β, IL-10) in aUC was significantly greater than in the others. Interestingly, the rise of IL-17 in the cUC was greater than in the qUC. Therefore Th17/Treg ratio was significantly higher in the cUC vs the others. Our impression is that Treg are involved in the maintenance of immune homeostasis in UC, suppress clinical relapse. It might be logical to assume that aUC patients require more Treg cytokines like IL-10, TGF-β to suppress mucosal inflammation. We also found that IL-17 featured severe types of UC, irrelevant of before or after operation. Such change may be used as a predictive biomarker for intractable UC.

T.71. Production of TNF-α by Paneth Cells is Associated with Intestinal Inflammation

Sabine Middendorp[1], Pieter van Lierop[2], Dicky Lindenbergh-Kortleve[2], Janneke Samsom[2], Hankje Escher[2], Edward Nieuwenhuis[1]. [1]Wilhelmina Children's Hospital-UMC Utrecht, Utrecht, Netherlands; [2]Erasmus MC-Sophia Children's Hospital, Rotterdam, Netherlands

Intestinal Paneth cells (Pc) produce anti-microbial defensins, lysozyme and pro-inflammatory TNF-α. Impaired Pc defensin production has been associated with intestinal inflammation. Elucidation of the mechanisms that regulate TNF-α production by Pc may identify a novel contributive pathway of mucosal inflammation. We determined lysozyme and TNF-α expression in Pc of pediatric Inflammatory Bowel Disease (IBD) patients, celiac disease patients and healthy controls. Additionally, we examined TNF-α and lysozyme expression in IL-10-/- mice under SPF and germ-free conditions as well as LPS-insensitive mice. We found that 45% of the Crohn's disease patients, 37% of the ulcerative colitis patients and 35% of the celiac disease patients expressed TNF-α in Pc, while ~19% of healthy duodenum was positive for TNF-α in Pc. In contrast, lysozyme was equally expressed by Pc in all patients and controls. We observed enhanced expression of TNF-α in Pc of IL-10-/- mice compared to WT mice that was absent in germ-free IL-10-/- mice. Finally, LPS-insensitive mice expressed less Pc-derived TNF-α compared to LPS-sensitive mice. In conclusion, TNF-α and lysozyme production by Pc are differentially regulated. TNF-α expression is associated with human mucosal inflammatory diseases, dependent on microbial stimulation and regulated by IL-10. Under comparable conditions, Pc lysozyme levels remain unaffected.

T.72. The 4G/5G Polymorphism of the Type-1 Plasminogen Activator Inhibitor (PAI-1) Gene Does Not Influence the Penetrating Behavior in Patients with Crohn's Disease in the Japanese Population

Naohisa Takeda, Ken Fukunaga, Tomonori Morinaga, Tomoko Hashimoto-Tamaoki, Nobuyuki Hida, Yoshio Ohda, Yoko Yokoyama, Koji Yoshida, Koji Kamikozuru, Iimuro Masaki, Risa Kikuyama, Kyoichi Kato, Kazutoshi Hori, Shiro Nakamura, Hiroto Miwa, Takayuki Matsumoto. Hyogo College of Medicine, Nishinomiya, Japan

Among Spanish patients with Crohn's disease (CD), the 4G/4G genotype of the 4G/5G polymorphism in the promoter region of the type-1 plasminogen activator inhibitor (PAI-1) gene has been reported to increase the probability of penetrating behaviors (B3) (AP&T, 2003). To assess the significance of the 4G/5G polymorphism in Japanese CD patients, 80 patients and 24 controls were examined. The CD phenotype was defined according to Vienna Classification. 71.3% of the CD patients showed the B3 behavior. The frequencies of the 4G/4G, 4G/5G and 5G/5G genotypes were similar between the CD group and the control group. In the patients with B3 CD, the frequencies of 4G/4G, 4G/5G and 5G/5G were 40.4% (23/57), 40.4% (23/57) and 19.3% (11/57), respectively. The frequency of B3 was higher in the patients with the 4G/4G genotype (85.2%, 23/27) than in the patients with the 4G/5G genotype (59%, 23/39). However, no significant differences were observed in either penetrating behaviors or perianal diseases between these two genotypes. These results indicate that the 4G/4G genotype of the PAI gene may not influence the penetrating behavior in Japanese CD patients. This may reflect, as in the case of the NOD2 gene, racial differences between Western and Asian populations.

T.73. Metabolic Phenotyping of the Crohn's Disease Like IBD Etiopathology in the TNFΔARE Mouse Model

Pia Baur[1], Lisa Gruber[1], Francois-Pierre Martin[2], Sebastiano Collino[2], Nabil Bosco[2], Philippe Guy[2], Serge Rezzi[2], Sunil Kochhar[2], Jalil Benyacoub[2], George Kollias[3], Dirk Haller[1]. [1]Technische Universität München, Freising, Germany; [2]Nestlé Research Center, Lausanne, Switzerland; [3]Alexander Fleming Biomedical Sciences Research Center, Vari, Greece

The consequences of inflammatory processes during the pathogenesis of IBD on systemic and gastrointestinal metabolism are largely unknown. Therefore, metabolic changes in key biological compartments were monitored over time in the TNFΔARE Crohn's disease mouse model. Chronic inflammation appeared from 8 weeks onwards, as assessed by histological scoring, and was associated with reduced body weight, adipocyte size and visceral fat mass. Non-targeted (1HNMR spectroscopy) and targeted (LC-MS) metabolomics was used to analyze site-specific variations related to the development of inflammation in tissues. Untargeted profiling of different gut sections at 24 weeks revealed significantly altered cholesterol and triglyceride metabolism. Targeted LC-MS analysis of the inflamed ileum over time highlighted significant changes in sphingolipid and phosphatidylcholine concurrent to the histological onset of inflammation. Similar metabonomic approaches were employed to explore the metabolic variations at systemic level. Liver from TNFΔARE mice showed elevated contents of amino acids and an altered lipid composition. Besides organ specific alterations, plasma of inflamed mice showed early changes in sphingolipids and phosphatidylcholines as revealed by targeted LC-MS. The observed modifications of lipid metabolism provide encouraging insights into IBD-related alterations of specific metabolic processes during inflammatory states and may help to further understand the disease mechanisms.

T.74. Antagonizing CD88 by PMX205 Protects Against DSS-induced Colon Damage in Mice

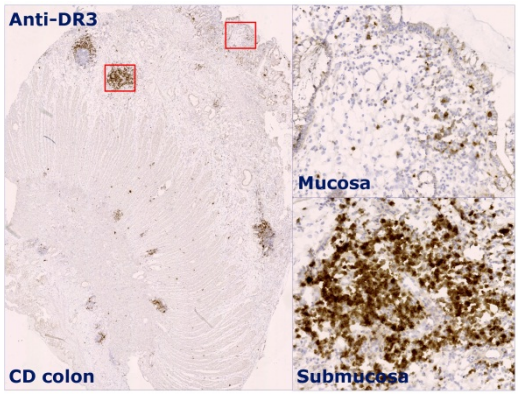
Umang Jain[1], Trent Woodruff[2], Andrew Stadnyk[1]. [1]Dalhousie University, Halifax, NS, Canada; [2]University of Queensland, Brisbane, QLD, Australia

Interest in the complement system as a promising therapeutic target for treating inflammatory bowel diseases has recently increased. PMX205, a pharmacological inhibitor of the primary C5a receptor (CD88), has been shown to have therapeutic efficacy in the delayed-type hypersensitivity model of colitis in rats. Here, we evaluated the effect of PMX205 in the murine DSS model of colitis. C57BL/6 mice with 3% DSS added to their drinking water for 5 days were orally gavaged daily with PMX205 in a prophylactic regimen. Histological scores, based on the extent of crypt damage, cell infiltration, edema and ulceration, were obtained from sections prepared from a longitudinal segment of their colon in a Swiss roll. The other longitudinal half was cultured and 24 hour supernatants were analyzed for cytokines. PMX205 (200 µg/mouse/day) significantly prevented DSS-induced body weight loss, rectal bleeding, colon shortening and histological damage. PMX205 significantly prevented the IL-12, IL-6 and MIP-2 upregulation evident in control mice. Fewer Ly6G positive cells (neutrophils) infiltrated the colon of PMX205 treated mice as compared to the control group. These results provide direct evidence that blocking CD88 inhibits proinflammatory changes in mice with an intact complement system, likely by blocking proximal events.

T.75. DR3 is Upregulated in Colon of Crohn's Disease Patients Compared to Normal Controls

Kirstine Roepstorff, Ditte Zerlang Christensen, Kristian Kjærgaard, Elisabeth Galsgaard. Novo Nordisk A/S, Maaloev, Denmark

DR3 (death receptor 3) is a member of the TNF receptor family. It is expressed by several cell types, including T cells, NK cells, and dendritic cells. Several studies have suggested that DR3 activation is an important driver of Crohn's disease (CD)[1]. In this study, we have investigated the expression of DR3 in intestinal tissue from CD patients (n=23) and normal controls (n=15) by immunohistochemistry. Tissue sections were stained with an anti-DR3 antibody and evaluated by a semi-quantitative grading system. The average histological score of DR3 expression was 3.3 for controls compared to 5.9 for CD ($p<0.0001$), showing significantly increased expression of DR3 in CD colon. In normal intestine, DR3-positive cells were predominantly found dispersed in the mucosal lamina propria and in lymphatic nodules as part of the gut-associated lymphoid tissue. In contrast, many DR3-positive cells were found in lamina propria as well as in submucosa and muscularis externa in CD intestine. Staining of serial sections for CD3 indicated DR3-expression on a subset of T cells in both CD and normal intestine. These data demonstrate increased infiltration of DR3-positive cells in the intestinal wall of CD patients. [1]Bamias et al., PNAS 2006 (103:8441-6). Takedatsu et al., Gastroenterology 2008 (135:552-67).



DR3-positive infiltrating immune cells in CD colon. Immunohistochemical staining for DR3 shows infiltration of all layers of the intestinal wall by DR3-positive immune cells in Crohn's disease.

T.76. The Role of Secretory Leukoprotease Inhibitor in Regulating Antimicrobial Responses in the Gastrointestinal Tract

Celia Menckeberg[1], Ytje Simons-Oosterhuis[1], Dicky Lindenbergh-Kortleve[1], Hankje Escher[1], Georg Kraal[2], Janneke Samsom[1]. [1]Erasmus MC, Rotterdam, Netherlands; [2]VU University Medical Center, Amsterdam, Netherlands

Crohn's disease (CD) is a chronic intestinal inflammation characterized by a deregulated mucosal immune response to microbial products. Secretory leukoprotease inhibitor (SLPI) is a potent inhibitor of microbially induced nuclear factor NF-κB activation. Although expression of SLPI was previously thought to be restricted to epithelial cells at the mucosa, we have shown that mucosal myeloid cells also highly express SLPI. The aim of this study was to examine SLPI expression and determine its function in CD pathogenesis. Immunohistochemical detection of SLPI in intestinal biopsies from CD patients showed SLPI positive foci in inflammatory infiltrates in a subgroup of patients. As immunohistochemical data suggested that SLPI was expressed by monocytic cells, the immune regulatory role of SLPI in the monocytic cell line Thp-1 was investigated. Knock-down of SLPI dramatically enhanced inflammatory interleukin-8 and tumor necrosis factor-α release by Thp-1 in response to stimulation with bacterial lipopolysaccharide. These data infer that SLPI is expressed in intestinal biopsies of a subgroup of CD patients and may act as a potent inhibitor of local inflammation. Elucidation of the role of SLPI in CD may contribute to understanding disease pathogenesis and yield a biomarker for a subgroup of patients with distinct disease activity.

T.77. Different Frequency of Lamina Propria CD4+LAP+ Cells in Crohn's Disease and Ulcerative Colitis

Antonella D'Ambrosio[1], Andrea Cossu[1], Massimo Sanchez[1], Anna Maria Pronio[2], Chiara Montesani[2], Mauro Di Camillo[2], Piero Vernia[2], Monica Boirivant[1]. [1]Istituto Superiore di Sanità, Roma, Italy; [2]University Sapienza, Roma, Italy

A CD4+CD25- regulatory T cell population expressing the surface TGF-β in its latent form LAP (Latency Associated Peptide) was previously identified and proved to be protective in murine models of colitis (J.Immunol. 2003,170:2516, J.Immunol. 2005,174:3237). In humans, we showed the presence of LP CD4+LAP+ T cells in patients with IPAA for ulcerative colitis (UC) (IBD 2008,14:662). We investigated the prevalence of LP

CD4+LAP+ T cells in IBD patients. We found that the majority of LP CD4+LAP+ were Foxp3 negative and that LP CD4+LAP+ CD25- sorted cells from controls were able to inhibit the proliferation of *in vitro* αCD3/28 stimulated autologous LP CD4+LAP- CD25- cells. LPMC isolated from surgically resected specimens from Crohn's Disease patients showed a reduced % of LP CD4+LAP+ while LPMC from UC patients showed an increased proportion of CD4+LAP+ T cells when compared to controls. The % of CD4+LAP+ cells was significantly higher in LPMC from biopsy specimens of UC active patients when compared to inactive patients who showed values comparable to controls (11.7 ± 2 active UC vs 3.5± 1.6 Controls; mean ± sem, p=0.005) Conclusions: In humans, LP CD4+LAP+ cells are mostly Foxp3- and display regulatory activity. These cells are selectively increased in UC.

T.78. Impact of Maternal Inflammation on Fetal Gut Programming and Susceptibility to Crohn's Disease-like Ileitis

Katharina Heller[1], Jana Hemmerling[1], George Kollias[2], Dirk Haller[1]. [1]Technical University of Munich, Freising- Weihenstephan, Germany; [2]Biomedical Sciences Research Center Alexander Fleming, Vari, Greece

Maternal stress during pregnancy has long-term consequences for disease risk of the offspring. We aim to investigate the impact of maternal inflammation on fetal gene expression in intestinal epithelial cells (IEC), using the heterozygous mouse model for Crohn's disease ileitis - TNFΔARE/+ mice. Female TNF+/+ (WT dam) were bred with male TNFΔARE/+ (ARE sire) and vice versa. WT and ARE offspring were generated from healthy WT and inflamed ARE dams, respectively. Offspring were sacrificed 17.5 days post conception (dpc), 1, 3 and 8 weeks postnatal. IEC from frozen ileal samples were captured by laser microdissection. Total RNA was extracted for gene expression analysis. Paraffin-embedded ileal tissue was analyzed pathologically. Maternal inflammation impacts global gene expression of ileal epithelium in 17.5 dpc fetuses with 2225 regulated genes in WT (FC of ±1.5, p-value <0.05). Reg3β and FABP was strongest regulated. Tissue pathology (WT: 0.39+/-0.31 vs. 0.50+/-0.14; ARE: 4.08+/-0.85 vs. 3.96+/-0.71) was unaffected in all offspring. Maternal inflammation strongly affects the gene expression of the fetal gut, but does not influence the susceptibility to Crohn's ileitis. These data indicate that maternal inflammatory signals shape the fetal gut program but are overwritten in the offspring by environmental triggers.

T.79. The Post-translational Oxidized Modification of Peroxiredoxin-VI in Ulcerative Colitis

Tomohisa Takagi, Yuji Naito, Kazuhiko Uchiyama, Hitomi Okada, Tomoko Oya-Ito, Megumi Takaoka, Katsura Mizushima, Yasuko Hirai, Toshifumi Tsuji, Munehiro Kugai, Hiroyuki Yoriki, Naohisa Yoshida, Kazuhiro Katada, Osamu Handa, Takeshi Ishikawa, Nobuaki Yagi, Naoki Wakabayashi, Hideyuki Konishi, Satoshi Kokura, Hiroshi Ichikawa, Toshikazu Yoshikawa. Kyoto Prefecural University of Medicine, Kyoto, Japan

Aims: Peroxiredoxin (Prx)-VI belongs to the 1-Cys Prx subfamily, which is one of nonselenium peroxidase family that is involved in cell defense against oxidative stress and in redox regulation of intracellular signaling. Our previous proteomics analysis for the inflamed intestinal mucosa in murine dextran sodium sulfated (DSS)-induced colitis revealed the decreased expression of Prx-VI. In this study, we investigated the expression of Prx-VI in the colonic mucosa in the patients with active ulcerative colitis (UC). Furthermore, we also investigated the posttranslational oxidized modification of Prx-VI in the intestinal inflammation. Materials and methods: The expression of Prx-VI mRNA and protein was determined by Realtime-PCR and western blotting in normal colon mucosa in human non-cancerous mucosa of patients with colon cancer and in inflamed mucosa obtained from patients with active UC. Furthermore, the expression of the oxidized-modified proteins was also determined by immunoblotting using monoclonal antibody against oxidized Prx-VI. In additional study, we measured the activity of peroxidase in oxidized Prx-VI. Results: The colonic expression of Prx-VI mRNA and protein was down-regulated in inflamed mucosa. Immunohistochemical study showed that Prx-VI expression was mainly localized in the epithelial cells. Furthermore, the oxidized Prx-VI was increased in the inflamed intestinal mucosa and the peroxidase activity of the oxidized Prx-VI was inhibited. Conclusion: In this study we showed decreased Prx-VI expression and increased oxidized Prx-VI in inflamed colonic mucosa. Our results indicate that the antioxidant function by Prx-VI is disrupted in the intestinal inflammation. Therefore, Prx-VI may be a therapeutic molecule for the intestinal inflammation.

T.80. SERPINB1 (Monocyte Neutrophil Elastase Inhibitor) is Related to the Pathogenesis of Ulcerative Colitis

Kazuhiko Uchiyama, Yuji Naito, Tomohisa Takagi, Katsura Mizushima, Ryusuke Horie, Hiroyuki Yoriki, Toshifumi Tsuji, Munehiro Kugai, Naohisa Yoshida, Kazuhiro Katada, Takeshi Ishikawa, Osamu Handa, Nobuaki Yagi, Satoshi Kokura, Toshikazu Yoshikawa. Kyoto Prefectural University of Medicine, Kyoto, Japan

Background and aims: SERPINB1 is known as monocyte neutrophil elastase (NE) inhibitor. In this study, we investigated the role of SERPINB1 in the pathogenesis of ulcerative colitis. Materials and Methods: The colonic expression of SERPINB1 mRNA and protein were determined in normal colon and inflamed mucosa from patients with ulcerative colitis (UC). Localization of SERPINB1 was identified by immunohistochemistry. We used YAMC cells, which is mouse normal colonic epithelial cells, to show the induction of NE by H2O2. Then we transfected mouse SERPINB1 gene and silenced NE to YAMC cells. Cellular survival was measured by WST-8 assay. Results: SERPINB1 mRNA and protein expression was significantly increased in colonic mucosa obtained from patients with ulcerative colitis. Immunohistochemical study showed that SERPINB1 expression was localized not only neutrophils and monocyte but also in the epithelial cells. NE was highly induced in YAMC cells by H2O2 treatment. SERPINB1 transfected and NE silenced YAMC cells showed significant resistant against H2O2 treatment. Conclusion: SERPINB1 protects colonic epithelial cells via inhibition of NE activity in colonic epithelial cells induced by H2O2 and upregulated in colonic mucosa of UC patients. These results indicate that SERPINB1 may be a novel marker and related to the pathogenesis of UC.

T.81. Is Ulcerative Colitis an Atypical Th2-mediated Disease Characterized by Excess Production of IL-13?

Paolo Biancheri[1], Ijeoma Joe-Njoku[1], Laura Rovedatti[2], Anna Vossenkaemper[1], Antonio Di Sabatino[2], Gino Corazza[2], Thomas MacDonald[1]. [1]Barts and the London School of Medicine and Dentistry, London, United Kingdom; [2]Fondazione IRCCS Policlinico S. Matteo, University of Pavia, Pavia, Italy

Background and Aims: Interleukin (IL)-13 is produced mostly by Th2 cells and appears to be involved in animal models of gut fibrosis. It has also been suggested that IL-13 is over-expressed in ulcerative colitis (UC). We have therefore investigated IL-13 production in the mucosa and muscle layers of patients with UC or Crohn's disease (CD). Methods: Biopsies and lamina propria mononuclear cells (LPMCs) from inflamed colon of 11 CD, 9 UC and 15 control patients were cultured *ex vivo* or with anti-CD3/CD28-antibodies. IL-13, IL-17 and interferon (IFN)-gamma production was measured by ELISA. Strictured and non-strictured muscle layer explants from 6 CD patients were cultured *ex vivo* and IL-13 and collagen production measured. Results: IL-13 production did not differ between CD, UC and control biopsies although IFN-gamma and IL-17 were significantly higher in CD and UC than in controls. Anti-CD3/CD28-stimulated LPMCs showed a small increase in IL-13 production without significant differences between the groups. Collagen production was higher in strictured CD, but IL-13 did not differ between strictured and non-strictured CD explants. Conclusions. IL-13 seems to play a minor role compared to IL-17 and IFN-gamma in mucosal inflammation in IBD and may not be involved in intestinal fibrogenesis.

T.82. Macrophages from Crohn's Disease Patients Exhibit Deficient Healing Functions

Dominique Velin[1], Fabrizia D'Angelo[1], Eric Bernasconi[1], Michel H Maillard[1], Catherine Pythoud[1], Daniel Bachmann[1], Pierre Michetti[2]. [1]CHUV, Lausanne, Switzerland; [2]La Source-Beaulieu, Lausanne, Switzerland

Background: We previously reported that myeloid cells can induce mucosal healing in a mouse model of acute colitis. Promotion of mucosal healing is becoming a major goal in the treatment of Crohn's disease. Our aim in this study is to investigate the pro-repair function of myeloid cells in healthy donor (HD), ulcerative colitis (UC) and Crohn's disease patients (CD). Methods: Peripheral blood mononuclear cells (PBMC) were isolated from blood samples by Ficoll density gradient. Monocytic CD14+ cells were positively selected and differentiated *ex vivo* into macrophages (Mφ). The repair function of PBMC, CD14+ monocytic cells and macrophages was evaluated in an *in vitro* wound healing assay. Results: PBMC and CD14+ myeloid cells were not able to promote healing. On the contrary, we observed that HD and UC Mφ promoted wound healing. Remarkably, CD Mφ were not able to promote wound healing. Conclusion: We showed that CD Mφ, unlike HD and UC Mφ, are defective in promoting wound healing. Our results are in keeping with the current theory of CD as an innate immunodeficiency. Defective Mφ may contribute to the mucosal healing defects in CD patients and to the subsequent chronic activation of the adaptive immune response.

T.83. Circulating 'Gut-**Tropic' Vδ2 T Cells are Altered in Crohn**'s Disease Patients and their Siblings

Neil McCarthy[1], Charlotte Hedin[1], Shaumick Bhattacharjee[1], Georgina James[1], Kevin Whelan[2], James Lindsay[1], Andrew Stagg[1]. [1]Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom; [2]King's College London, London, United Kingdom

Introduction: Crohn's disease (CD) is driven by Th1/Th17 responses to the gut microbiota. In primates, circulating Vγ9Vδ2$^+$ (Vδ2)T cells can produce both IFNγ and IL-17A, and they expand and accumulate in mucosal tissues upon *in vivo* activation, but a role for these cells in human CD is unknown. Method: Blood Vδ2T cells were analysed by flow-cytometry in CD patients (n=22) and their asymptomatic siblings (at increased risk of disease; n=14), or were activated *in vitro* with HDMAPP phosphoantigen (a synthetic analogue of a Vδ2T cell-specific microbial metabolite). Results: HDMAPP-stimulated Vδ2T cells proliferated, up-regulated 'gut-homing' integrin β7, and produced IFNγ and TNFα, but not IL-10, IL-17A or IL-22. Median Vδ2T cell numbers were significantly lower in CD patients than in controls (median 7,063 and 18,103ml^{-1} respectively; p=0.046) which was due to the effects of azathioprine immunosuppression in some CD patients (n=11; 812 Vδ2T cells ml^{-1}; 0.19% of total T cells) who displayed preferential loss of Vδ2T cells over αβT cells. Strikingly, asymptomatic siblings of CD patients had significantly higher numbers of anti-microbial Vδ2T cells in blood than healthy controls (18,103 and 37,903ml^{-1} respectively; p=0.049). Discussion: Vδ2T cell expansion may be driven by defective intestinal barrier function in CD and in 'at-risk' relatives, permitting translocation of microbial antigens and activation of circulating Vδ2T cells.

T.84. Regulation of NOD2 Signaling by CYLD

Diana Legarda, Adrian Ting. Mount Sinai School of Medicine, New York, NY

NOD2 is an intracellular PRR that detects MDP, a product of the bacterial cell wall, resulting in the activation of pro-inflammatory signal transduction pathways. NOD2 mutations are associated with an increased risk for Crohn's Disease (CD). Although several studies have examined the transmission of NOD2 signaling, little is known about its negative regulation. The attachment and removal of K63-linked polyubiquitin chains is one way of controlling protein-protein interaction and signal transduction events. One such deubiquitinating enzyme is CYLD. CYLD has been shown to be downregulated in colonic epithelial cells from CD and ulcerative colitis (UC) patients. The CYLD gene is located on chromosome 16q12, immediately adjacent to the NOD2 gene located at 16q21, which has been identified as a susceptibility locus for IBD. Therefore, given the documented role for CYLD as an anti-inflammatory molecule, the potential role for CYLD in CD, and the NOD2 polymorphisms associated with CD, we hypothesize that components of the NOD2 signal transduction pathway can be regulated by CYLD. This project is part of a larger study that will

allow us to clarify the contribution of CYLD to the regulation of NOD2 signaling and to examine the potential role of CYLD in the pathogenesis of CD.

T.85. Identification and Characterization of Adherent-invasive Escherichia Coli in Intestinal Mucosa of Chilean Patients with Crohn's Disease

Marjorie De la Fuente[1], David Díaz-Jimenez[1], Enzo Candia[1], Lucia Nuñez[1], Cristobal Suazo[2], Manuel Alvarez-L[3], Francisco Lopez-Kostner[2], Roberto Vidal[1], Rodrigo Quera[2], Marcela Hermoso Ramello[1]. [1]Universidad de Chile, Santiago, Chile; [2]Clínica Las Condes, Santiago, Chile; [3]P. Universidad Católica de Chile, Santiago, Chile

Adherent-invasive Escherichia coli (AIEC) strains have been suggested to play a role in the pathogenesis of Crohn's disease (CD). Our aim was to isolate and characterize these bacteria in Chilean CD vs. control patients, and compared them with the reference strain LF82, (Inflamm Bowel Dis.2008 14:1051). We studied mucosa from 17 patients with CD, 7 with other intestinal diseases (e.g., diverticulitis, irritable bowel syndrome) and 17 healthy control (colon cancer). AIEC were recovered from intestinal biopsies using the gentamicin protection assay. Bacterial isolates were analyzed by Pulsed field gel electrophoresis (PFGE) and virulence genes were characterized by PCR. AIEC strains were isolated in 8/17 CD, 1/17 controls and 5/7 patients with other pathologies. The mean CFU/biopsy in CD and other pathologies was higher compared to control patients (200.5, 139, 0.1 respectively). The studied strains were able to invade intestinal Caco-2 cells. The PFGE analysis shows that E. coli strains isolated from the same patients are genetically closely related, nevertheless, when comparing strains from different patients, including LF82, we observed a genetically heterogeneous population. The comparison of virulence genes, in contrast as previously described, two strains isolated from different CD patients harbored the gene daaE, typically of diffuse-adhering escherichia coli strains.

T.86. Secretion of Soluble TLR2 by Lamina Propia Mononuclar Cells is Increased in Ulcerative Colitis

Marcela Hermoso Ramello[1], Enzo Candia[1], David Díaz-Jimenez[1], Nancy Farfán[1], Lucia Nuñez[1], Patricia Langjahr[1], Marjorie De La Fuente[1], Francisco López-Kostner[2], Rodrigo Quera[2]. [1]Universidad de Chile, Santiago, Chile; [2]Clínica Las Condes, Santiago, Chile

Toll-like receptor 2 (TLR2) is a pattern recognition receptor that has been revealed to play key roles in inflammatory bowel diseases (IBD). Soluble TLR2 (sTLR2) variants have been shown to block TLR2-activated responses. Despite the roles of TLR2 in intestinal homeostasis, no study has elucidated the expression and cellular source of sTLR2 in IBD. Expression of TLR2 was determined in mucosa from IBD and control patients by RT-PCR and ELISA/immunoblot, respectively. The sTLR2 variant was analyzed by ELISA in conditioned medium from mucosa explants and lamina propria mononuclear cells (LPMCs) cultures. TLR2 and CX3CR1 expression in CD33+ cells was performed by FACS. Fractalkine (FKN) expression was also determined in mucosa from IBD and control patients by ELISA. High level of total TLR2 and transmembrane TLR2 were found in intestinal mucosa from UC compared to CD and control patients. The accumulation of sTLR2 was elevated in conditioned medium from intestinal mucosa and LPMCs from UC patients. Additionally, FKN levels, %TLR2+/CD33+ and TLR2+/CD33+/CX3CR1+ cells in LPMCs were higher in UC compared to CD and control patients. We conclude that sTLR2 is part of the intestinal mucosa innate immunity and seems to be produced by LPMCs regulating the inflammatory response in IBD.

T.87. RGS1 is a Key Regulator of Human T Cell Migration and a Potential Target for Therapy in IBD

Deena Gibbons[1], Lucie Abeler-Dorner[4], Tim Raine[3], Peter Irving[5], Anett Jandke[4], Melanie Wencker[4], Chris Rudd[3], Il-Young Hwang[2], John Kehrl[2], Adrian Hayday[1]. [1]King's College London, London, United Kingdom; [2]National Institutes of Health, Bethseda, MD; [3]University of Cambridge, Cambridge, United Kingdom; [4]Cancer Research UK, London, United Kingdom; [5]Biomedical Research Centre at Kings Health Partners, London, United Kingdom

How lymphocytes selectively respond to, or ignore, chemotactic signals in the gut is very poorly understood. Regulator of G protein signalling 1 (RGS1) might be a key regulator of cells in the gut, based on its very high differential expression in gut versus systemic mouse T cells. This study shows that RGS1 is elevated (50-100 fold) in human gut T cells compared to peripheral T cells. RGS1 gain-of-function profoundly reduces T cell migration to lymphoid homing chemokines whereas RGS1 loss-of-function enhances directional chemotaxis in gut T cells. RGS1 levels are further elevated in T cells derived from inflamed gut and colonic inflammation was significantly reduced if RAG2-/- mice were injected with Rgs1-/- T cells compared to those injected with wild type T cells. Stimulation of gut T cells isolated from RAG2-/- mice injected with either Rgs1-/- or wt T cells induced similar cytokine production, which suggests it is the inability of Rgs1-/- T cells to remain sequestered in the gut that reduces colitic pathology. This may be of significant interest in other diseases where T cells become sequestered and with which RGS1 has been associated in genome wide association studies namely celiac disease, multiple sclerosis and Type I diabetes.

T.89. Collagenous Colitis Patients Demonstrate a Th1/CTL-associated Gene Expression Profile with Increased Frequencies of Ki67+ Proliferating and CD45RO+ Activated/memory CD8+ and CD4+8+ Mucosal T Cells

Ashok Kumawat, Hilja Strid, Kristina Elgbratt, Nils Nyhlin, Curt Tysk, Johan Bohr, Elisabeth Hultgren-Hörnquist. Orebro University, Orebro, Sweden

Collagenous colitis (CC) is a chronic inflammatory bowel condition of unknown aetiology. Lamina propria lymphocytes (LPLs) and intraepithelial lymphocytes (IELs) isolated from mucosal biopsies from CC patients and healthy individuals were phenotypically characterized by flow cytometry. In CC patients, the frequencies of CD8+ and CD4+8+ double positive (DP) LPLs as well as IELs were increased compared to healthy controls. In

addition, the frequency of proliferating Ki67+CD8+ as well as activated/memory CD45RO+ CD8+ and DP LPL and IEL T cells were increased in CC patients. In contrast, the frequency of CD4+ LPLs was decreased. Despite this, the CD4+ population consisted of higher frequencies of CD45RO+ as well as Ki67+ cells. Quantitative RT-PCR on mucosal biopsies revealed increased expression of genes encoding T-bet, IFN-γ and IL-12, involved in maturation of Th1 and CD8+ T cells, in CC patients compared to healthy controls. In contrast to UC patients, there was no increased expression of genes involved in Th17 differentiation, i.e. IL-6, IL-17, ROR-γ, or IL-23. Preliminary data using PCR-array demonstrate >5 times increased expression of genes encoding IL-1α, IL-2, iNOS, CCR2, and HLA-DRβ, and more than 5 times reduced expression of TGF-β1, IL-2Rα and IL-5 in CC patients compared to healthy controls.

T.90. CEACAM5 is Upregulated in the Epithelium of Crohn's Disease Patients Under Inflammatory Conditions in Response to Proinflammatory Cytokines, such as IFN Gamma and IL-22

Vera Denmark[1], Jian Yu Xu[2], Stephanie Dahan[1], Lloyd Mayer[1]. [1]Mount Sinai School of Medicine, New York, NY; [2]James J. Peters VA Medical Center, New York, NY

Using a CEACAM5 specific Ab, T84.66, we had previously reported a lack of expression of CEACAM5 on the surface of gut epithelial cells in Crohn's disease (CD) patients. Here we investigate the effect of inflammation on CEACAM5 expression in Crohn's ileal and colonic epithelium. We observed an increase in CEACAM5 epithelial expression by immunofluorescent staining of colonic biopsies and by Western blot analysis of resected ileal and colonic specimens obtained from patients with active CD as compared to those with quiescent disease. The level of CEACAM5 expression correlated with the degree of histological inflammation. IFNγ and IL-22 induced the expression of CEACAM5 protein in epithelial cell lines. This upregulation of expression appeared to occur at the transcriptional level, as CEACAM5 mRNA levels were increased after treatment with IFNγ and IL-22. IL-17 did not appear to have a significant effect on CEACAM5 expression. Thus, the upregulation of epithelial CEACAM5 expression is likely driven by the specific cytokine milieu found in the gut mucosa during inflammation. Increased epithelial expression of CEACAM5 in CD might be one of the early signs of mucosal inflammation and, therefore, may be used as a potential marker of postoperative CD recurrence in the future.

T.91. Mucosal Addressin Cell-adhesion Molecule-1 (MAdCAM-1) is Down-regulated by Anti-TNF-alfa Antibodies in Inflammatory Bowel Disease (IBD)

Antonio Di Sabatino[1], Laura Rovedatti[1], Stefania Vetrano[2], Paolo Biancheri[3], Cristina Ubezio[1], Silvio Danese[2], Thomas MacDonald[3], Gino Roberto Corazza[1]. [1]Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy; [2]IRCCS Istituto Clinico Humanitas, Milano, Italy; [3]Barts and the London School of Medicine and Dentistry, London, United Kingdom

Background and aim: MAdCAM-1, expressed on gut endothelium, is critical in lymphocyte homing to the gut. MAdCAM-1 is up-regulated in active IBD and promotes the recruitment of circulating α4β7+ T cells leading to chronic bowel inflammation. TNF-α is known to up-regulate MAdCAM-1 expression in inflamed gut mucosa. On this basis, we explored in *ex vivo* experiments the influence of TNF-α-blockade on mucosal MAdCAM-1 expression in IBD. Methods: Colonic biopsies were collected from inflamed mucosa of 15 IBD patients and normal mucosa of 10 control subjects. Biopsies were cultured with 10μg/ml of infliximab or adalimumab or IgG1, in the presence or absence of the p38 mitogen-activated protein kinase inhibitor SB203580. MAdCAM-1 expression was determined on tissue homogenates by immunoblotting. Results: MAdCAM-1 expression was significantly higher in inflamed IBD than normal mucosa. A down-regulation of MAdCAM-1 was observed in IBD biopsies cultured with infliximab or adalimumab compared with IgG1-treated biopsies. SB203580 neutralised the down-regulatory effect of both infliximab and adalimumab on MAdCAM-1. Conclusions: Our findings show an *ex vivo* inhibitory effect of both infliximab and adalimumab on MAdCAM-1 expression in IBD via MAPK signaling. The amelioration of mucosal inflammation through the inhibition of T cell recruitment might be a novel mechanism of action of anti-TNF antibodies in IBD.

T.92. Methanandamide Modulates the Function of Myofibroblasts Isolated from Fibrostenosing Crohn's Disease (CD) Patients

Antonio Di Sabatino[1], Laura Rovedatti[1], Paolo Biancheri[2], Cristina Ubezio[1], Thomas MacDonald[2], Gino Roberto Corazza[1]. [1]Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy; [2]Barts and the London School of Medicine and Dentistry, London, United Kingdom

Background and aim: The endocannabinoid system is involved in liver fibrosis. To elucidate the role of cannabinoids in the fibrogenic process in CD, we explored the *in vitro* effects of methanandamide, the synthetic analogue of the major cannabinoid ligand anandamide, on collagen production and migration by intestinal myofibroblasts isolated from CD strictures. Methods: Myofibroblasts were isolated from surgical specimens collected from colonic strictured and unstrictured areas of 12 patients with fibrostenosing CD. Subconfluent monolayers of myofibroblasts were incubated for 24h with or without methanandamide. Collagen was measured on myofibroblast supernatants. A wound healing scratch assay was performed on myofibroblast monolayers grown in the presence or absence of methanandamide. Photographs were taken at time intervals using a digital camera attached to a light microscope. Results: Methanandamide significantly down-regulated collagen production by CD strictured and unstructured myofibroblasts. Methanandamide significantly improved migration of CD strictured and unstrictured myofibroblast in comparison to medium only. In unstimulated conditions CD strictured myofibroblasts showed a significantly lower migration capacity and higher collagen production compared with unstrictured CD myofibrobalsts. Conclusions: Our results highlight an anti-fibrogenic role of methanandamide in CD. Targeting the endocannabinoid system might represent a therapeutic strategy in counteracting intestinal fibrogenesis in CD.

T.93. The Role of Histone Deacetylase Inhibition in *ex vivo* and *in vitro* Models of IBD

Alexander Edwards, Rebecca Morgan-Walsh, Markus Gwiggner, Andrew Claridge, Graham Packham, Sylvia Pender. University of Southampton, Southampton, United Kingdom

Tissue destruction and inflammation of the mucosa are characteristic of inflammatory bowel diseases (IBD) including Crohn's Disease (CD) and Ulcerative Colitis (UC). Recent work showed that inhibition of histone deacetylases (HDAC) may have a protective anti-inflammatory effect in murine models of UC. We hypothesise that HDAC inhibitors (HDI) have anti-inflammatory effects in human IBD. Tissue biopsies from patients with IBD were isolated and cultured for 8 or 24hrs +/-FK228 (HDAC inhibitor) at a range of doses. Human gut fibroblasts were stimulated *in vitro* with TNF or IL-1B and treated with FK228. RNA and supernatants were analysed using qRT-PCR, microRNA arrays, western blotting and histology. FK228 increased Foxp3 and IL-10 gene expression while simultaneously decreasing CYLD, IFN-gamma, IL-17 and TNF-alpha gene expression in a CD biopsy model. The use of FK228 in a UC biopsy model led to a decrease in CYLD, IFN-gamma, MMP-3, MMP-10, TGF-beta, TIMP-1 and TIMP-3 while increasing TNF-alpha gene expression. In contrast with the CD biopsy, Foxp3 and IL-10 gene expression were decreased. *In vitro*, FK228 increased CYLD, Foxp3 and TGF-beta while decreasing TNF-alpha gene expression. Preliminary data suggest that HDI may have anti-inflammatory effects in IBD, although HDI may have different effects in CD and UC.

T.94. Novel Proteases Involved in the Function of Intestinal Fibroblasts in IBD

Rebecca Morgan-Walsh[1], Alexander Edwards[1], Siobhan O'Leary[1], Andrew Claridge[1], Markus Gwiggner[1], Nicholas Beck[2], Alexander Mirnezami[2], Fraser Cummings[2], Arlette Darfeuille-Michaud[3], Sylvia Pender[1]. [1]University of Southampton, Southampton, United Kingdom; [2]Southampton General Hospital, Southampton, United Kingdom; [3]Université d'Auvergne, Clermont-Ferrand, France

The etiology of inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis (UC) is still unknown. It is widely believed that these disorders are the result of inappropriate activation of the immune system to microflora derived antigens in genetically susceptible individuals. During IBD the epithelial layer of the gut can become damaged, enabling access of bacterial floral to the underlying tissues. We are therefore interested in the response of lamina propria myofibroblasts to stimulation by bacteria. A systemic review by the EU funded IBDase consortium resulted in the generation of a candidate gene list of novel proteases (P) and protease inhibitors (PI) which may be novel risk factors in IBD. Primary myofibroblasts were co-cultured with both a non-pathogenic E. coli and a pathogenic adherent invasive Escherichia coli (AIEC) isolated from a Crohn's patient. These cells were also stimulated with pro-inflammatory cytokines (IL-1β or TNF-α) or with TGF-β to re-capitulate the conditions in IBD *in vitro*. We have examined myofibroblast expression of the newly indentified P/ PI genes as well as that of the matrix metalloproteinases (MMPs) and their inhibitors the TIMPs, as dis-regulation of the MMP: TIMP balance can lead to a loss of tissue integrity *in vivo*.

T.95. Human Intestinal Fibroblasts Amplify the Inflammatory Response in Crohn's Disease by Inducing the Maduration of Dendritic Cells

Maica Masamunt Estrella, Jeroni Luna, Raquel Cabezon, Carolina España, Tiago Nunes, Daniel Benitez, Miquel Sans. Hospital Clínic i Provincial, Barcelona, Spain

Introduction: human intestinal fibroblasts (HIF) produce chemokines in response to pro-inflammatory stimuli, thus amplifying the inflammatory response. The aim of this study was to characterize the effect of TNFα and IL-17 in HIF from Crohn's disease patients and healthy controls, as well as the impact of HIF activation in the maturation of dendritic cells. Methods: Production of CCL20, MCP-1 and IL-8 by HIF in response to TNFα and IL-17 was quantified. Immature dendritic cells (iDCs) were obtained from peripheral blood monocytes and the effect produced by the supernatants of HIF cultures in their maturation and response to LPS were studied. Results: HIF from Crohn's disease present a basal production of CCL20 and IL-8 significantly higher than control HIF and simultaneous stimulation of HIF with TNFα and IL-17 results in a marked synergistic effect. HIF stimulation with TNFα and IL-17 does not modify the production of MCP-1. iDCs stimulation with supernatants of HIF cultures stimulated with TNFα and IL-17 increases the expression of CD86, CD80, CD83 and ICAM-1. Dendritic cells exposed to these supernatants show a marked response to LPS. Conclusion: TNFα and IL-17 induce pro-inflammatory chemokine production by HIF, which in turn, stimulate maturation of iDCs, amplifying the inflammatory response.

T.96. Peripheral Immune Response to Bacterial Stimulation is Reduced in Crohn's Disease and Ulcerative Colitis Patients Compared with Healthy Controls

Juliane Brueckner, Lloyd Mayer. Mount Sinai School of Medicine, New York, NY

Background: The role of the gut microbiota in IBD pathophysiology is incompletely understood. Methods: Peripheral APC:T cell co-cultures were stimulated with commensal Bacterial Antigen (BAg), extracted from endoscopic stool aspirates. Results: In normal peripheral APC:T cell co-cultures, addition of BAg resulted in significantly increased APC derived cytokines TNF, IL-6, IL-12/23 p40 and IL-1β as well as upregulation of APC expression of CD69 and CD25. In contrast, co-cultures with BAg in CD and UC patients revealed that TNF secretion was significantly reduced compared to normal controls, and IL-12/23p40 and IL-6 secretion and CD69 upregulation were markedly lower. T cell activation measured as IFNγ secretion and CD69 upregulation was found in normal but not in IBD patients. Interestingly, use of autologous serum instead of FBS markedly increased these findings. In contrast, when co-cultures were cultured with BAg derived from CD or UC patients as opposed to normal patients resulted in higher CD25 expression and a trend towards higher secretion of IL-1beta and IL-10 (p=ns). Conclusion: This functional study suggests an

impairment of innate immunity in IBD patients.

T.97. Differential Functional Response to Innate and Adaptive Immunostimulation for Nitric Oxide (NO) Production by Human Gastrointestinal Stem Cell (hGISC) Derived Non-transformed Epithelial Cells versus Malignant Colonic Adenocarcinoma Epithelial Cell (EC) Line HT29

Aparna Panja, Ansamma Joseph, David Harrison, Asit Panja. AlfaGene Bioscience Inc., Somerset, NJ

Increased NO production by intestinal EC is thought to play an important role in inflammatory conditions such as IBD. However, little is known with regards to regulation of NO production in the human intestinal epithelium. Therefore, to gain insight about NO production regulation by intestinal EC, we studied the effects of innate (LPS) and adaptive (IFN-γ) immunostimulatory agents on a novel hGISC derived non-transformed primary EC system and compared the results to those obtained from a colonic adenocarcinoma EC line, HT29. A macrophage cell line MHS served as a positive control. Cells were cultured with/without LPS (1µg/ml), IFN-γ(200 u/ml), or in combination for 24 hours. Culture supernatants were analyzed for NO content by Griess reaction assay. Our results show that neither LPS nor IFN-γ alone affect NO production by intestinal ECs. However, NO production by hGISC derived primary ECs was dramatically increased when LPS and IFN-γ were added in combination. In contrast, malignant HT29 cells do not show any difference in NO synthesis even when LPS (innate stimuli) and IFN-γ (adaptive stimuli) were combined. These findings may help further elucidate intestinal inflammatory disease mechanisms and lead to the development of new more efficient treatments.

T.98. Oral Anti-CD3 Prevents T Cell Mediated Mucosal Damage in Mouse Models of Enteropathy and Colitis

Katharina Forster[1], Ashleigh Goethel[1], Ryan Lam[1], Sheela Manek[1], Catherine Streutker[2], Kenneth Croitoru[3]. [1]University of Toronto, Toronto, ON, Canada; [2]St. Michael's Hospital, Toronto, ON, Canada; [3]Mount Sinai Hospital, Toronto, ON, Canada

Medical therapy that alters the natural history of inflammatory bowel disease (IBD) has not been identified. We explored the effect of feeding anti-CD3-specific antibody in the $CD4^+CD45RB^{high}$ T cell transfer colitis model and in a model of T cell induced enteropathy. Oral anti-CD3 protected SCID recipients of pathogenic T cells from developing colitis. Likewise, feeding anti-CD3 prevented T cell induced enteropathy after intraperitoneal challenge with anti-CD3. Analyses of spleen and mesenteric lymph node cells in both models showed no differences in total cell counts, percentages of $CD4^+$ and $Foxp3^+$ cells between the treatment groups. Thus the clinical effects were not caused by induction of $Foxp3^+$ Treg, depletion or limiting the expansion of T cells. Cytokine analyses *in vitro* and *in vivo* showed reduced INFγ and increased IL-10 suggesting anergy and/or induction of Treg cells as possible mechanisms. We also observed an increase in IL-6 in oral anti-CD3 treated mice after T cell activation, but no changes in IL-17 compared to hIgG fed controls. Feeding anti-CD3 is new strategy to induce changes in the immune system that are independent of specific Antigen in preventing colitis progression. This approach is of clinical relevance in treating IBD where the “triggering Antigen” is unknown. Funded by the CCFC.

T.99. The Human CD3-specific Antibody Otelixizumab is Effective in Down Regulating the T Cell Response in Inflammatory Bowel Disease (IBD) *in vitro*

Anna Vossenkaemper, Thomas MacDoanld. Barts and the London School of Medicine and Dentistry, London, United Kingdom

Crohn’s disease and ulcerative colitis are chronic inflammatory conditions of the intestine usually treated with corticosteroids, azathioprine or more recently with anti-TNF antibodies. As T cells are critical in driving inflammation in inflammatory bowel disease (IBD), we tested the ability of a new humanized anti-CD3 antibody, otelixizumab, to reduce proinflammatory cytokine production in human IBD samples. Otelixizumab with or without anti-CD28 did not induce proliferation of mucosal T cells from control or IBD tissues. However mucosal explants from Crohn’s disease patients showed reduced production of IFN-gamma and IL17, and increased IL10 secretion after treatment with otelixizumab. Otelixizumab also reduced IFN-gamma and IL17 secretion by lamina propria mononuclear cells (LPMCs) from Crohn’s disease and ulcerative colitis patients. LPMCs from Crohn’s disease patients cultured *in vitro* with otelixizumab showed reduced T-bet expression. Otelixizumab, however, did not reduce the production of IFN-gamma by colonic LPMCs stimulated with IL12, IL15, or PMA/ ionomycin. This work suggests that otelixizumab may induce immune deviation in activated T cells in IBD samples and therefore may be successful in the treatment of IBD.

T.100. Nfkb1 Inhibits LPS-induced Gene Expression through TPL2-dependent ERK Activation

Xixing Zhao, Yanyan Wang, Bruce Horwitz. Brigham and Women's Hospital, Boston, MA

The Nfkb1 gene codes for the NF-κB family member p50 and its precursor p105. Nfkb1 inhibits microflora-induced colitis and LPS-induced expression of IL-12 p40, however the molecular basis for Nfkb1-mediated suppression has not been definitively determined. Expression profiling of LPS stimulated Nfkb1-/- bone marrow-derived macrophages (BMDM) revealed elevated expression of a number of interferon (IFN)-responsive genes, in addition to increased expression of IL-12 p40. This was associated with increased secretion of IFN-β and augmented activation of STAT1. It has previously been suggested that activation of STAT1 can enhance IL-12 p40 expression, however, an IFN-β-specific blocking antibody that inhibits LPS-induced STAT1 activation did not inhibit expression of IL-12 p40. Nfkb1-/- macrophages exhibit a defect in LPS-induced ERK activation because of functional deficiency in the MAP3K TPL-2, which is stabilized by association with the C-terminal domain of p105. Expression of the C-terminal domain of p105 in Nfkb1-/- BMDM rescued LPS-induced ERK activation as expected, and interestingly inhibited expression of both IL-12

p40 and IFN-β. These results indicate that the C-terminal region of p105 inhibits the induction of IL-12 p40 and IFN-β by facilitating LPS-induced ERK activation.

T.101. Differential Degradation of Anti-tumor Necrosis Factor-alpha Agents by Matrix Metalloproteinase (MMP)-3 and MMP-12 in Inflammatory Bowel Disease (IBD)

Paolo Biancheri[1], Antonio Di Sabatino[2], Susanne Snoek[1], Ijeoma Joe-Njoku[1], Laura Rovedatti[2], Nadja Ahmad[1], Gino Corazza[2], Thomas MacDonald[1]. [1]Blizard Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, London, United Kingdom; [2]Fondazione IRCCS Policlinico S. Matteo, University of Pavia, Pavia, Italy

Background and aims: MMPs cleave human IgG releasing single chain 32kDa Fc monomers. Anti-TNF-alpha agents exert their activity in the inflamed IBD mucosa, a site rich in activated MMPs. We have therefore investigated the degradation of anti-TNF-alpha agents by MMP-3 and MMP-12. Methods: The structures of infliximab, adalimumab, certolizumab pegol and etanercept were explored by immunoblotting after co-incubation for 24h with MMP-3 or MMP-12. The ability of MMP-3- or MMP-12-treated antibodies to neutralise TNF-alpha was tested. The four drugs were co-incubated for 24h with mucosal homogenates from inflamed areas of 6 IBD patients and from normal gut of 6 controls and changes in their structure were detected by immunoblotting. Results: Infliximab, adalimumab and etanercept were degraded by MMP-3 and MMP-12, releasing 32kDa Fc monomers and F(ab)2, whereas certolizumab was not cleaved. Only etanercept lost its ability to inhibit luciferase production by TNF-alpha-stimulated HeLa 57A cells after treatment with MMP-3 or MMP-12. None of the anti-TNF-alpha agents were degraded by the control mucosa, whereas only etanercept was cleaved by IBD tissue. Conclusions: Etanercept is unable to neutralise soluble TNF-alpha after MMP-cleavage and is degraded *in vivo* by inflamed IBD mucosa. This may explain its inefficacy in IBD patients.

T.102. Myleoid Cells Promote Wound Repair through Secretion of Hepatocyte Growth Factor

Dominique Velin[1], Eric Bernasconi[1], Fabrizia D'Angelo[1], Michel Maillard[1], Catherine Pythoud[1], Daniel Bachmann[1], Pierre Michetti[2]. [1]CHUV, Lausanne, Switzerland; [2]La Source-Beaulieu, Lausanne, Switzerland

Background: Inflammatory bowel diseases (IBD) are characterized by chronic inflammation of the gut associated with impaired wound repair. We previously reported that myeloid cells can induce wound repair in the mouse model of dextran sulfate sodium (DSS) induced-colitis. In this study, we analysed the mechanism used by myeloid cells to promote wound repair. Methods: either we isolated myeloid cells from spleen and colon lamina propria or we generated bone marrow derived macrophages (BMM) from unmanipulated or colitic Balb/c mice and evaluated their repair functions in an *in vitro* wound repair assay. Results: spleen CD11b+, lamina propria CD11b+Gr1+ and CD11b+Gr1high cells and BMM promoted wound repair. We demonstrated that these myeloid cells produce Hepatocyte growth factor (HGF), a well known factor promoting wound repair both *in vivo* and *in vitro*. Inhibition of the HGF receptor or HGF myeloid cell production by siRNA inhibits at least 50 to 90% of the myeloid cell-induced wound repair. Lastly, we observed that myeloid cells accumulating in granulomas of DSS colitic mice produce HGF. Conclusion: we provide evidence that myeloid cells promote wound repair through HGF secretion. These results suggest that defective myeloid cells may contribute to the impaired wound repair in IBD patients.

T.103. Effects of Vitamin A in the Gut Homeostasis

Samara Medeiros, Flavia Loli, Andrezza Santiago, Andrea Alves, Rafael Pires, Rafael Rezende, Ana Faria. Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Introduction: Vitamin A has been reported to present regulatory effects on cell proliferation, antioxidant activity and ability of differentiate naïve T cells into regulatory T cells (Tregs). Objectives: Our aim in this study is to evaluate the effects of dietary deficiency and supplementation with vitamin A in murine models of inflammatory bowel disease. Methods and Results: We fed four different diets to C57BL/6 mice: normal diet (4000 UI vitamin A), vitamin A-free diet, and vitamin A-supplemented diets (10000 UI or 50000 UI vitamin A). After 7 weeks, colitis was induced by oral dextran sodium sulfate for 6 days, and immunological parameters were evaluated. Supplementation with both doses of vitamin led to an increase in frequency of Tregs in spleen, and mucosal lymphoid sites, whereas deficiency decreased Tregs in spleen. Supplementation also increased B1 cells in spleen, but reduced them in mesentheric lymph nodes. We observed a higher production of serum immunoglobulin A (IgA) and secretory IgA in mice supplemented and deficient of vitamin A. Conclusion: Vitamin A supplementation ameliorated clinical parameters in DSS-induced colitis and increased frequency of regulatory T cells. Its deficiency worsened colitis, suggesting that this vitamin is capable of reducing inflammation in the gut by an immunoregulatory mechanism.

T.104. PGLYRP2 as the Key Modulator of Enteric Mucosal Immune Response

Jooeun Lee, Stephen Girardin. University of Toronto, Toronto, ON, Canada

Nod1 and Nod2, which are members of the Nod-like receptor (NLR) family, detect bacterial peptidoglycan. Sensing of peptidoglycan by these Nod proteins is critical for the maintenance of intestinal homeostasis. Indeed, mutations in Nod2 are linked to Crohn`s disease (CD), a chronic form of inflammatory bowel disease that is characterized by Th1/17 dominated inflammatory responses. In addition to Nod1 and Nod2, peptidoglycan recognition proteins (PGLYRPs) were recently shown to participate in intestinal homeostasis. PGLYRP2, a peptidoglycan-cleaving amidase, is

expressed by cells within the intestinal epithelium, where they may act to regulate local concentrations of Nod ligands to promote intestinal homeostasis. We hypothesize that PGLYRP2 contributes to enteric immune homeostasis, at least in part through the modulation of Nod1/2 signaling. Our preliminary data indicate that PGLYRP2-deficient mice have reduced pathological changes (lower pathological scores and less visible colonic inflammation) as well as reduced level of IL-17 producing CD4+ T cells 7 days after infection with Citrobacter rodentium. Together, our work will shed light on the mechanisms by which these innate immune proteins induce immune control, ultimately leading us to identify novel therapeutic targets for the treatment of inflammatory bowel disease.

T.105. Critical Role of ER Chaperone Grp78 for the Maintenance of Intestinal T Cell Homeostasis

Jung-Su Chang[4], Sören Ocvirk[1], Emanuel Berger[1], Sigrid Kisling[1], Uli Binder[2], Arne Skerra[2], Amy Lee[3], Dirk Haller[1]. [1]Technical University of Munich, Freising-Weihenstephan, Germany; [2]University of Southern California Keck School of Medicine, Los Angeles, CA; [3]Taipei Medical University, Taipei, Taiwan

Defects in the endoplasmic reticulum (ER) stress associated mechanisms were recently implicated in the development of inflammatory bowel disease (IBD). We previously demonstrated in the $TNF^{\Delta ARE/+}$ mouse model for Crohn's disease-like ileitis that aberrant expression of ER chaperone Grp78 sensitizes intestinal epithelial cell (IEC) death. This is attributed to an aberrant cytotoxic CD8αβ+ IEL phenotype preferentially accumulating in the epithelium. In this study, we characterized the role of ER stress associated mechanisms that contribute to intestinal T cell homeostasis in $TNF^{\Delta ARE/+}$ mouse. We identified a critical activity of Grp78 as T cell intrinsic factor that mediated CD8αβ+ IEL homeostasis in $TNF^{\Delta ARE/+}$ mice. Heterozygous $Grp78^{-/+}$ mice revealed an attenuated granzyme B-dependent cytotoxicity of CD8αβ+ T cells against IEC, suggesting a critical activity of Grp78 in maintaining a cytotoxic phenotype. A deficient granzyme B production was associated with a defect in IL2-mediated proliferation of $Grp78^{-/+}$ CD8αβ+ T cells. Adoptively transferred $Grp78^{-/+}$ CD8αβ+ T cells showed a decreased frequency to accumulate in the intestine of $RAG2^{-/-}$ recipient mice. This suggests that Grp78 intrinsically controls intestinal T cell homeostasis and promotes uncontrolled CD8αβ+ IEL cytolytic activity against IEC that may further exacerbate disease manifestation in chronic intestinal inflammation.

T.106. The Role of Gammadelta T Cells in Progress of Colorectal Adenocarcinoma

Shunji Matsuda. NHO Ehime Hospital, Tohon, Japan

The role of gamma-delta T cells (dg T cells) in gut epithelium on colorectal adenocarcinoma has not been clarified yet. In this study, the T cells in human colorectal carcinoma tissues were analysed by immunological staining using monoclonal antibodies. Transformation of epithelial cells and progress of colorectal carcinoma in knockout mice were also examined. The results of these experiments were (1)gd T cells were decreased inside colorectal carcinoma tissues comparing with normal colorectal epithelium. The decrease of gd T cells was significant in highly-moderately differentiated cancer tissues. (2)The ratio of gd T cells in epithelium which is distant from cancer tissues were higher than that in marginal epithelium. (3)Aberrant crypt foci were observed in every mouse strains 1-1.5 month after AOM-administration. The number of ACF was highest on gd T cell-knockout mice. (4) Colorectal adenocarcinoma was observed only on gd T cell- knockout mice 5-9 months after AOM-administration. These results suggest that gd T cells play a role in suppression of progress of colorectal adenocarcinoma.

T.107. HIF-dependent Effector T Cell Regulation in Inflammatory Bowel Disease

Eoin McNamee, Eric Clambey, Joseph Westrich, Holger Eltzschig. University of Colorado, Aurora, CO

During inflammatory bowel disease the inflamed mucosa becomes hypoxic due to increased metabolic demand and a paucity of available oxygen. Stabilization of hypoxia-inducible factor (HIF) occurs within the cellular milieu of the inflamed intestine and directly affects the leukocyte transcriptome. However, the effect of hypoxia-dependent signaling pathways on effector T cell function, critical for IBD pathogenesis, is poorly defined to date. To address this we isolated murine CD4+ T cells subsets and exposed them to ambient hypoxia *in vitro* (1% O2; 6h). Of note, hypoxia markedly repressed expression of the transcription factor Tbet (Tbx21; 55-fold), the master regulator of CD4+ TH1 T cells. In addition, these transcriptional findings were mirrored at the protein level after 48h. In a proof of principal *in vivo* experiment, mice exposed to whole body hypoxia (10% O2; 24h) displayed suppressed splenic Tbet+ effector T cells. Furthermore, pharmacological stabilization of HIF attenuated all indices of experimentally induced colitis while conversely, selective T cell-HIF-1α deletion exacerbated disease compared to WT counterparts. Based on these preliminary studies, we hypothesize that mucosal T cell-HIF stabilization serves to attenuate experimental IBD by repressing TH1 effector function.

T.108. Study of the Mechanisms Exerted by Fructooligosaccharides from Yacón in an Intestinal Infection Model

Eva María del Mar Vélez[2], María Bibas Bonet[2], Natalia Castillo[1], Oscar Mesón[2], Alfredo Grau[2], Gabriela Perdigon[1]. [1]Cerala-Conicet, San Miguel de Tucumán, Argentina; [2]Universidad Nacional de Tucumán, San Miguel de Tucumán, Argentina

Fructooligosacharides (FOS), obtained from Yacón roots, have a prebiotic effect when used as a dietary supplement. We studied if FOS from Yacón can prevent enteric infection against salmonella typhimurium and the mechanism involved. BALB/c mice were divided into 4 groups: normal control, Basal (45 days with FOS-340mg/kg/day), infection control (IC-without FOS administration) and treated group (TG-45d with FOS+s. typhimurium); IC and TG groups were challenged at days 15, 30 and 45 with s. typhimurium. Translocation to liver and spleen, total and specific s-IgA, IgA+, TLR4+, CD206+, IL6+, TNFα, IFNγ and MIP 1α + cells were analyzed after challenge. We found protection only at 30 days of FOS administration with an

increase in the total s-IgA but not in the specific s-IgA levels for TG compared with IC group. In TG as regard IC group the N° IgA+, TLR4+, CD206+, IL6+ and MIP 1α+, TNFα and IFNγ cells were increased. We demonstrate that FOS from Yacón roots prevent s. typhimurium infections up to 30 days of administration through non-specific immunity with increased total s-IgA, expression of TLR4 and CD206 receptors and IL6+ and MIP1α + cells, that would improve immunological barrier mechanisms against s. typhimurium infection.

T.109. The Immunomodulatory Drug FTY720 Prevents Clearance of Citrobacter Rodentium Infection in Mice

Carola Murphy, Lindsay Hall, Grainne Hurley, Aoife Quinlan, Fergus Shanahan, Kenneth Nally, Silvia Melgar. University College Cork, Cork, Ireland

Background: The sphingosine-1-phosphate (S1P) agonist FTY720 prevents lymphocyte migration to sites of pathology and has shown great efficacy in both human and animal models of autoimmunity and transplantation. However, its clinical use may increase the risk of opportunistic infections, particularly in the gastrointestinal tract. We investigated the impact of FTY720 treatment in the Citrobacter rodentium model of colitis. Methods: Mice were gavaged with vehicle or FTY720 (3mg/kg) for 6 days pre-infection. Post-infection dosing was continued every 2nd day up until day 12 (D12). Mice were culled on D8 (peak-infection) and D14 (late infection/clearance). Throughout the study faecal viable counts were enumerated. At necropsy, immune cell phenotyping was performed on blood (FACS). C. rodentium colonisation was detected using bioluminescence imaging (BLI). Colons were weighed and measured and splenic CFUs were enumerated. qRT-PCR and immunofluorescent staining was performed. Results: FACS confirmed peripheral blood lymphopenia in FTY720-treated animals. CFU counts and BLI revealed inability of FTY720-treated mice to clear the infection by D14 in contrast to vehicle-treated animals. Results were supported by clinical and histological signs of colonic inflammation. Gene expression analysis revealed a deficient host immune response in drug-treated mice. Conclusion:Treatment with FTY720 impairs the mucosal immune response to bacterial infection.

T.110. **γδ T Cell Subsets Differentially Modulate αβ T Cell Responses to** Enteric Virus Infection

Lijuan Yuan, Ke Wen, Guohua Li, Tammy Bui, Fangning Liu, Yanru Li, Jacob Kocher. Virginia Polytechnic Institute and State University, Blacksburg, VA

Intestinal γδ T cells may play an important role in modulating innate and adaptive immune responses to enteric viruses. We evaluated γδ T cell subset (CD2+CD8-, CD2+CD8+ and CD2-CD8-) responses to rotavirus infection (frequencies, tissue distribution, TLR2, TLR3, TLR9, IFN-γ, IL-10, TGF-β and FoxP3 expression) in gnotobiotic pigs. We also studied cytokine-producing profiles of the three subsets and their influence on αβ T cell proliferation and cytokine production in sort-purified cocultures. Rotavirus infection induced a significant expansion of the intestinal CD2+CD8+ subset and significant increases in frequencies of FoxP3-expressing CD2+CD8+ γδ T cells in ileum, spleen and blood whereas the CD2+CD8- subset showed the highest increases in TLR2, TLR3 and TLR9 expression in ileum of gnotobiotic pigs. The CD2+CD8- subset had significantly increased frequencies of IFN-γ expression postinfection in pigs and significantly enhanced IFN-γ production by CD4+ T cells in the cocultures. The CD2+CD8+ subset produced significantly higher levels of IL-10 than CD8- subsets and significantly enhanced IL-10 and TGF-β production by CD4+ and CD8+ αβ T cells *in vitro*. Thus, the CD2+CD8- subset contributed to anti-viral immune responses by promoting CD4+ T cell proliferation and IFN-γ production. The CD2+CD8+ subset exerts regulatory T cell function in maintaining and restoring intestinal homeostasis upon enteric virus infection.

T.111. Activation of B Lymphocytes in the Gut Associated Lymphoid Tissue of Weaned Mice After Intragastric Inoculation of Shiga Toxin-producing Escherichia Coli

Romina Fernández-Brando[1], Gabriel Cabrera[1], Ariela Baschkier[2], Elizabeth Miliwebsky[2], María Mejías[1], Cecilia Panek[1], María Ramos[1], Marta Rivas[2], Marina Palermo[1]. [1]Instituto de Medicina Experimental Academia Nacional de Medicina, Buenos Aires, Argentina; [2]Instituto Nacional de Enfermedades Infecciosas, Carlos G. Malbrán, Buenos Aires, Argentina

After intragastric inoculation of E.coli O157:H7 Stx+ strains (125/99)in mice, we observed an early decrease of B lymphocytes (BLym) percentage (%) in Peyer's patches (PP), with a simultaneous increase in mesenteric lymph nodes (MLN) compared to controls: mice inoculated with a E.coli O157:H7 Stx- strain (605/03) or PBS (Ctrl). We aim to further study the BLym activation and trafficking. Flow cytometry analysis showed BLym activation in 125/99- and 605/03-inoculated mice: at 12 h post-inoculation as an increased CD69+ BLym % from PP(mean±SD,n) (Ctrl=31.3±2.3,3; 605/03=43.1±4.5,3; 125/99=50.3±10.8,3; $p<0.05$), and at 24 h as a decreased CD62L MFI in Blym from MLN (Ctrl=1.6±0.2,2; 605/03=0.6±0.2,3; 125/99=1.2±0.1,3; $p<0.005$; the same tendency was observed for TLym, and for B and TLym from PP. After 12 h of i.v. injection of allogenic CFSE+Lym only 125/99-inoculated mice showed an increased % of B220+CFSE+ BLym in MLN(Ctrl=12.6±0.8,4; 605/03=13.1±2.7,2; 125/99=14.7±1.9,4; $p<0.05$). Additionally, 125/99- and 605/03-inoculated mice showed an increased % of IgA+-BLym in PP(Ctrl=10.21±3.5,10; 605/03=13.6±2.6,15; 125/99=15.6±3.1,11; $p<0.05$), but only 125/99-inoculated mice showed an increased % of IgA+-BLym in NLM(Ctrl=5.9±1.7,5; 605/03=6.5±1.8,7; 125/99=8.00±1.2,11; $p<0.05$).Although both E.coli strains induced GALT activation, our results suggest that only the Stx-producing strain was able to induce trafficking of BLym and to increase IgA+BLym in MLN.

T.112. Effect of Toll-like Receptor-2 (TLR-2) Deficiency on Transcriptomic Profile in DSS-colitis

Violeta Lorén[1], Elisabet Pedrosa Tapias[1], Cristina Sánchez[1], Patricia Romero[1], Eduard Cabré[1], Eugeni Domènech[1], Josep Mañé[1], Ildefonso Cases[2], Juanjo Lozano[1]. [1]Institut d'Investigació Germans Trias i Pujol. Ciberehd, Badalona, Spain; [2]Institute for Personalize and Predictive Medicine of

Cancer, Badalona, Spain

Background and aim: TLR-2 plays a pivotal role in intestinal homeostasis. Given the etiopathogenic relationship between inflammatory bowel disease and pattern recognition receptors polymorphisms, we characterized colonic gene expression profiles in TLR-2 knockout (KO) and wild type (WT) mice with DSS colitis. Methods: We studied vehicle-treated WT (n=3), DSS-treated WT (WTDSS, n=5), and DSS-treated KO mice (KODSS, n=5). Five days after starting oral DSS or vehicle treatment, mice were sacrificed. RNA from colonic samples was used for genome-wide microarray analysis (CodeLinkTM). Selected genes from microarrays data analysis (false-discovery rate<0.01 and fold change>2) by LIMA-R package were undertaken to hierarchical clustering and gene ontology by GeneCodis and GSEA software, and PubMed database. Results: As compared to WT, WTDSS mice showed 129 and 148 up- and down-regulated genes, respectively, while only 14 and 3 genes were up- and down-regulated in KODSS. Pathway-based enrichment analysis of up-regulated genes in WTDSS identified genes related to extracellular matrix activity, while up-regulated genes in KODSS were linked to cell cycle. Down-regulated genes in both groups involved oxidative, xenobiotic and retinol metabolism. Conclusions: 1. DSS-colitis favours extracellular-matrix-receptor interaction. 2. This does not occur in presence of TLR2 deficiency where increased cell proliferation predominates.

T.113. Innate Activation of Early Th17 Responses During Infectious Colitis is Micro-flora Dependent

Kaoru Geddes[1], Stephen Rubino[1], Catherine Streutker[2], Joao Magalhaes[3], Lionel Le Bourhis[3], Stephen Girardin[1], Dana Philpott[1]. [1]University of Toronto, Toronto, ON, Canada; [2]St. Michael's Hospital, Toronto, ON, Canada; [3]Institut Curie, Paris, France

The nod-like receptors (NLRs) Nod1 and Nod2 play a pivotal role in regulating homeostasis at mucosal surfaces, as evidenced by the association of variants of Nod1 with asthma and Nod2 mutations with Crohn's disease. In addition to NLRs, Th17 cells have emerged as important mediators of inflammatory responses at mucosal surfaces and have been implicated in various inflammatory conditions. In the Salmonella colitis model we found that mice deficient for both Nod1 and Nod2 have a significantly reduced Th17 response by 24 hours post infection. This early response was shown to be dependent on IL-6 as the Th17 cells did not develop in IL-6 knockout mice or following IL-6 depletion. These Th17 cells have a memory-like phenotype and express high levels of CD44, CD69, CCR6 and CCR7 and require specific antigen since chimeric mice lacking MHCII in the hematopoietic compartment no longer had a Th17 response. Interestingly, germ-free mice were also lacking this Th17 response suggesting that the cognate antigen for these Th17 cells is derived from a commensal peptide that may be conserved in the pathogen. Thus Nod1 and Nod2 appear to modulate IL-6 to quickly activate Th17 cells whose cognate antigen is derived from normal flora.

T.114. Dendritic Cells Contribute to Early Invasion of Peyer's Patches by Salmonella Typhimurium

Chee Han Lim[1], Sabrina Voedisch[1], Michaela Friedrichsen[1], Mikael Rhen[2], Oliver Pabst[1]. [1]Hannover Medical School, Hannover, Germany; [2]Karolinska Institute, Stockholm, Sweden

M cell mediated uptake, active sampling by gut dendritic cells (DC) and direct invasion of epithelial cells have been shown to support Salmonella invasion of gut tissue. Yet the contribution of these individual mechanisms to the overall infection process remains unresolved. Here we exploit stochastic variations in the representation of individually tagged Salmonella to determine the number of bacteria seeding Peyer's patches directly after oral infection. We show that only 20-30 bacteria set up the infection in each Peyer's patch of C57BL/6 wild type mice. To dissect the contribution of the DC to Peyer's patch invasion, we depleted DC in transgenic mice carrying the diphtheria-toxin receptor under the control of CD11c promoter. We found almost 50% reduced invasion events in DC-depleted compared to non-depleted mice. CX3CR1-deficient mice showed similar invasion frequencies compared to wild type mice, suggesting that Salmonella uptake into Peyer's patches is not helped by CX3CR1-dependent dendrite formation. Consistently, confocal microscopy revealed CD11c-positive CX3CR1-negative cells closely associated with the follicle associated epithelium of Peyer's patches. In conclusion, we reveal an unexpected role of DC in Salmonella uptake into Peyer's patches and propose a novel method to dissect different aspect of Salmonella pathogenicity.

T.115. LPLUNC1 Modulates Innate Immune Responses to V. Cholerae Infection

Sarah Shin[1], Taher Uddin[2], Robert Citorik[1], Edward Ryan[1], Stephen Calderwood[1], Firdausi Qadri[2], Regina Larocque[1], Jason Harris[2]. [1]Massachusetts General Hospital/Harvard Medical School, Boston, MA; [2]International Centre for Diarrheal Disease Research, Dhaka, Bangladesh

Vibrio cholerae causes a severe diarrheal disease called cholera. Recent studies indicate that components of the innate immune system, including long palate, lung and nasal epithelium clone protein 1 (LPLUNC1), play a role in V. cholerae infection. LPLUNC1 is a protein with a previously uncharacterized function that is structurally similar to bactericidal/permeability increasing protein (BPI). We initially hypothesized that LPLUNC1 may have a bactericidal effect on V. cholerae; however, we were unable to demonstrate a dose-dependent bactericidal effect of LPLUNC1 *in vitro*. Using HEK cells expressing toll-like receptors (TLRs), we found an immunomodulatory function of LPLUNC1 in that addition of purified LPLUNC1 protein resulted in a dose-dependent decrease in LPS-driven TLR4 signaling. The inhibitory effect of LPLUNC1 was TLR4-specific, as LPLUNC1 did not affect lipoprotein-mediated TLR2 activation. Immunostaining on duodenal biopsies showed expression of LPLUNC1 in intestinal Paneth cells of cholera patients. Our results demonstrate that LPLUNC1 is expressed in the small intestine and may play a role in modulating the host inflammatory response to V. cholerae infection.

T.116. The Clonal Repertoires of Virus-specific CD8+ T Lymphocytes are Shared in Mucosal and Systemic Compartments During Chronic SIV Infection in Rhesus Monkeys

Piya Sircar, Kathryn Furr, Norman Letvin. Beth Israel Deaconess Medical Center, Boston, MA

Since mucosal tissues play a fundamental role in early HIV/SIV infection, it is crucial to understand the virus-specific responses in mucosal tissues to facilitate devising strategies to prevent and control these infections. We have employed TCR repertoire analyses to define the clonal composition of a dominant SIV epitope-specific CD8+ T cell population in mucosal and systemic compartments of SIV-infected rhesus monkeys during both acute and chronic infection. We show that the CD8+ T cell repertoire in mucosal tissues of uninfected rhesus monkeys is oligoclonal, while the repertoire in blood is polyclonal. Early after infection, the SIV-specific CD8+ T cell clonal repertoire is distinct in mucosal compartments and peripheral blood. However, we observed a narrowing of the clonal repertoire in all sampled anatomic compartments as infection progressed from acute to chronic, and there was comparable clonal diversity in all anatomic compartments. We showed during chronic infection that the same clonal populations of virus-specific CD8+ T cells are present in all compartments. These data indicate that the SIV-specific CD8+ T cells in systemic and mucosal sites have a shared clonal origin and are capable of both responding to infection in the systemic circulation and trafficking to mucosal tissues. We have also determined the effectiveness of systemic prime-boost vaccine regimens in generating clonally diverse virus-specific CD8+ T cell responses in mucosal compartments.

T.117. Control of Intestinal Homeostasis by Tristetraprolin-mediated mRNA Decay

Franz Kratochvill, Florian Ebner, Pavel Kovarik. University of Vienna, Vienna, Austria

Although expression of proinflammatory genes is needed to protect the host, prolonged and dysbalanced inflammation is harmful. Balanced immune responses are required for the maintenance of intestinal homeostasis. Stability and decay of messenger RNAs are fundamental parameters involved in inflammatory gene regulation. Many proinflammatory mRNAs contain AU-rich elements in their 3' untranslated regions interacting with stabilizing and destabilizing proteins. Tristetraprolin (TTP) is one of the best known mRNA-destabilizing proteins. Mice lacking TTP exhibit complex inflammatory syndrome including cachexia, dermatitis and arthritis. Data recently obtained in our laboratory revealed that TTP targets 1/3 of inflammation-induced mRNAs to degradation in macrophages. As such, TTP appears to be a key factor controlling immune homeostasis. Detailed analysis of TTP-mediated mRNA decay in inflammatory diseases was so far impossible due to severe inflammatory phenotype of TTP-deficient mice. To circumvent this we generated mice with conditional TTP deletion and address the role of myeloid TTP in a model of experimental colitis. Animals displayed increased level of basal inflammatory signaling in the gut before induction of colitis. Unexpectedly, mice lacking myeloid TTP exhibit a significant degree of protection against induced colitis. We currently examine the precise mechanism of beneficial effects that lacking TTP-mediated mRNA decay reveals during experimental colitis.

T.118. Antibody-dependent HIV-1 Neutralization in Sera and External Secretions

Qing Wei, Zina Moldoveanu, Wen-Qiang Huang, Jiri Mestecky. University of Alabama at Birmingham, Birmingham, AL

HIV-1 infection is dominantly a mucosal disease and evaluating mucosal immune responses is highly relevant. To determine Ig isotypes of HIV-1-specific antibodies, sera, cervicovaginal and rectal lavages (CVL, RL) were analyzed by ELISA, western blot, and neutralization assay. IgG specific for gp160, gp120, or gp41 were detected in all sera but IgA to at least one glycoprotein were found in 59% of sera. All samples had the ability to neutralize at least one of three HIV-1 viruses (SF162, YU2, NL4.3). Selective depletion of IgG resulted in a significant decrease in IC50 even in samples with strong IgA binding to HIV-1 glycoproteins. HIV-1 neutralization was reduced or became undetectable when both IgG and IgA were removed. In corresponding CVL and RL, HIV-1-specific IgG antibodies were directed at three glycoproteins and IgA antibodies toward two glycoproteins. Neutralization results indicated that IgG in CVL had greater ability to reduce virus infectivity compared to IgA. However, the difference in HIV-1 neutralization before and after Ig depletion was not observed in RL implying that innate factors other than antibodies were present. These data demonstrate that neutralizing antibodies are mainly of the IgG isotype in both sera and secretions of HIV-1 infected women.

T.119. Partial Reconstitution of Resident and Circulating Gut-homing CD4 T Cells in HIV-1+ Subjects Treated with Raltegravir-containing Regimen Commencing in Either Primary or Chronic Infection

John Zaunders[1], Kersten Koelsch[1], Michelle Bailey[2], Katherine Marks[1], Nabila Seddiki[2], Yin Xu[2], Mark Danta[3], Christoph Boesecke[2], Linda Gelgor[2], David Cooper[2], Anthony Kelleher[2]. [1]St. Vincent's Centre for Applied Medical Research, Darlinghurst, NSW, Australia; [2]University of New South Wales, Sydney, NSW, Australia; [3]St. Vincent's Hospital, Sydney, NSW, Australia

Untreated HIV-1 infection leads to loss of CD4 T cells from gut-associated lymphoid tissue (GALT). We hypothesised that early commencement of antiretroviral therapy containing the integrase inhibitor Raltegravir would improve recovery of CD4 T cells. Seven primary (PHI) and eight chronically (CHI) HIV-1 infected subjects provided biopsies from five sites (rectum; left and right colon; terminal ileum; and duodenum) before and after 52 weeks of therapy. CD4+ CD45+EpCam- lymphocytes in enzymatically digested biopsies, and gut-homing (integrin beta7+) memory CD4 T cells in blood, were counted by flow cytometry and compared by Mann-Whitney test to HIV-negative controls. Baseline biopsy CD4 lymphocytes and blood beta7+ CD4 T cells in PHI were higher than in CHI (98,830 vs 30585 cells/site; p=0.002 and 48 vs 20 cells/µl; p=0.018, respectively). Biopsy CD4 lymphocytes and blood beta7+ CD4 T cells were significantly correlated (rho=0.55, p=0.03). After therapy, CHI biopsy CD4 counts and blood beta7+

CD4 T cells increased, but remained less than HIV-uninfected controls (56174 vs 214,685; p=0.04 and 37 vs 69; p=0.009, respectively). After therapy, PHI biopsy and blood beta7+ CD4 counts were 119,563 and 43, respectively. Greater depletion of CD4 T cells from GALT in CHI compared with PHI suggests that early treatment may better prevent irreversible loss of these cells.

T.120. Stat3 Activation in Intestinal Epithelial Cells Controls C. Rodentium Infection

Nadine Wittkopf, Ulrike Billmeier, Claudia Günther, Eva Martini, Stefan Wirtz, Markus Neurath, Christoph Becker. Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany

We have previously shown that mice lacking the ability for activation of the transcription factor Stat3 in intestinal epithelial cells (IECs) show decreased secretion of antimicrobial peptides and deregulated homeostasis. We now investigated the role of intestinal epithelial Stat3 activation during gastrointestinal infections. We could show that infection of wildtype mice with Citrobacter rodentium, a murine model pathogen for attaching and effacing Escherichia coli such as EHEC and EPEC in men, resulted in rapid activation of Stat3 in IECs. Citrobacter-infected wildtype mice showed strong induction of antimicrobial peptides concurrent with epithelial activation of Stat3. Mice with an IEC-specific deletion of Stat3 (Stat3-IEC-KO) revealed an increased susceptibility to Citrobacter infection as indicated by elevated bacterial load in the gut and more severe colitis. Histological examination of colonic tissue samples from Citrobacter-infected Stat3-IEC-KO mice demonstrated enlarged infiltration of immune cells, enhanced mucosal hyperplasia and increased epithelial apoptosis. Strikingly, Stat3-IEC-KO mice showed unexpected invasion of Citrobacter into other organs such as lung, liver and kidney, indicating a defective mucosal barrier. These data implicate a protective role for Stat3 activation in IECs during Citrobacter infection by controlling bacterial growth by production of antimicrobials and suppression of apoptosis for maintaining the epithelial barrier integrity.

T.121. Limited Usefulness of Intestinal Fatty Acid Binding Protein (I-FABP) as a Marker of Enterocyte Damage in HIV-1 Infection

Marius Trøseid[1], Piotr Nowak[2], Babilonia Barqasho[2], Andreas Lind[1], Anders Sönnerborg[2], Dag Kvale[1]. [1]Oslo University Hospital, Oslo, Norway; [2]Karolinska University Hospital, Stockholm, Sweden

Chronic immune activation driven by microbial translocation from a damaged gut plays a fundamental role in HIV-1 progression. Intestinal fatty acid binding protein (I-FABP), a marker of enterocyte damage, was recently found elevated in HIV-1 positive individuals and was associated with lower CD4+ cell counts and insignificantly elevated in patients who died (Sandler et al., J Infect Dis 2011). To evaluate the clinical applicability of I-FABP in chronic HIV-1 infection we measured I-FABP in two different laboratories with the currently available ELISA kit (Hycult, Cell Sciences). Although the background optical density of the ELISA was unusually high (0.3-0.6), 3 out of 4 patients hospitalised for severe infectious diarrhoea had detectable I-FABP levels in accordance with previous reports. In contrast, only 4 (16%) out of 25 snap frozen plasma samples from chronically HIV-1-infected individuals without treatment had detectable I-FABP levels (range 84-1720 pg/mL, 1:1 dilution). I-FABP measured by this ELISA kit may be relevant in detecting severe intestinal damage but have been generally undetectable in controls, in keeping with our results from chronic HIV infection. Thus, the clinical applicability for detecting enterocyte damage in HIV is limited given the low sensitivity and possibly the high background of the currently available assay.

T.122. Dysregulation of Mucosal Humoral Responses in HIV-1-infected Individuals

Zdenek Hel, Paul Goepfert, Jiri Mestecky. University of Alabama at Birmingham, Birmingham, AL

HIV-1 infection is characterized by a rapid depletion of memory CD4+ T cells in mucosal tissues, most prominently in the intestinal mucosa, during the acute phase of infection. Since CD4+ T cells play a critical role in the regulation of class switching, somatic hypermutation, and transepithelial transport of antibodies, their profound depletion from mucosal tissues is likely to result in perturbations of production and secretion of mucosal antigen-specific immunoglobulins. We determined humoral responses in serum and external secretions of HIV-1-infected patients and uninfected control subjects. Levels of immunoglobulins specific to common bacterial (E. coli and S. typhi LPS, flagellins F2 and Cbir1,) and food (OVA, BGG, and yeast mannan) antigens were determined. Our results indicate that: 1) HIV-1-infected individuals display increased levels of total IgA, IgM and IgG immunoglobulins in plasma, particularly IgA isotype, and an increase of IgM in external secretions; 2) the IgG/IgM and IgA/IgM ratios of specific antibodies to most mucosal antigens in external secretions progressively decrease in the course of HIV-1 infection; and 3) levels of plasma IgG and IgA specific to multiple food and bacterial antigens correlate with the level of activation of CD4+ and CD8+ T cells in HIV-1-infected patients. Decreased IgA/IgM ratio in intestinal secretions of HIV-1-infected subjects is consistent with a lack of help for IgM to IgA class switch recombination in gut mucosa. In conclusion, the obtained results suggest that HIV-1 infection is associated with significant perturbations of immunoglobulin secretion at mucosal surfaces.

T.123. Immune Reconstitution Inflammatory Syndrome in Whipple's Disease is Induced by Unregulated T Cell Reconstitution After Initiation of Treatment

Verena Moos, Katina Schinnerling, Anika Geelhaar, Kristina Allers, Julian Friebel, Thomas Schneider. Charité, Berlin, Germany

Background: Whipple's disease, an infection caused by Tropheryma whipplei, can be treated effectively with antimicrobials. Corresponding to other infectious diseases, an immune reconstitution inflammatory syndrome (IRIS) might occur in Whipple's disease after the start of antimicrobial

treatment. However, the immunological mechanisms favoring the development of IRIS are largely unknown. Methods: Following definition of immune reconstitution inflammatory syndrome, we determined immunological parameters in 17 patients with Whipple's disease IRIS and 45 normal patients with Whipple's disease. Macroscopic and histologic features of IRIS were described. T cell reconstitution, activation, Th1 reactivity, and regulatory activity in the peripheral blood and CD4+ infiltration in the duodenal mucosa were measured before and after start of antimicrobial treatment and when IRIS was manifest, respectively. Results: Reconstitution of CD4+ T cells and CD4+ activation was more pronounced in Whipple's disease patients developing IRIS compared to Whipple's disease patients without IRIS. In contrast, the numbers of regulatory T cells and the regulatory capacity laged behind the rapid T cell regeneration at the time point IRIS was manifest. Thus, Th1 reactivity was rigorously restored, and activated memory T cells invaded tissues where the infection with T. whipplei was manifest, inducing a massive inflammatory reaction and severe tissue damage. However, T. whipplei-specific Th1 reactivity did not contribute to IRIS in Whipple's disease. Conclusion: IRIS in Whipple's disease is favored by low CD4+ counts at the start of antimicrobial treatment and a rapid reconstitution and activation of T cells that is not well balanced by the regeneration of regulatory T cell capacity.

T.124. Clostridium Difficile Mediated Effects on Intestinal Epithelia

Nazila Jafari[1], Elaine Allan[1], Bertrand Vernay[1], Richard Stabler[2], Brendan Wren[2], Mona Bajaj-Elliott[1]. [1]University College London, London, United Kingdom; [2]London School of Hygiene and Tropical Medicine, London, United Kingdom

Clostridium difficile infection (CDI) is a major cause of hospital infection, morbidity and mortality however asymptomatic carriage has also been reported. C. difficile virulence is associated with production of toxins (A, B and binary). The role of toxins has been well studied but how the gastrointestinal mucosa responds to bacteria itself is unknown. In the present study we compared and contrasted the effect of C. difficile [R20291, 630 (A+B+) and M68, CF5 (A-B+)] strains on human intestinal epithelial cells (IECs) physiology with the aim to delineate bacterial-mediated effects on IEC. Inter strain variation in bacterial adherence to IEC was noted however adherence did not correlate with the magnitude of downstream cellular responses, as measured by transepithelial electrical resistance (TEER), antimicrobial immunity or the rate of apoptosis. R20291 was most potent, M68 (A-B+) strain was found to be as cytotoxic as (A+B+) strains. In contrast, CF5 (A-B+) strain had the least effect on barrier integrity, cell death and proinflammatory cytokine production. Similar findings were also observed in an ex-vivo model of infection in human colonic explants. The differences in host responses noted during infection may be indicative of their pathogenic potential.

T.125. Structure-function Analyses of Lipooligosaccharide (LOS) Discriminates Campylobacter Jejuni Phylogenetic Clades

Holly Stephenson[1], Constance John[2], Ozan Gundogdu[3], Dominic Mills[3], Nick Dorrell[3], Brendan Wren[3], Mona Bajaj-Elliott[1], Gary Jarvis[2]. [1]Institute of Child Health, UCL, London, United Kingdom; [2]University of California, San Francisco, San Francisco, CA; [3]London School of Tropical Medicine & Hygiene, London, United Kingdom

Livestock and environmental sources (e.g. contaminated water) combine to make C. jejuni-mediated gastroenteritis a global health burden. Genetic analysis of 111 C. jejuni strains identified two separate phylogenetic clades, distinguished by isolation from either livestock or environmental sources (Champion et al. 2005). Presently, how different sources of infection may alter disease pathogenesis remains unclear. As TLR4 engagement is critical in recognition of C. jejuni, we hypothesized that variation in lipooligosaccharide (LOS) structure may show an association with the source of infection. LOS was isolated from 14 C. jejuni strains (7 environmental, 7 livestock) by hot-phenol extraction, and MALDI-TOF mass spectrometry (MS) on the full length, de-O-acylated, and lipid A moiety was performed. Using a TLR4 reporter (HEK) cell line and THP-1 monocytic cells, we found that isolated LOS differentially activated TLR4-mediated signaling events, dependant on the phylogenetic clade of the strain. The MALDI-TOF MS analyses did not reveal any apparent correlation between the degree of phosphorylation or amidylation of the lipid A and the TLR4 activation. The role of variability in the oligosaccharide moiety in the differential biological responses is under investigation.

T.126. Adaptability of Mucosal IL-17/IL-22 T Cell Responses to Bacterial Infection

Stephen Rubino, Kaoru Geddes, Joao Magalhaes, Dana Philpott, Stephen Girardin. University of Toronto, Toronto, ON, Canada

The cytokines IL-17 and IL-22 have emerged as critical mediators of mucosal defense to bacterial pathogens in the gastrointestinal tract, and these cytokines can be produced by a wide variety of innate and adaptive immune cells. Recently, we have described that the enteric pathogen Citrobacter rodentium induces an early cecal IL-17A/IL-22 response in CD4+T helper cells that is dependent on signaling from the innate immune receptors Nod1 and Nod2. We sought to determine whether this early Th17 response required antigen presentation by the major histocompatibility complex II (MHCII) for full induction. Indeed, at both early (day 4) and late (day 9) phases of C. rodentium infection, the mucosal Th17 was completely ablated in mice adoptively transferred MHCII-lacking hematopoietic cells (MHCIIKO/WT) compared to WT/WT mice. Unexpectedly, the global IL-17A/IL-22 responses was not reduced in MHCIIKO/WT lamina propria as there was a 100-1000 fold increase in the number of IL-17A+IL-22+CD8+CD4-TCRβ+ (Tc17 cells) that was compensating for much of the production IL-17 and IL-22. Together, these results indicate that proper activation of CD4+T cells responses are required to dampen IL-17A and IL-22 expression from atypical cellular sources such as Tc17 cells, thus demonstrating the plasticity of IL-17A/IL-22 responses to enteric challenges.

T.127. Stress-induced Colitis in TIMP-3 Genetic Modified Mice

Beatriz Valle-Argos[1], Elena Vorobeva[1], Siobhan O'Leary[1], Dylan Edwards[2], Hideaki Nagase[3], Sylvia Pender[1]. [1]University of Southampton, Southampton, United Kingdom; [2]University of East Anglia, Norwich, United Kingdom; [3]Imperial College London, London, United Kingdom

Idiopathic inflammatory bowel disease (IBD) is a chronic relapsing condition. Patients with long term colitis history often experience relapse after complete remission with unknown reasons. Stress, adverse life events, and depression are suggested as triggers for relapse. The aim of this study is to investigate the effects of stress on the immune responses and regulation of matrix metalloproteinases (MMPs) which play an important role in tissue injury in the gut. Transient colitis in C57/BL6 and Timp-3 genetic modified mice was induced by 2,4,6-trinitrobenzenesulphonic acid (TNBS) and resolved by 6 weeks. Colitis was then reactivated by a combination of restraint and sonic stress followed by a sub-optimal dose of TNBS which usually did not cause any colitis at this dose. Histological analysis of colonic sections was performed. The inflammatory cytokines and protease gene expression were evaluated using real-time PCR. We have shown that 50% of the WT mice had reactivated colitis after stress induction. The relapse was related to the upregulation of MMPs. All stress-induced colitis in WT mice required previous colitis. Moreover, we also studied the effect of stress-induced colitis in TIMP-3 genetic modified heterozygots mice. Almost 80% of the Timp-3+/- mice had stress-induced colitis even without previous colitis. We will define the mechanism especially the role of immune response and Timp-3 in stress induced colitis. This study is supported by IPODD EU consortium.

T.128. Enteropathogenic Escherichia Coli (EPEC) Type III Secretion System Potentiates Murine Dendritic Cell (DC) IL-**1β Production via** Caspase-1 Activation

Nayani Pramanik[1], Mark Lucas[1], James Kaper[2], Alan Phillips[1], Claire Bryant[3], Mona Bajaj-Elliott[1]. [1]University College London, London, United Kingdom; [2]University of Maryland, Baltimore, MD; [3]University of Cambridge, London, United Kingdom

Enteropathogenic Escherichia coli (EPEC) is a leading cause of acute and chronic diarrhoea in developing nations, principally affecting children < 2 years of age. The interaction of EPEC with the gastrointestinal (GI) mucosal immune system remains largely uncharacterised. A recent study by Lebeis et al 2009 indicated a critical role for IL-1 receptor signalling in Citrobacter rodentium (murine model for EPEC) mediated disease pathogenesis. Dendritic cells (DC) are critical innate microbial sensors that dictate downstream adaptive immunity. In the present study we aimed to investigate the signalling events that regulate IL-1β production in murine DC in response to EPEC infection. An intact type III secretion system (T3SS) was found to be necessary for optimal IL-1β release as absence of T3SS or loss of pore formation by the T3SS abrogated cytokine production. Interestingly, certain members of the inflammasome complex were dispensable for T3SS induced IL-1β secretion. Ongoing experiments indicate a potential role for ASC in EPEC-mediated IL-1β release. Better understanding of this complex pathway may highlight novel therapeutic targets for infection control and highlight strategies for vaccine development for this major enteropathogen.

T.129. Immune Response to Taenia Solium Calreticulin During Experimental Taeniosis in the Golden Hamster

Fela Mendlovic[2], Mayra Cruz-Rivera[1], Guillermina Avila[1], Sonia Leon Cabrera[1], Ana Flisser[1]. [1]Universidad Nacional Autonoma de Mexico, Mexico, Mexico; [2]Universidad Anahuac del Norte, Mexico, Mexico

Taenia solium causes two important diseases in humans. Most research has focused on the metacestode stage since it causes neurocysticercosis, one of the main parasitic diseases of the central nervous system. However the tapeworm carrier is the main risk factor for acquiring neurocysticercosis and little is known about the immunity induced in the small intestine. Calreticulin is a ubiquitous protein involved in cellular Ca2+ homeostasis, present in E/S products in several helminth infections and shown to induce predominantly Th2 responses. We cloned and expressed T. solium calreticulin (TsCRT) as a functional Ca2+-binding protein. We hypothesize that TsCRT induces a humoral and cellular immune response both locally and systemically during taeniosis. Our results indicate that lymphocytes from mesenteric lymph nodes of infected hamsters do not proliferate in response to TsCRT or a total adult sonicate. Moreover, the response to Con A is importantly diminished during infection. The systemic specific response is not affected since splenocytes proliferate upon stimulation, and anti-TsCRT antibodies are present in 50% of infected hamsters. These data suggest that infection with the adult T. solium tapeworm modulates the mucosal immune response during experimental taeniosis. We are currently determining the cytokine profile induced.

T.130. Inhibition of IL-**1β**-induced COX-2 and IL-8 Expression by Histone Deacetylase Inhibitor Trichostatin A (TSA) in A549 Human Alveolar Epithelial Cells

Yuh-Lin Wu, Meng-Lun Lu. National Yang-Ming University, Taipei, Taiwan

Disturbed inflammation is closely related to pulmonary diseases. Pro-inflammatory cytokine interleukin (IL)-1β is able to upregulate expression of several inflammatory mediators, including cyclooxygenase (COX)-2 and IL-8. Previous studies have reported that histone deacetylase can regulate COX-2 and IL-8 expression. This project aimed to clarify whether histone deacetylase inhibitor trichostatin A (TSA) may alter IL-1β-regulated COX-2 and IL-8 expression in A549 human lung alveolar epithelial cells. By employing western blotting and ELISA, TSA was able to attenuate IL-1β-induced COX-2 and IL-8 expression and the secretion of prostaglandin E2 and IL-8. Meanwhile, TSA appeared to facilitate COX-2 protein degradation in a proteasome-dependent pathway. With RT-PCR, TSA was found to reduce IL-1β-mediated COX-2 and IL-8 mRNA expression. However, promoter analysis of COX-2 and IL-8 genes indicated that TSA did not seem to inhibit IL-1β-induced COX-2 or IL-8 transcription. In addition, by monitoring the

mRNA stability profiles, TSA appeared to promote IL-8 mRNA degradation. Inhibitors of MAP kinases, NF-κB, and PI3K demonstrated that p38, JNK and PI3K were critical mediating IL-1β-induecd COX-2 and IL-8 expression. Biochemical analyses further delineated that TSA was capable of dampening IL-1β-stimulated JNK and PI3K phosphorylation. Our results suggest that TSA can suppress IL-1β-incurred inflammatory molecules expression, at least in part via attenuation of JNK and PI3K activation.

T.131. Reversing the Damaging Effects of Carbohydrate and Lipid Rich Diets by Probiotic Bacteria and Green Tea Extract

Fahri Bayiroglu[1], Turan Karaca[2], Salih Kaya[4], Mecit Yoruk[2], Leyla Aslan[2], Bahat Comba[2]. [1]Ağrı İbrahim Çeçen University, Ağrı, Turkey; [2]Yuzuncu Yil University, Van, Turkey; [3]Ondokuz Mayis University, Samsun, Turkey; [4]Dicle University, Diyarbakir, Turkey

Carbohydrate and lipid rich diets cause many diet associated diseases, cancer and obesity. Long term exposure of high calorie diets was shown to cause morpho-functionally alterations in alimentary tract (Comp B.Physiol A 1991, 99:651). Intestinal immune system was weakened and became prone to many diseases connected to chronic inflammation (Inflamm B.Dis 2005,11:154). It is suggested these alterations of intestinal immune system could be prevented by probiotic bacteria (PB) and green tea extracts comprising polyphenolic compounds. It is known little how these high calorie diets breaks intestinal canal and how these treatments renovate broken intestinal tissue. To elucidate these mechanisms, rats were fed carbohydrate and lipid rich diets with lyophilized PB (L.casei Shirota) and green tea extract (GTE). In our work, number of lymphocytes in small intestine mucosal area increased significantly as either high calorie diets application, but GTE tends to decrease these cells in either group (Table1). But probiotics caused fluctuations in small intestines. Paneth cell counts increased especially in jejunum and ileum sections with GTE applications in both groups (Figure1). Treatments caused significant increases in IL-12, Il-1β and TNF-α levels in either diet groups (Figure2,3 and 4). Conclusively, high calorie diets shown to cause inflammation in gut wall, abovementioned treatments recruit intestinal mucosal immunity for fighting these challenges.

T.132. HDAC Dependent Regulation of the IL-6/STAT3 Pathway in T Helper Cells

Rainer Glauben[1], Elena Sonnenberg[1], Martin Wetzel[1], Paolo Mascagni[2], Martin Zeitz[1], Britta Siegmund[1]. [1]Charité Berlin, CBF, Berlin, Germany; [2]Italfarmaco spa, Cinisello Balsamo, Italy

Histone modifications represent a promising new approach in cases where cell functions are to be modulated as in autoimmune diseases or cancer. While several histone deacetylase (HDAC) inhibitors are currently in clinical cancer studies, we demonstrated an additional anti-inflammatory potency in murine colitis models. Furthermore, when naïve T helper cells were polarized in the presence of ITF2357, the generation of FoxP3$^+$ cells could be enhanced, the polarization to the pro-inflammatory Th17 cells suppressed. In parallel, we demonstrated a dose-dependent down-regulation of the IL-6 receptor on naïve CD4$^+$ T cells treated with ITF2357. This effect could be observed on the mRNA expression level and on the protein level via flow cytometry. These results were confirmed in murine colitis models where the IL6R expression was diminished on naïve T cells within the lymphnodes, paralleled by a significant reduction of Th17 cells in the lamina propria of ITF2357-treated animals. Consequently, HDAC inhibition resulted in a reduced amount of activated/phosphorylated STAT3 in T cells identifying the IL-6/STAT3/IL-17 pathway as an important target of HDAC inhibitors. Thus, the present study demonstrates that inhibition of HDAC exerts an anti-inflammatory potency by modulating T cell differentiation, representing a novel therapeutic strategy for chronic (intestinal) inflammation.

T.133. **An IKKβ**-IL-17 Axis Regulates the Adjuvant Activity of Edema Toxin via Sublingual Route

Junbae Jee, Astrid Bonnegarde, Haley Steiner, Michael Fial, Prosper Boyaka. Ohio State University, Columbus, OH

Cytokines secreted by antigen-presenting cells (APCs) and helper T (Th) cells are known to induce secretory IgA Abs. However, their contribution to the mucosal adjuvant activity of Bacillus anthracis edema toxin (EdTx) has not been thoroughly investigated. As it was shown in previous study using intranasal immunization, EdTx had an adjuvant activity for sublingually co-administered Yersinia pestis F1-V, resulting in promoting F1-V specific serum IgG Ab titers. However, serum and mucosal IgA Ab, i.e. saliva and fecal responses were not enhanced in C57BL/6 mice. To address the role of cytokines from APCs and Th cells, we examined the mucosal adjuvant activity of EdTx in mice with IκB-kinase β-deleted macrophage (IKKβΔMΦ) and IL-17A KO mice. Both naïve IKKβΔMΦ and IL-17A KO mice showed enhanced number of IgA Ab-secreting cells in cervical lymph nodes (CLNs). After sublingual immunization with EdTx, the mucosal IgA Ab-responses were enhanced in IKKβΔMΦ and IL-17A KO mice, with higher increase measured in IKKβΔMΦ mice. In addition, EdTx enhanced NF-κB phosphorylation and proliferation of CLN cells in IKKβΔMΦ, but not IL-17A KO mice. In conclusion, the CLN-microenvironments in IKKβΔMΦ and IL-17A KO mice differently shape systemic and mucosal immune responses induced by EdTx as a sublingual adjuvant.

T.134. Increased Susceptibility to Autoimmune Gastritis in TSLPR-deficient Mice

Hisayo Nishiura[1], Norihiko Watanabe[1], Masahiro Kido[1], Nobuhiro Aoki[1], Satoru Iwamoto[1], Ryutaro Maruoka[1], Steven Ziegler[2], Tsutomu Chiba[1]. [1]Kyoto University, Kyoto, Japan; [2]Benaroya Research Institute, Seattle, WA

Thymic stromal lymphopoietin (TSLP) is involved in the pathogenesis of allergic inflammation in the gastrointestinal tract and promotes Th2-type intestinal immunity against helminth infection. In addition, TSLP regulates Th1-type inflammation in a mouse model of colitis, suggesting that TSLP plays crucial roles in intestinal immune homeostasis. Although autoimmune gastritis (AIG), mediated by inflammatory Th1 responses, develops in the

gastric mucosa, it is not clear whether TSLP is involved in regulating these responses in AIG. To examine the roles of TSLP in the development of AIG, we used BALB/c mice thymectomized 3 days after birth (NTx mice). We found that in AIG-bearing mice, TSLP was expressed in the inflamed stomach and that the serum anti-parietal cell Ab levels in neonatal thymectomized TSLPR-deficient (NTx-TSLPR-/-) mice were significantly elevated over those in NTx-TSLPR+/+ mice. In addition, NTx-TSLPR-/- mice exhibited earlier onset of AIG than that observed in NTx-TSLPR+/+ mice. The rapid development of AIG in NTx-TSLPR-/- mice resulted in more aggressive CD4+ T cell infiltration and more severe loss of parietal and chief cells in the progression phase of AIG, accompanied by enhanced Th1 responses. In conclusion, these data suggest that TSLP negatively regulates the development of AIG.

T.135. Comparison of Immunologic Properties of Cellular and Soluble Constituents of Breast Milk of Healthy and Allergic Mothers

Jiri Hrdy[1], Olga Novotna[1], Ingrid Kocourkova[2], Ludmila Prokesova[1]. [1]Charles University in Prague, Prague, Czech Republic; [2]Institute for the Care of Mother and Child, Prague, Czech Republic

Breastfeeding is believed to suppress allergy development. The differences between milk of healthy and allergic mothers and the effect of milk on cord blood momonuclear cells (CBMC) were studied using 3H thymidine incorporation, ELISPOT, flow cytometry, qPCR and cocultivation of milk cells with CBML in Transwell system. CBML of children of allergic mothers poses higher both spontaneous and polyclonally stimulated proliferation activity in comparison with children of healthy mothers. High concentration of soluble colostrum/milk constituents of both healthy and allergic mothers suppresses proliferation of CBML after stimulation with polyclonal activators. However, we detected increased immunoglobulin production (ELISPOT) after polyclonal activation of CBML in the presence of colostrum/milk. We did not proved significant differences in gene expression of Th1/Th2 cytokines between colostral cells of healthy and allergic mothers but a trend to increased levels of Th2 cytokines in allergic group was quite obvious. Soluble products of milk cells influenced cytokine expression in CBML only marginally. In conclusion, maternal milk influences CBML activation but there is no significant difference in the effect of colostrum/milk of healthy and allergic mothers. On the other hand, there is a substantial difference in the CBML reactivity of children of healthy and allergic mothers.

T.136. The Expansion of T Follicular Helper Cells During Chronic Salmonella Exposure Mediated the Generation of Autoimmune Hypergammaglobulinemia in MyD88-deficient Mice

Hyun-Jeong Ko[1], Hyungjun Yang[2], Sun-Young Chang[2], Sang-Uk Seo[2], Mi-Na Kweon[2]. [1]Kangwon National University, Chuncheon, Republic of Korea; [2]International Vaccine Institute, Seoul, Republic of Korea

Oral administration of recombinant attenuated Salmonella Typhimurium vaccine (RASV) strain in MyD88$^{-/-}$ mice resulted in chronic inflammation accompanying increased germinal center (GC) reaction and hypergammaglobulinemia with anti-dsDNA Ab in sera and the deposition of immune complex in the kidney, suggesting onset of autoimmunity. CD4^{+} T cells expressing ICOS, PD-1, IL-21, and CXCR5 were dramatically increased in these mice, indicating the expansion of follicular helper T (Tfh) cells. Of note, the depletion of CD4^{+} T cells completely blocked the generation of polyclonal IgG Ab in sera after oral RASV challenge and blockade of PD-1 and ICOS significantly reduced the hypergammaglobulinemia. Inflammatory myeloid cells expressing CD11b and Gr-1 were drastically expanded in the spleen of MyD88$^{-/-}$ mice after oral RASV challenge and produced high level of IL-6 after *in vitro* restimulation with RASV. Further, B cells from MyD88$^{-/-}$ mice expressed IL-21 after stimulation with RASV, and hence they might be the source of IL-21 for the initial differentiation of Tfh cells in chronic Salmonella infection. Overall, these results suggest that generation of Tfh cells by persistent Salmonella infection triggered autoimmune hypergammaglobulinemia in a PD-1- and ICOS-dependent manner.

T.139. Acceleration of Intestinal Inflammatory Responses by Extracellular Nucleotides-mediated Mast Cell Activation

Yosuke Kurashima, Takeaki Amiya, Jun Kunisawa, Hiroshi Kiyono. University of Tokyo, Minato-ku, Tokyo, Japan

Mast cells (MCs) are effector cells in allergic and inflammatory diseases, but their precise roles in intestinal inflammation remain unknown. Here we show that ATP-mediated activation of mast cells through P2X7 purinergic receptors plays a pivotal role in the induction of inflammatory responses in the colon. Treatment with a P2X7 purinoceptor-specific antibody inhibited MC activation and subsequent intestinal inflammation. Similarly, intestinal inflammation was ameliorated in MC-deficient KitW-sh/W-sh mice, and reconstitution with wild-type, but not P2x7-/-, MCs resulted in recovered susceptibility to inflammation. In the P2X7 purinoceptor-mediated activation, MCs recognized ATP including retrogressively generated one from ADP through the action of ecto-adenylate kinase on MCs. Therefore, inhibition of P2X7 receptor as well as adenylate kinase, an ecto-enzyme to convert ADP to ATP, reduced ADP-induced mast cell activation. The ATP-P2X7 purinoceptor-mediated activation of MCs induced the production of not only inflammatory cytokines but also chemokines and leukotrienes to recruit inflammatory cells for subsequent exacerbation of intestinal inflammation. These findings reveal the pivotal role of P2X7 purinoceptor-mediated MC activation in both initiation and exacerbation of intestinal inflammation.

T.140. Influence of Metals onto sIgA, IgA1 and IgA2 Production in Saliva and onto Cytokine Production by Lymphocytes in Patients Undergoing Implantation Therapy in Dentistry

Stepan Podzimek, Milan Tomka, Jarmila Prochazkova. Charles University, Prague, Czech Republic

The purpose of the study was to compare sIgA, IgA1 and IgA2 production in saliva before and after implantation therapy and to compare *in vitro*

cytokine production by lymphocytes stimulated by mercury and titanium antigens in patients undergoing implantation therapy in dentistry. Saliva from 21 patients was collected before implantation therapy and saliva from 9 patients after therapy and sIgA, IgA1 and IgA2 antibody production was established using RID method. Lymphocytes from 15 patients were stimulated by mercury and titanium antigens and production of 39 cytokines was established using Quantibody INF-3 array. Patients which reacted to titanium antigen and therefore zirconia implant was used in these patients, we found non-significant increase in sIgA, IgA1 and IgA2 production in saliva after implant therapy. On the other hand, in patients tolerating titanium, we did not find any differences in sIgA, IgA1 and IgA2 production before and after implantation. When compared to non-stimulated lymphocytes, lymphocyte culture stimulated by mercury antigen produced significantly increased levels of eotaxin-2, MIP-1a and MIP-1b, lymphocyte culture stimulated by titanium antigen produced significantly increased levels of IL-1ra and significantly decreased levels of IL-10. The study was supported by the grant of Czech Ministry of Health nr. NS 10577-3.

T.141. Omega-3 Fatty Acid (FA) Has Inhibitory Effects on Endometriosis via Downregulation of Inflammatory Cytokine Signaling

Kensuke Tomio, Kei Kawana, Aki Yamashita, Satoko Kojima, Seisuke Sayama, Takeshi Nagamatsu, Tomoyuki Fujii. University of Tokyo, Tokyo, Japan

Omega-3 polyunsaturated fatty acids (omega-3 FA) are well-known to involve anti-inflammation on the mucosa. Endometriosis is the most common disease in genital mucosa and associates with chronic inflammation against ectopic endometrium. We addressed anti-inflammatory effect of omega-3 FA on endometriosis by using mice model Fat-1 transgenic mice expressing an omega-3 FA desaturase converting omega-6 FA into omega-3 FA were used for endometriosis mice model. In this model, mice endometrium obtained from estradiol-supplemented donor was minced and injected into the peritoneal cavity of other recipient mice. Fat-1 or the littermate mice (WT) were used for the endometriosis model. Four mice for each group were sacrificed at every week (1-4 wks after injection). Peritoneal endometriosis lesions at each time point were analyzed pathologically as well as used for microarray gene expression. In Fat-1 group, the number of endometriosis lesions were significantly less than WT group ($p=0.028$). mRNA levels of interleukin-1beta, interleukin-6, metalloproteinases, prostaglandinE synthase and nuclear factor kappa B decreased in the Fat-1 group. Our data indicate omega-3 FA inhibited initiation of endometriosis through the anti-inflammatory effect and have a potential for prevention and treatment of endometriosis.

T.142. Non-toxic Concentration of Acetyl Salicylic Acid Can Induce ROS-dependent Increase of Small Intestinal Epithelial Cell Permeability

Akifumi Fukui, Yuji Naito, Osamu Handa, Kazuhiro Katada, Kazuhiko Uchiyama, Tomohisa Takagi, Nobuaki Yagi, Satoshi Kokura, Toshikazu Yoshikawa. Kyoto Prefectural University of Medicine, Kyoto, Japan

Background: Recent advance in endoscopy such as video capsule endoscopy and double balloon endoscopy enabled us to look inside the small intestine in more detail. Consequently, the acetyl salicylic acid (ASA)-induced small intestinal mucosal injury has been of great interest to gastroenterologists since the incidence of this disease is considerably higher than previously believed. However, the mechanism by which ASA-induced small intestinal injury is not clear yet. Objective: To investigate the mechanism by which low concentration of ASA induces small intestinal epithelial cell injury. Method: ASA was added to differentiated Caco-2 monolayer, a model of small intestine, and cell death was quantified by MTT assay and LDH release in the cell culture supernatant. As a functional study, the permeability of the Caco-2 monolayer was assessed by measuring transepithelial electrical resistance (TEER) and the flux of FITC-conjugated dextran across the monolayer. Moreover, tight junction protein (Claudin-1, ZO-1) expression was assessed by western blotting. Reactive oxygen species (ROS) production in Caco-2 was evaluated by redox-sensitive fluorogenic probes using a fluorometer and was confirmed morphologically by a confocal microscopy. Result: ASA induced cell death of Caco-2, both in dose and time dependent manners. However, lower concentration of ASA was able to increase the permeability of Caco-2 monolayer without affecting cell viability. This concentration of ASA also induced ROS production within 1hr and decreased Claudin-1 and ZO-1 expression in Caco-2. Conclusion: Taken together, low concentration of ASA-induced increment of cell permeability and ROS production that is independent of apoptosis, is one of the mechanisms by which ASA-induced small intestinal mucosal injury.

T.143. Pro-inflammatory Immunity and Intestinal Homeostasis

Gerard Eberl. Institut Pasteur, Paris, France

The intestine contains an estimated 1,014 symbiotic bacteria, thus 10-100 times more cells than our own body. These bacteria are vital for our own development, nutrition and protection, but can also endanger our health if not contained properly. The task of containing this vast microbiota, and maintaining intestinal homeostasis, falls to the immune system, which deploys a complex arsenal of tissues, cells and molecules to prevent invasion and select for the right bacterial residents. A particular set of immune cells, the RORγt+ innate lymphoid cells (ILCs), are programmed to develop and function ahead of the bacterial colonization of the gut. These cells are the prime producers of IL-17 and IL-22, both critical cytokines to boost the defense capacity of the intestinal epithelium and to recruit phagocytes that eliminate penetrating bacteria. Upon colonization with symbiotic bacteria, this program is repressed; upon damage and infection, it is amplified to force a return of the intestine to homeostasis. When these cells are absent altogether, the intestine tips over to fatal intestinal immunopathology caused by bacterial penetration.

T.144. Immunomodulatory Effects of Milk and Soy Protein-based Diets in the Interleukin-10 Gene-deficient Mouse

Alison Hodgkinson[1], Natalie McDonald[1], Matthew Barnett[2], Colin Prosser[3], Nicole Roy[2]. [1]AgResearch, Hamilton, New Zealand; [2]AgResearch, Palmerston North, New Zealand; [3]Dairy Goat Co-operative Ltd., Hamilton, New Zealand

Gastrointestinal homeostasis is maintained by interactions between host, microbiota and diet. If the balance is perturbed, inflammatory disease can result. We studied the effects of soy, cow milk or goat milk protein-based diets on gene expression levels of cytokines, chemokines and their receptors in intestinal tissue of Il10 gene-deficient mice. Five week-old Il10-/- and C57BL/6J control mice were fed diets (modified AIN-76A) containing 20% protein from soy, cow milk or goat milk for 6 weeks. Mice were then sacrificed and colonic tissues (n = 6 per group) were analysed for mRNA expression of 84 targeted inflammatory genes. Il10-/- mice fed soy-based diet gained weight similar to C57BL/6J control mice and showed little change in inflammatory gene expression levels. In contrast, the Il10-/- mice fed milk-based diets lost weight and developed diarrhoea. In mice fed cow milk, 25% of the genes were up-regulated (range 3 to 62-fold; P>0.01), compared with 14% (range 3 to 31-fold; P>0.01) for mice fed goat milk. Milk-based diets induced expression of inflammatory genes in Il10-/- mice, with cow milk inducing a greater number of genes at higher expression levels compared with goat milk. In contrast, soy protein-based diets ameliorated development of intestinal inflammation in Il10-/- mice.

T.145. The Regulation of T Cell Activity by Decidual Cells via PD-1:B7-H1/-DC Interaction

Seisuke Sayama, Takeshi Nagamatsu, Aki Yamashita, Satoko Kojima, Kensuke Tomio, Kei Kawana, Tomoyuki Fujii. Tokyo University, Tokyo, Japan

B7-H1 and B7-DC are inhibitory costimulatory ligands, which suppress T cell activation by binding to their common corresponding receptor, programmed death-1 (PD-1). PD-1 signaling negatively modulates T cell functions and is involved in the maintenance of peripheral tolerance. This study aimed to investigate the significance of PD-1:B7-H1/B7-DC interaction in the fetomaternal immune regulation. The expression of PD-1 ligands was examined in human decidual samples. Flowcytometric analysis demonstrated B7-H1 on both of decidual macrophages (DMs) and stromal cells (DSCs), while B7-DC only on DSCs. This expression pattern was shared between early and term decidua. Peripheral monocytes obtained from pregnant women were negative for B7-H1, showing clear contrast with DMs. The stimulation with IFN-γ and TNF-αenhanced PD-1 ligand expression on DMs and DSCs. Real-time PCR revealed this alteration is based on the up-regulation at mRNA level. In co-culture study between T cells and DSCs, IFN-γ production from T cells is increased in the presence of blocking antibody for PD-1 signaling, suggesting that DSCs have an ability to suppress T cell cytokine production via PD-1:B7-H1/-DC interaction. Our findings implied that PD-1 ligands on decidual cells might play an important role in the prevention of dangerous T cell activation against allogeneic fetal antigen, contributing to successful pregnancy.

T.146. Altered Immunoregulatory Profile During Anti-TNF Treatment of Patients with Inflammatory Bowel Disease

Jeanette Grundström[1], Ludvig Linton[1], Sarah Thunberg[1], Helena Forsslund[1], Izabella Janczewska[1], Ragnar Befrits[2], Marianne van Hage[1], Guro Gafvelin[1], Michael Eberhardson[1]. [1]Karolinska Institutet, Stockholm, Sweden; [2]Karolinska University Hospital, Stockholm, Sweden

Inflammatory bowel disease (IBD) can be treated by anti-TNF therapy, but the immuno-regulatory mechanisms of the treatment are unknown. To investigate this, peripheral blood lymphocytes and intestinal biopsies from IBD-patients treated with anti-TNF were analysed by flow cytometry. Regulation of peripheral blood mononuclear cell (PBMC) proliferation was analysed by blocking of IL-10, TGFβ or depletion of CD25+ cells in antigen-stimulated cultures. No changes in regulatory T cells (Treg) were observed in peripheral blood throughout treatment. Mucosal CD4+CD25+ cells decreased after two weeks of treatment, and thereafter increased. Simultaneously, the percentage of CD69+ cells among these cells increased throughout treatment. There was also an increase of mucosal Th1 cells after treatment compared to week two of treatment. Further, mucosal CD25+TNFRII+ cells were decreased after treatment compared to before. Before treatment, PBMC baseline proliferation was increased when IL-10 was blocked. However, after treatment IL-10 blocking had no effect whereas depletion of CD25+ cells resulted in increased proliferation. Our data do not support that anti-TNF treatment act through restoration of Treg activity in IBD patients, but rather leads to induction of effector T cells. However, although total numbers of Tregs are not altered, the Treg subset composition seems to change during the course of treatment.

T.147. Resistin-**like Molecule (RELM)α Promotes Th17** Cell Responses and Bacterial-induced Colitis

Meera Nair[1], Gregory Sonnenberg[1], Yurong Du[1], Kirk Bergstrom[2], Bruce Vallance[2], David Artis[1]. [1]University of Pennsylvania, Philadelphia, PA; [2]University of British Columbia, Vancouver, BC, Canada

The pathogenesis of inflammatory bowel disease is associated with the exaggerated expression of proinflammatory cytokines including IL-17A, and identifying the factors that promote cytokine expression may provide new ways to treat this disease. Employing citrobacter rodentium as a model of infection-induced intestinal inflammation, which is physiologically similar to the onset of IBD in patients, we identify a critical role for REsistin-Like Molecule (RELM)α in promoting intestinal inflammation. Citrobacter infection resulted in significant increases in RELMα expression both locally in the colon and systemically in the serum. Further, abrogation of RELMα expression using RELMα-/- mice ameliorated infection-induced inflammation. RELMα-/- mice exhibited reduced leukocyte activation in the infected colons and reduced citrobacter-specific Th17 cell responses. Conversely, recombinant RELMα treatment reestablished intestinal inflammation and immune cell activation in infected RELMα-/- mice. To test if the proinflammatory effects of RELMα were through the promotion of Th17 cell responses, infected WT and IL-17A-/- mice were treated with recombinant RELMα. In contrast to RELMα-treated WT mice, RELMα treatment of IL-17A-/- mice did not promote citrobacter-induced inflammation. Together, these data support a pathogenic role for RELMα in inducing inflammation at mucosal surfaces, in part through the promotion of Th17 cell

responses.

T.148. Features of Mucosal Topic Early Inflammation

Liangzhu Li[1], Yinyin Ben[1], Songhua Yuan[1], Yu Yang[1], Aiping Liu[2], Jianqing Xu[2], Xiaoyan Zhang[1]. [1]Shanghai Public Health Clinical Center, Shanghai, China; [2]Fudan University, Shanghai, China

Current existing models fail to identify the topical early inflammation and subclinical injury, and unable to predict the mucosal toxicity induced by microbicides. The present study, four representative microbicide candidates including cellulose sulfate (CS), Nonoxynol-9 (N-9), carraguard and tenofovir, were used to develop a new preclinical safety evaluation system. After treated with the candidates, though no obvious vaginal mucosa damage was observed by traditional histopathological examination, slight disruptions at cell junctions in epithelial cells layers were identified with occludin immunofluorence in situ, and proportion of early apoptotic-cells increased greatly, which suggested that CS could cause the increase of permeability in epithelial cells layers. In addition, application of CS in mouse's genital tract recruits inflammatory cells, including γδT cells, NK cells and macrophage, into the topical vaginal mucosa, and increased the secretion of a panel inflammatory cytokines (TNF-α, IL-10 and IFN-γ). Cytokines induced by N -9 featured with TNF-α and IL-6.However, no mucosal disruption or aberrant topical immune activation was observed for both carraguard and tenofovir. All these data are in accordance with the results of clinical tests. It demonstrated that our new safety evaluation system is able to identify minor epithelial barrier disruption and aberrant activation of topical mucosal immune system.

T.149. Immunomax /active Hexose Correlated Compound Induces *in vitro* Production of Interleukin-12 and Interferon-α and γ by PBMC-derived Mononuclear Cells from Subjects with Recurring Herpes Simplex Type 1 and 2 Infections

George Drannik[1], Anna Drannik[2]. [1]Ukrainian Center for Clinical Immunology, Kiev, Ukraine; [2]McMaster University, Hamilton, ON, Canada

We showed that subjects with recurrent HSV-1&2 infections secrete lower levels of IL-12, IFN-α and γ, suggesting that such individuals could benefit from immunostimulatory therapy. Active hexose correlated compound (AHCC) is the active part of Immunomax and represents a polymolecular compound from fungi Lentula edodes with high content of α-1,4-glucans. AHCC can potentially modify immune-inflammatory responses *in vivo*, and is used in Japan in cancers and viral infections. We attempted to characterize the effect of AHCC on the ability of PBMC- MNC from individuals with recurrent HSV-1&2 to produce interleukin (IL)-12 and interferon (IFN)-α & γ. After standard isolation, 1.5x10^6 c/ml of PBMC-MNC were incubated in culture medium (control group - spontaneous production) or in presence of 0.64 mg of AHCC as a stimulant (experimental group - induced production). We show that in presence of AHCC, PBMC-MNC secreted significantly higher levels of IL-12 (29.3±0.9 - 77.4±3.8 pg/ml), IFN-α (16. 4±1.5 - 53.0±3.3 pg/ml), and IFN-γ (44.2±3.2 - 92.1±5.4 pg/ml), with p<0,001) over 24 h of stimulation. Collectively, our results highlight the potential of AHCC to be recommended as an alternative treatment for individuals with frequently recurring HSV infections but in remission in order to promote immune rehabilitation and decrease HSV recurrence frequency.

T.150. Arginine and Glutamine Decrease IgE Dependent Cytokine Expression in Human Mucosal Mast Cells

Sandra Lechowski, Stephan Bischoff, Axel Lorentz. University of Hohenheim, Stuttgart, Germany

Introduction: Arginine (Arg) and glutamine (Gln) are essential amino acids with immunomodulatory functions, e.g. pharmacological doses of Arg and Gln caused a reduced cytokine-release in colonic biopsies from patients with active Crohn's disease. Mast cells (MC), among other functions, participate in the couse of intestinal inflammation. Here, we examined effects of Arg and Gln on mediator release and cytokine expression of human mucosal MC. Methods: MC were isolated from intestinal tissue by mechanic and enzymatic digestion, purified by positive selection, and cultured with SCF and IL-4. Leukotriene C4 (LTC4) and β-hexosaminidase were measured by ELISA and enzymatic assay, mRNA-expression was assessed by real-time RT-PCR, and signalling-molecule-activation was determined with Proteome ProfilerTM Array. Results: Following over night incubation with combined pharmacological doses of Arg (2 mmol/l) and Gln (10 mmol/l), IgE dependent mRNA expression for CXCL8, CCL2, CCL4 and TNF as well as activation levels of 18 out of 48 signaling molecules were decreased compared to low doses of Arg (0.1 mmol/l) and Gln (0.6 mmol/l). LTC4 was reduced in response to pharmacological doses of Arg and Gln whereas β-hexosaminidase was not affected. Conclusion: Immunomodulatory nutrients such as Arg and Gln are capable of modifying IgE-dependent cytokine-expression of human mucosal MC.

T.151. Antioxidant, Anti-inflammatory and Immunomodulating Properties of an Enzymatic Protein Hydrolysate from Yellow Field Pea Seeds (Pisum sativum L.)

Tri Vuong[1], Fatou Ndiaye[2], Jairo Duarte[1], Rotimi Aluko[3], Chantal Matar[1]. [1]University of Ottawa, Ottawa, ON, Canada; [2]University of Moncton, Moncton, NB, Canada; [3]University of Manitoba, Winnipeg, MB, Canada

Enzymatic protein hydrolysates of yellow pea seed has been shown to possess high antioxidant and antibacterial activities. The aim of this work was to confirm their antioxidant, anti-inflammatory and immunomodulating activities. The pea protein hydrolysate (PPH), after a 12h pre-treatment, showed significant inhibition of NO, TNF-α- and IL-6 production by LPS/IFN-γ-activated-macrophages up to 20%, 35% and 80%, respectively. Oral administration of PPH in mice enhanced the phagocytic activity of their peritoneal macrophages and stimulated the gut mucosa immune response. The number of IgA+ cells was elevated in the small intestine lamina propria, accompanied by an increase in the number of IL-4+, IL-10+, and IFN-γ+ cells. This was correlated to up-regulation of IL-6 secretion by small intestine epithelial cells (IEC), probably responsible for B-cell terminal

differentiation to IgA secreting cells. Moreover, PPH might have increased IL-6 production in IECs via the stimulation of Toll-like receptors (TLRs) family, especially TLR2 and TLR4 since either anti-TLR2 or anti-TLR4 was able to completely abolish PPH-induced IL-6 secretion. Thus, enzymatic protein degradation confers antioxidant, anti-inflammatory, and immunomodulating potentials to pea proteins and the resulted peptides could be used as an alternative therapy for the prevention of inflammatory-related diseases.

T.152. Polaprezinc Protects Rat Intestinal Epithelial Cells from the Cytotoxicity of Acetylsalicylic Acid Through the Upregulation of Heat Shock Protein 70

Osamu Handa, Yuji Naito, Qin Ying, Akifumi Fukui, Ryusuke Horie, Toshifumi Tsuji, Hiroyuki Yoriki, Kazuhiko Uchiyama, Takeshi Ishikawa, Tomohisa Takagi, Nobuaki Yagi, Satoshi Kokura, Toshikazu Yoshikawa. Kyoto Prefectural University of Medicine, Kyoto, Japan

Background/aims: Besides the activation of immune system, the upregulation of heat shock protein (HSP) in cells is one of defense mechanisms against apoptotic stimuli. To protect the small intestine from mucosal injury induced by acetyl salicylic acid (ASA) is one of critical issues in the clinical field. Polaprezinc (PZ), an anti-ulcer drug, provides gastric mucosal protection against various irritants. In this study, we investigated the protective effect of PZ on ASA -induced apoptosis of rat intestinal epithelial cell line (RIE1). Methods: Confluent RIE1 was incubated with 70μM PZ for 24h, and stimulated with 15mM ASA or not for the next 15h. Subsequent cellular viability, cell death and HSP70 expression were assessed. Further, we employed HSP70-specific small interfering RNA (siRNA) in some experiments. The production of reactive oxygen species (ROS) was also assessed by redox sensitive fluoro-probes. Results: ASA significantly induced apoptosis of RIE1, which was significantly suppressed by PZ. Although PZ increased HSP70 expression in RIE1, PZ could not suppress ASA-induced apoptosis in HSP70 silenced RIE1. Moreover, protective ability of PZ on ASA-induced ROS production was significantly inhibited in HSP70-silenced RIE1. Conclusion: PZ-increased HSP70 expression could be one of mechanisms by which PZ suppresses ASA-induced small intestinal injury.

T.153. IL-33 Attenuates Development and Perpetuation of Chronic Intestinal Inflammation by Dampening Th1 Responses

Philipp Gross, Nadja Dunger, Werner Falk, Claudia Hofmann. University Hospital, Regensburg, Germany

Interleukin-33 (IL-33) is the newest member of IL-1 family. Recent evidence shows the importance of IL-33 in autoimmune and inflammatory diseases. To elucidate the impact on inflammatory bowel diseases we studied the effect of IL-33 during the induction period of chronic dextran sodium sulfate (DSS)-induced colitis and after establishment of chronic inflammation. For induction of chronic colitis BALB/c mice received 4 cycles of DSS. Animals were treated with 0.1-1 μg murine IL-33 i.p. between the DSS cycles (intermediate treatment, i.t.) or after the onset of disease (post treatment, p.t.). Evaluation of intestinal inflammation was performed by histologic analysis, cytokine secretion of mesenteric lymph node cells, determination of myeloperoxidase (MPO), NF-κB, and CREB activity. Both approaches resulted in a significant reduction of inflammatory colon contraction, amelioration of disease scores, suppression of IFN-γ, and a shift to TH2-associated cytokines. NF-κB activity in mesenteric lymph node and lamina propria mononuclear cells was reduced upon IL-33 treatment, while an increase of MPO-activity was determined. Summarizing IL-33 has extenuating effect in chronic DSS-induced colitis: Excessive Th1-directed cytokine responses are shifted towards Th2-like immune reactions and general inflammation parameters are reduced. Augmented neutrophil activity may protect against the translocation of pathogenic bacteria through the damaged epithelium.

T.154. Eosinophil Activity in the Intestinal Mucosa of Diarrhea-prone Irritable Bowel Syndrome Patients

Maria Vicario, Cristina Martinez, Beatriz Lobo, Meritxell Guila, Ana Maria Gonzalez-Castro, Marc Pigrau, Mar Guilarte, Carmen Alonso, Ines deTorres, Fernando Azpiroz, Javier Santos. Vall d'Hebron Research Institut. Ciberehd, Barcelona, Spain

Background: The eosinophil (Eo), a multifunctional leukocyte, has been identified as a stress-dependent source of corticotrophin-releasing factor (CRF) in the intestine. In irritable bowel syndrome, a stress-sensitive disorder, the contribution of Eos to its pathophysiology remains unknown. Methods: Healthy (H, n=18) and age-matched, non-allergic, non-celiac participants fulfilling diarrhea-prone IBS (IBS-D, n=21) Rome III were included. Mucosal jejunal biopsies were obtained by Watson's capsule. Eos counts were performed after major basic protein (MBP) staining. Eos ultrastructure was assessed by transmission electron microscopy and CRF was identified by immunogold labeling. The expression of genes involved in Eo chemotaxis, activation and secretory activity was quantified by PCR. Results: The number of MBP+ cells were similar in both groups (H:44±7; IBS-D:78±19 per mm2) with increased fragmentation of cytoplasmic granules in IBS-D. The level of CRF labelling in the Eos granules was significantly increased in IBS-D than in H ($P<0.05$). Gene expression of eotaxins, SCF, ECP, and secretory carrier membrane proteins SCAMP-1/2/3 were significantly decreased, while the synaptosomal-associated protein SNAP-23 was increased in IBS-D respect to H ($P<0.05$). Conclusion: The restricted activation profile of jejunal Eos with increased CRF production in IBS-D may have a critical role in the pathophysiology of this disorder.

T.155. Platelets are Efficiently Recruited by Intestinal Epithelial Cells upon Infectious Triggering and Release RANTES *in vitro*

Hind Hamzeh-Cognasse[1], Pauline Damien[1], Fabrice Cognasse[2], Florence Grattard[1], Anne Carricajo[1], Philippe Berthelot[1], Bruno Pozzetto[1], Olivier Garraud[2]. [1]Université de Lyon, Université de Saint Etienne, Jean Monnet, Saint Etienne, France; [2]Etablissement Français du Sang, Saint Etienne, France

Blood platelets have been shown to produce inflammatory mediators upon endogenous or infectious triggering. Platelets could efficiently be recruited

to, and translocate through, the inflammatory intestinal mucosa. Platelet-derived microparticles can play an important role in inflammatory diseases such as arthritis with the presence of platelet-derived microparticles in synovial fluid. These observations strongly suggest an active contribution of platelets and/or platelet-derived microparticles to inflammation at mucosal sites. Therefore, we propose that upon inflammatory or infectious triggering, epithelial intestinal cells recruit platelets. We established an *in vitro* procedure to study, in real-time, the migration of CFSE vital fluorescent stained platelet towards conditioned media, in a Transwell chamber. We observed that platelets migrated towards a recombinant chemokine gradient only when they were associated to autologous leukocytes and that increasing numbers of leukocytes associated to platelets enhanced platelet migration. We also showed that conditioned media from Caco2 intestinal cells stimulated with heat-inactivated bacteria, such as E. coli or S. flexneri could efficiently recruit leukocyte-associated platelets, and that platelets could contribute to local immune response by releasing RANTES after migration. Thus, this study describes an immunoregulatory function of recruited platelets within the intestinal mucosa, emphasizing their role as an active component of the inflammatory response.

T.156. IL-33 Induces Pro-fibrogenic Gene Expression and Myofibroblast Hypertrophy: Potential Role in Inflammation-associated Intestinal Fibrosis and Stricture Formation

Benedetta Mattioli, Luca Pastorelli, Carlo De Salvo, Daniele Corridoni, Rekha Garg, Theresa Pizarro. Case Western Reserve University, Cleveland, OH

Fibrosis is a major complication of chronic gut inflammation, such as in IBD patients and SAMP1/YitFc (SAMP) mice, a spontaneous model of Crohn's disease-like ileitis. We evaluated the role of IL-33, recently reported to be increased in IBD and SAMP, as a potential mediator of intestinal profibrotic events. Histologic/trichome analysis, ST2 (IL-33 receptor) IHC, and qRT-PCR for profibrogenic genes were performed on SAMP and control AKR mice. Exogenous IL-33 was administered to SAMP/AKR, and ilea evaluated as above. Microarray analyses was done on SAMP/AKR ilea and IL-33-stimulated subepithelial myofibroblasts (SEMFs). SAMP displayed macroscopic/microscopic features resembling human intestinal fibrosis, first observed at 20 wks and reached peak levels by 50 wks of age. mRNA expression of coll-1, coll-3, IGF1, and CTGF was dramatically elevated in SAMP vs. AKR ilea. Robust ST2 staining was present within the inflamed lamina propria of SAMP, localized to SEMFs. IL-33 treatment induced marked hypertrophy of the muscularis propria, and potently increased profibrotic mRNA gene expression. Microarray data from SAMP ilea and IL-33-treated SEMFs displayed a global increase in profibrogenic gene expression. These data suggest an important role for IL-33 in intestinal fibrosis, and represents a potential target for the treatment of IBD-associated fibrosis and stricture formation.

T.157. Interferon-λ Signalling Drives Pathogenic Immune Responses During Microbiota-induced Septic Peritonitis

Stefan Wirtz, Tamuna Mchedlidze, Limei Shen, Markus Neurath. University of Erlangen-Nuremberg, Erlangen, Germany

Recent studies demonstrated that IFN-λs are an important part of TLR-mediated host responses in viral infections and showed association of reduced IFN-λ production with rhinovirus-induced asthma exacerbation. However, the role of these novel cytokines in bacterial induced diseases remains poorly understood. Here we investigated the role of IFN-λ dependent immune-responses in bacterial infections. Initially, we analyzed IFN-λ expression in mice systemically subjected to the intestinal microbiota. As a result qPCR/ELISA analysis demonstrated a strong upregulation of IFN-λ expression in serum/organs that depended on the presence of TLR4 and the IRF3 transcriptional activator. Furthermore, mice deficient in IFN-λ signaling, subjected to cecal-ligation-and-puncture (CLP) showed significantly increased survival compared to controls. Survival post CLP model was associated with highly decreased systemic spread of infection, inflammatory responses and organ injury. Similar results were obtained in a model of LPS-induced septic-shock, where high apoptosis in IFN-λ receptor expressing intestinal epithelial cells was observed. In line with these observations, a transgenic-overexpression strategy leading to increased serum-levels of IFN-λ3 resulted in increased systemic bacterial loads, tissue injury and mortality in these mice. In conclusion, the present data suggest that activation of IFN-λ/IFN-λR signaling contributes to bacterial sepsis and this could have implications for human disease.

T.158. Interleukin-1 Mediates Intestinal Inflammation by Promoting the Accumulation of Innate and Adaptive IL-17A Producing Cells

Margherita Coccia, Oliver Harrison, Mark Asquith, Kevin Maloy. University of Oxford, Oxford, United Kingdom

IL-1 is a potent pleiotropic cytokine modulating both innate and adaptive immunity. Despite the very high levels of IL-1 observed in the intestines of patients suffering from Inflammatory Bowel Disease, little is known about its contributionto chronic intestinal inflammation. Here, we used two complementary models of chronic colitis to address the role of IL-1 in driving innate and adaptive immune responses in the intestine. Our results show that IL-1 can drive intestinal inflammation in both innate and adaptive models of colitis. Moreover, we identified a crucial role for IL-1 in promoting the accumulation of IL-17A-producing cells in the inflamed intestine. Our data place IL-1 in the complex proinflammatory network required for intestinal inflammation and open the way for the investigation of therapeutic strategies targeting IL-1 for the treatment of inflammatory conditions of the intestine.

T.159. Crohn's Disease Intestinal Fibrosis is Associated with Infiltrating NK Cells Producing IL-13, which Downregulates Extracellular Matrix Degradation

Christine Whiting[2], Jennifer Bailey[2], John Tarlton[2], Iain Peters[2], Gabriella Koning[1], Amanda Williams[2], Morgan Moorghen[3], Paul Sylvester[2], Chris Probert[2], Paul Bland[1]. [1]Gothenburg University, Gothenburg, Sweden; [2]University of Bristol, Bristol, United Kingdom; [3]St. Marks Hospital, London, United Kingdom

Inflammation-induced fibrosis is a serious complication of inflammatory bowel disease, particularly Crohn's disease (CD), in which it induces stricture formation, necessitating resection of affected bowel. The mechanisms are unknown. Fibrosis in other tissues has been linked to T cell IL-13 and we have investigated the hypothesis that IL-13 amplifies the TGFβ-driven fibrosis in CD. We have investigated resected tissue for parameters of extracellular matrix synthesis and degradation, and have analysed mechanisms using tissue-derived mesenchymal cells and explants. Fibrosis, mostly confined to muscle and submucosal layers was associated with increased TGFβ, but remodelling MMP-2 was not elevated. IL-13 transcription was highest in fibrotic muscle and IL-13 receptors α1 and α2 were both upregulated in fibrotic muscle. A prominent population of large granulated cells was associated with inner fibrotic muscle. These cells were CD45+CD3-CD56+/-KIR+Rα1+Rα2- and, following laser capture, were found to transcribe high levels of IL-13, but only low levels of IFNγ or TGFβ. *Ex vivo* studies showed that while IL-13 did not directly stimulate collagen synthesis, it suppressed the synthesis of MMP-2. We propose that infiltration of a subpopulation of NK cells is crucial to the amplification of muscle fibrosis in CD through secretion of IL-13, which downregulates collagen degradation mechanisms.

T.160. Transcriptomic Alterations in Drug Metabolism and Steroid Hormone Biosynthesis in Crohn's Disease Patients

Yamile Zabana[1], Eduard Cabré[1], Josep Mañé[2], Violeta Lorén[2], Eugeni Domènech[1], Miriam Mañosa[1], Jaume Boix[1], Marta Piñol[1], Juanjo Lozano[1], Elisabet Pedrosa Tapias[2]. [1]Hospital Universitari Germans Trias i Pujol. Ciberehd, Badalona, Spain; [2]Institut d'Investigació Germans Trias i Pujol. Ciberehd, Badalona, Spain

Introduction: A differential gene expression profile is described in inflammatory bowel disease. Resectional surgery is a cornerstone in the management of Crohn's disease (CD). This subgroup of patients is not characterized with a functional genetic approach. Objectives: To describe the functional genetic characteristics of the intestinal tissue of CD patients who had undergone resectional surgery. Methods: Ileal inflamed tissue of 20 CD patients was collect from ileo-cecal intestinal resection. Controls were patients with normal ileal tissue, without CD requiring ileo-cecal resection. Human whole genome microarray (Codelink) was perfomed. Profile comparison and functional analysis of human mRNA was done with LIMA-R Package, GeneCodis, gene set enrichment analysis (GSEA) and informatics database consultation. Results: GSEA analysis showed that CD patients had enrichment of extracellular matrix, collagen and negative regulation of cell differentiation. With FDR restriction to values of < 0.0001, CD patients presented 536 down-regulated and 248 up-regulated genes. Down-regulated genes were involved in drug metabolism (cytochrome P450), steroid hormone biosynthesis, and tight junction. By the other hand, up-regulated genes were those involved in chemokine, leukocyte transendothelial migration, adipocytokine and TGF-beta signaling pathways. Conclusions: These preliminary results suggest new deficient pathways in CD pathophysiology as those involved in drug metabolism and steroid hormone biosynthesis.

T.161. The Inhibitory Effect of Triticum Aestivum Herba Extract on Colon Tissue Injury in Ulcerative Colitis Animal Model

Sun-Hee Lee[1], Sung-Won Lim[1], Young-Mi Lee[2], Dae-Ki Kim[1]. [1]Chonbuk National University Medical School, Jeonju, Republic of Korea; [2]Wonkwang University and Wonkwang, Iksan, Republic of Korea

Ulcerative colitis (UC) is a typical inflammatory intestinal disease belong to the inflammatory bowel diseases (IBD) and characterized by erosion, mucosal ulceration and infiltration of inflammatory cells. To investigate the inhibitory effect of methanol extract of Triticum aestivum herba (TA-Ex) on UC in mice. UC was induced in female Balb/c mice (19-20g) by providing 3% DSS in drinking water. TA-Ex was orally administrated daily for a weeks. Saline was used negative control and 5-aminosalicylic acid(ASA) as positive control. Oral administration of TA-Ex significantly attenuated clinical signs of UC including body weight, colon length and state of diarrhea. Moreover, TA-Ex reduced significantly myeloperoxidase(MPO) activity and expression of COX-2 and TNF-a in DSS-stimulated distal colon tissues. Histological study showed that TA-Ex suppressed markedly infiltration of inflammatory cells, mucosal cell disruption, and expression of NF-κB in colon tissues compared with that of negative control. The inhibitory effect of TA-Ex was similar to that of ASA. Therefore, we suggest that TA-Ex might be useful for a potential therapeutic agent for UC.

T.162. Therapeutic Effect of Deoxyschisandrin on Experimental Mouse Colitis Induced by Dextran Sulfate Sodium

Sung-Won Lim[1], Sun-Hee Lee[1], Young-Mi Lee[2], Kang-Soo Lee[1], Dae-Ki Kim[1]. [1]Chonbuk National University Medical School, Jeonju, Republic of Korea; [2]Wonkwang University and Wonkwang-Oriental Medicines Research Institute, Iksan, Republic of Korea

Ulcerative colitis (UC) belong to inflammatory bowel diseases (IBD) is immune-mediated chronic disorders and characterized by mucosal ulceration, upregulation of pro-inflammatory cytokines, and infiltration of immune cells. We previously demonstrated that dextran-sulfate sodium (DSS)-induced colitis have been suppressed by oral treatment of extract of Schizandrae chinensis, which is a famous traditional Asian medical plant widely used as an antistress, anti-aging, and neurological performance-improving herb. In this study, we investigated therapeutic effect of deoxyschisandrin (DSC), which is a lignan compound purified from Schisandra chinensis, to protect mice from development of UC. Animal model of colitis was established by providing 3% DSS for 5days. DSC was orally administered daily. DSC administration led to a significant recovery of body weight and colon length,

and attenuation of diarrhea revealed in DSS-induced colitis. In histochemistry assay, DSC administration markedly decreased tissue damage, infiltration of inflammatory cells and the level of NF-κB expression in colon tissue. In addition, DSC inhibited the expression of inflammatory factors including TNF-α and MPO. These results indicate that DSC can significantly enhance recovery of normal colonic function throughout suppression of inflammatory response, implicating that DSC maybe is useful for a therapeutic agent for UC.

T.163. Clindamycin Therapy Results in Profound and Long-lasting Decreases in Microbial Flora Diversity During Clostridium Difficile Infection in Mice

Charlie Buffie, Irene Jarchum, Joao Xavier, Eric Pamer. Memorial Sloan-Kettering Cancer Center, New York, NY

Clostridium difficile is the most common cause of hospital-acquired diarrhea. C. difficile-associated colitis commonly develops in patients who receive antibiotics, particularly clindamycin. Antibiotics can alter the density and composition of the intestinal microbial flora, but the mechanism by which antibiotics lead to increased susceptibility to C. difficile is not clear. Here, using a mouse model of C. difficile colitis, we characterized the effects of clindamycin and C. difficile infection on intestinal microbial diversity over a 30-day period using 16s rDNA pyrosequencing. Clindamycin treatment and infection was followed by a 3 to 4 day period of weight loss and 40% mortality by day 5 post-infection. Surviving mice regained weight and recovered from diarrhea, but intestinal colonization by C. difficile was maintained up to day 30. Our results demonstrate that while a single dose of clindamycin treatment resulted in only a moderate, transient decrease in bacterial density, the diversity of the microbial flora was dramatically decreased for the entirety of the 30-day period. Thus, we demonstrate that brief clindamycin therapy has a profound and long-lasting impact on the intestinal microflora and may have important implications for C. difficile colitis susceptibility and relapse.

T.164. Ileal Injury in Two Mice Models of Microbial Sepsis: Probable Causes and Possible Protective

Hossam Ebaid. King Saud University, Riyadh, Saudi Arabia

Sepsis incidence and deaths are rising everyday despite the numerous researches and new therapeutic advance. Sepsis is associated with ileal injury. This study aims to determine the causes of ileal injury in two models of sepsis and the possible protective role of phytic acid. Results showed an increased inflammatory and lymphocytic cells' influx associated with apoptotic index decrease. The inflammation was accompanied by hyper-mucus secretion, villar atrophy, necrosis and desquamation with each infection and was much severe in LPS. Most enterocytes of the infected mice lost their microvillar brush border and had destructed organelles. The morphometric studies recorded significant decrease in all examined measures (villar, enterocyte and microvillar heights, crypt depth and the goblet cells number) after four weeks of the onset of the experiment with both models. Phytic acid had the ability to attenuate ileal injury in the two models of sepsis after four weeks of its administration where its supplementation can greatly minimize the histopathological and cytological complications and morphometric alterations resulted from bacteria or its endotoxin. Its administration for four weeks was better for inducing its ameliorative effect via increasing mucus secretion, decreasing apoptotic index, attenuating the inflammatory and lymphocytic cells' count or increasing the renewal of the crypt cells and villar epithelial cells proliferation.

T.165. Interferons and the Transcription Factor T-bet Reprogram Th2 Commitment to Permit Protective Anti-viral Response

Ahmed Hegazy[1], Michael Peine[1], Caroline Helmstetter[1], Isabel Panse[1], Anja Fröhlich[1], Andreas Radbruch[2], Max Löhning[1]. [1]Charité - University Medicine Berlin, Berlin, Germany; [2]German Rheumatism Research Center (DRFZ), Berlin, Germany

CD4+ T cells can differentiate into several effector subsets, including classical Th1 and Th2 cells. Cytokine signals induce the expression of lineage-specifying transcription factors, which promote alternative T cell differentiation programs. T-bet and GATA-3 are the master transcription factors of Th1 and Th2 cell differentiation, respectively. Knowledge on the plasticity of CD4 T cell lineages, especially of Th2 cells, is limited. To challenge the commitment of Th2 cells and to investigate the functional relevance of Th2 cell plasticity *in vivo*, we have established an adoptive transfer system of Th2 and Th1 cells reactive to lymphocytic choriomeningitis virus (LCMV). In the absence of viral infection, Th2 memory cells remained GATA-3+ and produce Th2, but not Th1, cytokines upon reactivation. Upon LCMV infection, the majority of the Th2 cells started to express IFN-γ, and many of these IFN- γ+ cells co-expressed IL-4 and other Th2 cytokines. In addition, the GATA-3+ Th2 cells adopted a GATA-3+T-bet+ “Th2+1” phenotype at the single-cell level. Remarkably, this GATA-3+T-bet+ and IL-4+IFN- γ+ phenotype was stably maintained *in vivo* for months after the resolution of the LCMV infection, suggesting ‘‘lineage-like’’ properties of Th2+1 cells. Th2 cell reprogramming required TCR stimulation, combined type I and type II interferon and IL-12 signals, and T-bet. Virus-induced T-bet induction in virus-specific Th2 cells was crucial to prevent viral persistence and fatal immunopathology. Taken together, our findings provide a molecular concept for the long-term balanced coexistence of Th2 and Th1 lineage characteristics in single memory T cells and may open new avenues for novel therapeutic interventions to modulate inefficient, immunopathological, or autoimmune CD4 T cell-dependent immune responses.

T.166. Interleukin-1 Gene Polymorphisms Associated with Reflux Oesophagitis in the Czech Population

Petra Linhartová[1], Filip Marek[2], Zdeněk Kala[2], Lydie Izakovičová-Hollá[1]. [1]Masaryk University, Brno, Czech Republic; [2]University Hospital Brno-Bohunice, Brno, Czech Republic

Reflux Oesophagitis (RO) is the subset of gastro-oesophageal reflux disease (GORD) with inflammation of the lower oesophagus due to acid gastric contents regurgitation. Polymorphisms in the interleukin-1 (IL-1) genes play a role in inflammatory diseases though the modulation of cytokine levels.

The aim of this study was to associate the three variants in the IL-1 cluster with RO in the Czech population. In this case-control association study 131 patients with RO and 165 controls were genotyped using PCR-RFLP or PCR method for IL-1 gene polymorphisms [IL-1α (-889C/T), IL-1β (+3953C/T), and IL-1RN (IL-1 receptor antagonist, VNTR)]. No significant differences were found in the allele and genotype frequencies of all polymorphisms between patients with RO and controls. Complex analysis revealed differences in IL-1 haplotype frequencies; the haplotype C(IL-1α-889)/C(IL-1β+3953)/1(IL-1RN*1 allele) of the IL-1 gene cluster was significantly more frequent (54.8% vs. 43.7%, p=0.023) and the haplotype C(IL-1α-889)/C(IL-1β+3953)/2(IL-1RN*2 allele) less frequent (11.9% vs. 21.2%, p=0.020) in RO patients than in controls. Although no significant role of single IL-1 variants in RO was found, our results suggest that different IL-1 haplotypes may be associated with increased or decreased susceptibility to RO. This study was supported by the project NPVII 2B06060, 1M0528 and IGA NT11405-6.

T.167 Impact of Ruminococcus Gnavus E1 on the Mucus Carbohydrate Profile

Josette Perrier, Fabien Graziani, Cendrine Nicoletti, Michel Fons, Thierry Giardiana. Université Paul Cézanne Aix-Marseille III, Marseille, France

The microbiota plays a fundamental role on the health of the host, impacting his physiology, metabolism and protecting against pathogens. In response, the intestinal cells modulate the carbohydrate profile of the mucus. To study this molecular cross-talk in the intestinal mucosa with the microbiota, we have used as a model Ruminococcus gnavus E1, isolated from the dominant microbiota of a healthy adult, Gram-positive, strict anaerobic. Our studies clearly show *in vivo* (on gnotobiotic animals) and *in vitro* (on Caco-2 and HT29-MTX) an upregulation of the expression of genes coding for intestinal fucosyltransferases and galactosyltransferases, in the in the epithelial cells also confirmed by microscopical analyses using lectine-FITC. This variation of expression of mRNA coding for different glycosyltransferases proves the re-initiating programme of mucus protection layer by the dominant commensal R. gnavus E1. Bry L, et al., Science 1996, 273: 1380; Freitas M,et al., Histochem Cell Biol. 2002, 118: 149; Hooper LV, et al., Science 2001,291: 881; Sonnenburg JL, et al., Science 2005, 307: 1955.

T.168 Microbial Functions at the Human Gut Mucosal Interface: An -omic Overview of the Crohn's Disease Microbiome

Stanislas Mondot[1], Patricia Lepage[1], Jessica Coehlo[2], Marie Joossens[3], Anne Lavergne-Slove[2], Trent Northen[4], Benjamin Bowen[4], Joël Doré[1], Philippe Marteau[2], Marion Leclerc[1]. [1]INRA, Jouy-en-Josas, France; [2]AP-HP Lariboisiere Hospital, Paris, France; [3]Catholic University Leuven, Leuven, Belgium; [4]Lawrence Berkeley National Laboratory, Berkeley, CA

Objectives: The gut microbiota plays a crucial role in Crohn's disease (CD). However, functions of gut mucosal microorganisms are poorly understood. The present study investigated metagenome, metatranscriptome and metabolome of the microbial communities in luminal and mucosal compartments of the human gut. Methods: Luminal and mucosal compartments were sampled from ileo-caecal resection specimens of CD patients. Genomic DNA, cDNA and 16S rDNA were sequenced using 454-FLX-Ti pyrosequencing. Bacterial diversity was assessed; DNA and cDNA sequences were assigned to functional metabolic pathways (COGs and KEGGs). Metabolomic was performed using Imaging Laser desorption/ionization mass-spectrometry (AB Sciex; Foster City, CA). Bacteriophages loads and morphotypes were characterized. Results: The main bacterial genera were similar between luminal and mucosal compartments (Prevotella, Bacteroides, Faecalibacterium, Roseburia). From a metatranscriptomics point of view, luminal content and mucosa were less similar. Functions significantly associated to the mucosal microbiota in CD were Cofactors and Vitamins' Metabolism and Translation, ribosomal structure and biogenesis. Furthermore, several metabolites with mass between 200 and 1150Da were highly abundant and spatially distributed in the ulcerated and non-ulcerated areas of resected tissues. Virus-like particles were counted at $>10^9/mm^3$ mucosa. Non-ulcerated areas were dominated by a few bacteriophages morphotypes. Conclusion: These results, combining metagenomics with metatranscriptomics and imaging metabolomics, provide the first insights to characterize potential mechanisms underlying functional dysbiosis in CD.

T.169. Chemokine and Cognate Receptor Expression in the Stomach of Mice Vaccinated Against Helicobacter Pylori Infection

Michael Mozer, Malin Sundquist, Jan Holmgren, Sukanya Raghavan. University of Gothenburg, Gothenburg, Sweden

Vaccination is an attractive approach for the control of H. pylori-related diseases like chronic gastritis, peptic ulcers and gastric cancer. We have studied the mechanisms guiding immune cells to the stomach and subsequent elimination of the bacteria in mouse model of H. pylori infection. C57BL/6 mice were vaccinated with H. pylori lysate antigens and a mucosal adjuvant and challenged with live H. pylori bacteria. Stomach tissue was taken at various time-points post challenge to evaluate bacterial colonization, analyze chemokine gene expression by RT-PCR and influx of cells by flow cytometry. Protection against H. pylori infection in vaccinated mice was first evident 7 days after infection and sustained for 21 days. A significant up-regulation of chemokine gene expression attracting neutrophils (CXCL2, CXCL5), T cells (CXCL10, CXCL11) and dendritic cells (CCL19), and their corresponding receptors CXCR2, CXCR3 and CCR7 coincided with vaccine-induced protection. Flow cytometric analysis revealed a sequential accumulation of CD4+ T cells, neutrophils and CD103+ dendritic cells in the gastric lamina propria of vaccinated mice post-challenge. In conclusion, this study has provided us insights into mechanisms guiding immune cells important for protection against H. pylori infection to the stomach, which should assist in the design of an efficacious vaccine.

Poster Presentations: Friday, July 8
Authors Present: 13:00-14:30

F.1. Microbial Immune Subversion and Disruption of Host Homeostasis in the Periodontium
George Hajishengallis, Jennifer Krauss. University of Louisville Health Sciences Center, Louisville, KY

Periodontal health represents a dynamic balanced state where proinflammatory and antimicrobial activities are optimally regulated to keep bacteria at bay while preventing unwarranted host responses. This homeostatic balance or 'physiological inflammatory state' may be disrupted, however, by pathogens that subvert the host response. Porphyromonas gingivalis appears to be a keystone periodontal pathogen which modifies immune selective pressure in ways that promote the microbial adaptive fitness while causing collateral tissue damage in the periodontium. In this regard, we have shown that P. gingivalis subverts key components of innate immunity, such as Toll-like receptors (TLRs), complement, and their crosstalk pathways. *In vitro* and *in vivo* studies show that P. gingivalis instigates a crosstalk between the complement C5a receptor (C5aR) and TLR2 in leukocytes leading to increased induction of bone-resorptive cytokines. Paradoxically, the same crosstalk impairs the killing of P. gingivalis by neutrophils or macrophages, attributed to selective inhibition of specific antimicrobial responses, such as nitric oxide generation in macrophages. Consistent with these findings, C5aR-deficient or TLR2-deficient mice display increased resistance to inflammatory bone loss and can better control P. gingivalis infections compared to wild-type controls. In conclusion, P. gingivalis induces C5aR-TLR2 crosstalk which impairs host immunity and promotes periodontal tissue destruction.

F.2. TPL-2 Contributes to Intestinal Pathology by Regulating NF-**κB** Activity through Nuclear Export of P50 Homodimers
Maria Lawrenz[1], Alexander Visekruna[2], Ulrich Steinhoff[2]. [1]MPIIB, Berlin, Germany; [2]Philipps-Universität, Marburg, Germany

NF-κB activation is a hallmark of IBD and is controlled by proteasomal degradation of IκBα and p105 which retain NF-κB dimers in the cytoplasm. Activation of TPL-2-ERK also requires degradation of p105 to release TPL-2 which phosphorylates MEK and ERK subsequently. We found that patients with Crohn's disease have enhanced activation of TPL-2-ERK in the inflamed intestine. We aimed to study the role of TPL-2-ERK in DSS-colitis. TPL-2-/- mice developed reduced intestinal inflammation during DSS-colitis as measured by body weight loss. Lack of TPL-2 did not affect cellular infiltrates into the colon but dramatically reduced the ability of these cells to produce IL-1β, TNFα and IL-6. We found that TPL-2 controls NF-κB activation and thereby regulates cytokine production. Mechanistically, TPL-2 is involved in the nuclear export of NF-κB p50 homodimers, which is a prerequisite for efficient DNA binding of NF-κB heterodimers. In Summary, lack of TPL-2 strongly attenuates intestinal inflammation in a murine colitis model. Accordingly, treatment of wt animals with the TPL-2 kinase inhibitor was sufficient to reduce intestinal inflammation. Thus, the TPL-2 kinase might be a promising target for anti-inflammatory treatment since it modulates NF-κB activation after immune stimulation while leaving NF-κB mediated protective cell functions undisturbed.

F.3. Rotavirus Infection and Type III Interferon
Masatake Asano[1], Daisuke Omagari[1], Itaru Moro[2], Kazuo Komiyama[1]. [1]Nihon University School of Dentistry, Tokyo, Japan; [2]Nihon University Advanced Research Institute for the Sciences and Humanities, Tokyo, Japan

Rotaviruses, members of the Rotaviridae family, are nonenveloped viruses with segmented double-stranded RNA genomes. Rotavirus has been identified as the major etiologic agent of diarrhea in infants and young children and the most common cause of severe gastroenteritis in developing countries. The main target of rotavirus is the intestinal epithelial cells (IECs). To get insight into the genes expressed specifically by rotavirus infection, we performed microarray analysis using the human colon-derived adenocarcinoma cell line, HT-29. The data demonstrated that rotavirus infection can induce the expression of type III interferon (IFN) mRNA. To confirm the microarray data, we performed real-time PCR and ELISA to detect the expression and production of type III IFN. Type III IFN induction was detected in both HT-29 and another IEC line, T84, but not in Caco-2 cell. The peak production of type III IFN was detected at 6 h post infection. The production was decreased thereafter and no type III IFN was detected at 24 h. We further performed the small interfering RNA (siRNA) experiments to know which receptor can sense the infection of rotavirus. The results indicated that retinoic-acid inducible gene I (RIG-I) is the responsible sensor for the detection of virus infection in HT-29 cells.

F.4 Splenic IgM+ B Cells are Found to be IgA+ in the Small Intestine
Manuela Buettner, Desiree Weiberg, Ulrike Bode. Hannover Medical School, Hannover, Germany.

We previously showed that the spleen is involved in inducing of an immune response after oral antigen application. Furthermore, a high level of IgA+ cells was seen in the small intestine after dissection of the mesenteric lymph nodes (mLN). Therefore, it was studied, whether the spleen is involved in the induction of IgA+ cells in the small intestine after an oral immune response. Ovalbumin combined with choleratoxin primed splenic IgM+ B cells of Ly5.1 mice were isolated and injected into wildtype mice. The presence and the isotype of these cells in various tissues were detected after application of ovalbumin. The results show that these cells were present in the mLN, the Peyer´s patches (PP) and the intestine itself. Most of the isolated IgM+ B cells which migrated into the spleen were still IgM+. This illustrates that these cells did not change their isotype. Surprisingly,

previously isolated IgM+ B cells, now in the small intestine, and also in the PP had changed to IgA or IgG positive. The data show that spleen B cells are not only able to enter the intestine and the associated lymphatic tissue after antigen application, but also have a different isotype in these tissues.

F.5. Stromal/Extracellular Matrix-associated Cytokines Regulate Tolerogenic DC-T Cell Effector Responses in Normal Intestinal Mucosa and Pro-inflammatory DC-T Cell Responses in Crohn's Disease Mucosa

Lesley Smythies, Kayci Huff, Anna Fox, Jamie Cannon, Jayleen Grams, Richard Stahl, Philip Smith. University of Alabama at Birmingham, Birmingham, AL

To elucidate the role of stromal cells and their product, extracellular matrix (ECM), in mucosal immunoregulation, we have shown that intestinal stroma regulates local macrophage (JCI 2005 115:66; JBC 2011, 285:19593) and effector T cell function (Mucosal Immunology 2011, EPub). However, little is known about stromal/ECM regulation of intestinal dendritic cells (DCs). Therefore, we “mucosalized” DCs by exposing monocyte-derived DCs (M-DCs) to stroma-conditioned media (S-CM) generated from isolated stroma from normal intestinal mucosa or Crohn’s disease intestinal mucosa, as we have described (Refs above). After inducing M-DC maturation by exposure to Crohn’s disease-associated CBir1 flagellin, normal S-CM induced a tolerant M-DC profile reflected in (a) minimal production of IL-12 and IL-6, (b) high aldehyde dehydrogenase (ALDH) activity and (c) generation of T cells that proliferated poorly and released low levels of IFN-γ. In contrast, Crohn’s disease S-CM induced a pro-inflammatory M-DC profile with (a) significantly increased production of IL-12 and IL-6, (b) reduced ALDH activity and (c) generation of T cells that strongly proliferated and released abundant IFN-γ. These findings uncover a role for the stroma/ECM in the immunoregulation of mucosal DC-T cell effector function and implicate the stroma/ECM in the loss of homeostasis that characterizes inflammation in Crohn’s disease.

F.6. Mast Cell Toll-like Receptor 2 Signaling is Crucial for Effective Killing of Francisella Tularensis

Bernard Arulanandam[1], Annette Rodriguez[1], M. Guentzel[1], Jieh Yu[1], Thomas Forsthuber[1], Michael Berton[2], James Chambers[1]. [1]University of Texas at San Antonio, San Antonio, TX; [2]University of Texas Health Sciences Center, San Antonio, TX

Mast cells provide critical defense mechanisms against microbial respiratory pathogens. We recently demonstrated that mast cells inhibit francisella tularensis intramacrophage replication primarily via interleukin-4 (IL-4) secretion. Here, mast cell recognition and control of Francisella replication was investigated using lucifer labeled francisella with confocal and live cell microscopy. Toll-like receptor 2 (TLR2) was crucial for mast cell innate immune responses, including trafficking and early accumulation of lysosomal associated membrane protein 2 (LAMP2), MHCII, and the hydrolytic enzyme cathepsin L. Importantly, $TLR2^{-/-}$ mast cells lacked detectable IL-4 and showed a 2 to 3 log increase of F. tularensis type A (SCHU S4) and live vaccine strain replication compared to WT and $TLR4^{-/-}$ mast cells. $TLR2^{-/-}$ mast cells also exhibited a delay in enhanced expression and membrane localization of LAMP2 and cathepsin L, which coincided with host cell destruction. Recombinant IL-4 rescued $TLR2^{-/-}$ mast cells and promoted killing of bacteria. Notably, WT mice challenged intranasally with francisella revealed mast cells infiltrating the lungs in close proximity to mCherry labeled francisella, supporting an *in vivo* role for mast cells during pulmonary infection. Collectively these results suggest that optimal mast cell mediated innate immune responses require TLR2 signaling and subsequent IL-4 production for killing of francisella.

F.7. Dextran Sodium Sulfate Induced Colitis Leads to Transcriptional Modulation of Pattern Recognition Receptors and T Cell Polarization

Bin Zheng[1], Mary Morgan[1], Henk van de Kant[1], Johan Garssen[2], Gert Folkerts[1], Aletta Kraneveld[1]. [1]Utrecht University, Utrecht, Netherlands; [2]Danone Research, Wageningen, Netherlands

Studies from germ-free animals reveal that intestinal microflora is important in intestinal inflammation. Since pattern recognition receptors (PRRs) are responsible for the detection of bacteria, PRRs may contribute to intestinal diseases, like inflammatory bowel disease (IBD). In this study, the dextran sodium sulfate (DSS) model of colitis was used to survey the mRNA expression of PRRs along the length of the colon during colitis. Additionally, T cell responses in the colon were examined by measuring the mRNA expression of both T cell subset-associated master transcription factors and cytokines. The expression of the majority of PRRs was increased and was correlated with the degree of inflammation. The inflamed regions were also characterized by a robust Th1/Th17 response. These results emphasize the potential capacity of PRRs to influence ongoing inflammation and provide a basis for future research focusing on PRRs as a therapeutic avenue for IBD and other intestinal diseases.

F.9. Significantly Altered Expression of Innate Pattern Recognition Receptors (PRRs) in Cervical Mononuclear and Epithelial Cells of HIV-exposed Uninfected Commercial Sex Workers in Kenya

Xiao-Dan Yao[1], Richard Lester[2], Robert Were Omange[3], Bethany Henrick[1], Walter Jaoko[4], Charles Wachihi[4], Terry Ball[3], Francis Plummer[3], Kenneth Rosenthal[1]. [1]McMaster University, Hamilton, ON, Canada; [2]British Columbia Center for Disease Control, Vancouver, BC, Canada; [3]Public Health Agency of Canada, Winnipeg, MB, Canada; [4]University of Nairobi, Nairobi, Kenya

Immune activation plays a critical role in HIV disease progression. Indeed, the key feature distinguishing pathogenic from non-pathogenic SIV infections is the lack of aberrant immune activation in naturally infected simian hosts. Here, we assessed mucosal innate signaling in the genital tract of HIV-1 resistant (HIV-R) compared to HIV-susceptible (HIV-S) and HIV-positive (HIV-P) commercial sex workers. Our results showed that expression of selected PRRs, including TLR2, RIG-I, Mda5 and UNC93B were significantly reduced in cervical mononuclear cells (CMCs) of HIV-R compared to HIV-S and HIV-P groups. TLR7 and TLR8 also showed significantly reduced expression in CMCs of HIV-R compared to HIV-S and

HIV-P in repeated assessments over time. *In vitro* stimulation of CMCs demonstrated reduced cytokine responses to LPS, ssRNA and imiquimod in HIV-R group. Interestingly, TLR3 and TLR7 mRNA and downstream NFκB protein were significantly increased in cervical epithelial cells (ECs) from HIV-R compared to HIV-P. Lastly, CVL from HIV-R had lower levels of inflammatory cytokines, IL-1β, IL-8 and RANTES. These data suggest that cervical mononuclear cells of HIV-R women have suppressed innate responses that may prevent immune activation and inflammation, whereas their cervical ECs express elevated viral RNA sensors capable of eliminating HIV upon exposure.

F.10. Control of Rip2 Dependent Innate Immune Activation by GEF-H1

Joo-Hye Song[1], Zhao Yun[1], Carmen Alonso[1], Isabel Ballaster[1], Bayasi Guleng[1], Seiji Arihiro[1], Peter Murray[2], Koichi Kobayashi[3], Hans-Christian Reinecker[1]. [1]Massachusetts General Hospital and Harvard Medical School, Boston, MA; [2]St. Jude Children's Research Hospital, Memphis, TN; [3]Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA

Background: Guanine Nucleotide Exchange Factor H1 (GEF-H1) is encoded by the arhgef2 gene and a component of signaling by the Nucleotide-binding domain, Leucine-Rich repeat containing receptor C (NLRC) family of microbial pattern recognition receptors. Rip2 is a member of the receptor-interacting protein (RIP) family of kinases, which have emerged as essential sensors of NLR and Toll like receptor signaling for the activation of NF-κB. Results: We demonstrate that GEF-H1 is required for the activation of NF-κB by Rip2 following NOD2 activation. GEF-H1 was part of NOD2 and Rip2 containing immunocomplexes and significantly enhanced NF-κB (3.2-fold) and TNF-α(15-fold) and IL-6 (11-fold) expression compared to NOD2 and Rip2 signaling alone. GEF-H1, Rip2 and NOD2 co-localized in an endosomal compartment during signal transduction. GEF-H1 was able to activate NF-κB activation in NOD2-deficient but not Rip2-deficient macrophages. Surprisingly, GEF-H1 mediated tyrosine phosphorylation of Rip2, which also occurred during signaling by the NOD2 but not in the presence of the 3020insC variant of NOD2 associated with Crohn's disease. Rip2 mutants lacking the tyrosine target of GEF-H1 mediated phosphorylation were unable to mediate NF-κB activation in RIP2 deficient macrophages and fail to transduce NOD2 signaling. Conclusion: GEF-H1 is required downstream of NOD2 as part of Rip2 containing signaling complexes and was responsible for tyrosine phosphorylation of Rip2. Thus, GEF-H1 connects tyrosine kinase function to NLR signaling and is fundamental to the regulation of microbial recognition by ubiquitous innate immune mechanisms mediated by Rip2 kinase.

F.11. Oxidative Respiratory Burst of Neonatal Monocytes and Neutrophils in Response to Gram-negative and Gram-positive Microorganisms and TLR Ligands

Ana Lúcia Lessa-Marques, Camila Quinello, Simone Corrêa-Silva, Marco Antonio Cianciarullo, Magda Carneiro-Sampaio, Patricia Palmeira. University of São Paulo, São Paulo, Brazil

Because of immature immune function, the newborn is susceptible to intestinal and systemic infections. Klebsiella pneumoniae, Escherichia coli, Pseudomonas and Staphylococcus aureus are major pathogens of neonatal infections in developing countries. The aim is to investigate if cord blood monocytes and neutrophils present a developmental deficiency in their capacity to generate an oxidative burst in response to Gram-positive and negative rods and to TLR-2 and TLR-4 ligands. Until now, monocytes and neutrophils from cord blood of term infants and from peripheral blood of adult volunteers were exposed to E. coli, S. aureus, LPS, Pam3CSK4 and PMA, and the intracellular hydrogen peroxide levels was analyzed by the DHR assay. The oxidative burst of monocytes and neutrophils exposed to Pam or LPS was extremely low and at similar levels comparing neonates and adults. Hydrogen peroxide production by neutrophils and monocytes exposed to E. coli was similar between adult and cord samples. Cord blood monocytes and neutrophils produced higher hydrogen peroxide levels in response to PMA and S. aureus as compared with adult ones. A deficiency in generation of an oxidative burst may not contribute to the increased susceptibility of neonates to infections caused by Gram-positive and Gram-negative pathogens. Financial Support: FAPESP (2009/54246-5).

F.12. Activation of Toll-like Receptors and NOD-like Receptors Provides a Mechanism for Enhanced IgG4 Responses in Autoimmune Pancreatitis

Tomohiro Watanabe, Tsutomu Chiba. Kyoto University Graduate School of Medicine, Kyoto, Japan

IgG4-related disease is a recently recognized disease entity affecting multiple organs, including the pancreas, biliary tracts, and salivary glands. Although IgG4-related disease is characterized by systemic IgG4 antibody responses and by infiltration of IgG4-expressing plasma cells, the innate immune responses leading to adaptive IgG4 antibody responses are poorly understood. In this study, we determined the innate immune responses leading to IgG4 antibody production. Activation of the nucleotide-binding oligomerization domain (NOD) 2 in monocytes from healthy controls induced IgG4 production by B cells in a B cell-activating factor (BAFF)-dependent and T cell-independent manner. In addition, peripheral blood mononuclear cells from patients with IgG4-related disease produced a large amount of IgG4 upon stimulation with NOD-like receptor (NLR) and Toll-like receptor (TLR) ligands; this enhancement of IgG4 production was associated with BAFF induced by NLR and TLR ligands. Monocytes from patients with IgG4-related disease induced IgG4 production by B cells from healthy controls upon stimulation with NLR and TLR ligands. These studies thus establish that abnormal innate immune responses against microbial antigens underlie the immuno-pathogenesis of IgG4-related disease.

F.13. Intestinal Macrophages in Health and Disease

Calum Bain, Aude Aumeunier, Allan Mowat. University of Glasgow, Glasgow, Scotland

Macrophages (mf) are essential for maintaining intestinal homeostasis, but are also one of the principal drivers of the pathology associated with IBD. How mf can play such contrasting roles remains unclear, due in part to the complexity of discriminating mf from other intestinal myeloid cells such as dendritic cells. We have used multi-parameter flow cytometry to characterise colonic lamina propria (CLP) myeloid cells. We show that in the resting colon the majority of mf are F4/80highCD11b+MHCIIhighCX3CR1high and are unresponsive to TLR stimulation. However resident mf produce IL10 and TNFα constitutively, indicating that they are responding continuously to their microenvironment in a balanced manner that avoids overt inflammation. During acute DSS colitis there is an influx of F4/80+CD11b+CD11cnegCX3CR1int TLR-responsive mf, which come to dominate the inflamed LP. These cells derive from Ly6Chi monocytes that are recruited via a CCR2-dependent mechanism and are necessary for the development of pathology. Interestingly, a small number of F4/80+CD11b+CD11cnegCX3CR1int mf can be found in the normal colon, raising the question of how these cells are related to the resident mf. By defining these relationships, we aim to understand if targeting inflammatory mf could be a selective means of treating disease without interfering with physiological processes.

F.14. Anti-**influenza Effects of γ**-PGA in Mx1+/+ Congenic C57BL/6 Mouse

Jong-Soo Lee[1], Ho-Jin Moon[1], Young-Ki Choi[1], Haryoung Poo[3], Moon-Hee Sung[2], Chul-Joong Kim[1]. [1]Chungnam National University, Daejeon, Republic of Korea; [2]BioLeaders Corporation, Daejeon, Republic of Korea; [3]KRIBB, Daejeon, Republic of Korea

The IFN-induced resistance factor Mx1 is a critical component of innate immunity against Influenza A viruses (IAV) in mice. So, it will be effective anti-influenza drug that materials can induce the Mx1 protein such as a type-I IFN. Regular laboratory mice are carrying the defective alleles of the Mx1 gene. So, Mx1+/+ congenic mice which are mimic the innate immune system of human, are developed and they are highly resistant to infection with IAV. In this study, we used a mouse-adapted IAV strain, which is unusually virulent in Mx1+/+ mice and we assessed the anti-influenza effects of high molecular weight of ploy-gamma-glutamic acid (HM-γ-PGA). Inoculation of HM-γ-PGA enhanced the antiviral state of mice and when challenging with mouse-adapted IAV with control group, control group mice exhibited typical clinical symptoms of influenza infection, whereas HM-γ-PGA-treated mice showed no significant clinical symptoms with zero mortality. The present data demonstrated that HM-γ-PGA has the ability to enhance antiviral state in Mx1+/+ mice through the induction of IFN-mediated defense mechanism especially Mx1 and can be used as a possible agent for the influenza [This research was supported by the Technology Development Program for Agriculture and Forestry (110057-03), National Agenda Project grant from KRCFST].

F.15. **LTβR**-independent Differentiation of Intestinal IL-22-producing NKp46+ Innate Lymphoid Cells

Naoko Satoh-Takayama, Sarah Lesjean-Pottier, Shinichiro Sawa, Christian Vosshenrich, Gerard Eberl, James Di Santo. Institut Pasteur, Paris, France

The natural cytotoxicity receptor NKp46 is an activating receptor expressed by several distinct innate lymphoid cell (ILC) subsets, including NK cells, some γδ T cells and intestinal RORγt+IL-22+ cells (NCR22 cells). NCR22 cells may play a role in mucosal barrier function through IL-22-mediated production of anti-bacterial peptides from intestinal epithelial cells. Previous studies identified a predominant proportion of NCR22 cells in gut cryptopatches (CP), lymphoid structures that are strategically positioned to collect and integrate signals from luminal microbes. Still, whether CP or other lymphoid structures condition NCR22 cell differentiation is not known. Programmed and inducible lymphoid tissue development requires cell-surface expressed lymphotoxin (LT)α1β2 heterotrimers (provided by lymphoid tissue inducer (LTi) cells) to signal LTβR+ stromal cells. Here we analyzed NCR22 cells in LTβR-deficient Ncr1GFP/+ mice that lack organized secondary lymphoid tissues. We found that NCR22 cells develop in the absence of LTβR, become functionally competent and localize to the lamina propria under steady-state conditions. Following infection of LTβR-/- mice with the gram- pathogen citrobacter rodentium, IL-22 production from NCR22 cells was not affected. These results indicate that organized lymphoid tissue structures are not critical for the generation of an intact and fully functional intestinal NCR22 cell compartment.

F.16. Sheep, a Model to Study Intestinal NKp46+ Cell Subset Functions in Neonatal Infections

Francoise Drouet[1], Timothy Connelley[2], Preben Boysen[3], Daniela Pende[4], Anne Storset[3], Fabrice Laurent[1]. [1]INRA, Nouzilly, France; [2]School of Veterinary Studies, Edinburgh, United Kingdom; [3]Norwegian School of Veterinary Science, Oslo, Norway; [4]Istituto Nazionale per la Ricerca sul CancroScience, Genova, Italy

Natural killer (NK) cells likely participate to fight pathogens at mucosal surfaces. Sheep have been used as a model to study their role in uterine mucosa, though only “NK-like cells” were described until we first characterized ovine NK cells as CD16+ /CD14- cells (1) overlapping the ovine-specific-NKp46+ population (2). We then isolated lymphocytes from lamb tissues to characterise NKp46+ cells from intestine, gut associated lymphoid tissues (G.A.L.T.) and mesenteric lymph nodes (MLN). Preliminary data indicated that contrary to spleen and MLN in which CD16 was expressed on 100% and 90 % of NKp46+ cells respectively, the NKp46+CD16- subset represented more than 50% of NKp46+ cells in gut mucosa and G.A.L.T., with a lower perforin content. Studying the direct cytotoxicity of NKp46+ cells from blood versus MLN, we observed a lower percentage of cell lysis with the latter, suggesting the presence of non cytotoxic cells beside classical NK cells. Altogether, these data suggest that the “NK-22”

subset recently described in human and mouse (non cytotoxic and possibly involved in tissue repair) has likely an equivalent in this species. Thus, sheep would represent a useful model of adequate size to study the intestinal response to pathogens in neonates. 1. J. Elhmouzi-Younes, P. Boysen, D. Pende, A.K. Storset, Y. Le Vern, F. Laurent, F. Drouet. Ovine CD16+/CD14- blood lymphocytes present all the major characteristics of Natural Killer cells. Vet. Res. 2010 Jan-Feb;41(1):4. DOI : 10.1051/vetres/2009052. 2. T. Connelley, A. K. Storset, A. Pemberton, N. MacHugh, J. Brown, H. Lund, W. I. Morrison. NKp46 defines ovine cells that have characteristics corresponding to NK cells. Vet. Res. 2011 in press.

F.17. Functional Complexity of HIV-specific T Cell Responses Detected in the Female Genital Tract of Chronically HIV-infected Women

Alfred Bere, Lynette Denny, Willem Hanekom, Wendy Burgers, Jo-Ann Passmore. University of Cape Town, Cape Town, South Africa

Background: T cells that produce cytokines to HIV antigens can be detected in the cervical mucosa of chronically HIV-infected women. The aim of this study was to investigate the frequency, specificity and broad functionality of cervical mucosal T cell responses and to compare these with responses detected in blood. Methods: Cervical cytobrush-derived and blood-derived T cells from 16 chronically HIV-infected women were expanded for 14 days with Dynal anti-CD3/CD28 beads and then assessed for responses to HIV Gag peptides using intracellular cytokine staining (CD107a, IFN-γ, TNF-α, and MIP-1β). Results: Following expansion of cervical and blood-derived T cells, the functional profile (CD107a, IFN-γ and TNF-α) of CD8+ T cells correlated significantly between compartments in response to Gag stimulation. Although the majority of Gag-responses at the cervix were monofunctional, HIV Gag-specific polyfunctional CD8+ T cells were detected at the cervix (median of 1.96%) of HIV-infected women. Conclusion: HIV Gag-specific blood and cervical T cells during chronic infection were largely monofunctional with polyfunctional T cells being detected in women with high blood CD4 count and low plasma viral load. HIV-specific Gag T cell responses detected in blood were predictive of similar responses at the cervix during HIV infection.

F.18. The Pathogenicity of Salmonella Enterica Serovar Typhimurium SL1344 is Coupled to Invasiveness and not the Ensuing Immune Response

Rikke Brandt[1], Anne Petersen[1], Susanne Brix[1], Tine Licht[1], Hanne Frøkiær[2]. [1]Technical University of Denmark, Lyngby, Denmark; [2]Copenhagen University, Frederiksberg, Denmark

Immune evasion by Salmonella spp. is promoted by bacterial invasion and redirection of cellular processes within phagocytes. We compared the pathogenicity of two genetically very similar S. Typhimurium strains; S. Typhimurium DT120 (DT120) and S. Typhimurium SL1344 (SL1344), both causing a typhoid-like illness in a mouse model. We evaluated the bacterial concentration in cecum, spleen and liver, as well as neutrophil numbers and viability in spleen and bone marrow upon oral or intravenous administration of the two bacterial strains. Oral administration of SL1344, but not DT120, resulted in massive neutrophil infiltration of the spleen, paralleled with neutrophil depletion in the bone marrow at day five post infection, and most mice died before day 8. A large number of neutrophils, both in the spleen and bone marrow reservoir, were apoptotic or necrotic. Oral administration of SL1344 gave rise to significantly higher bacterial counts in spleen and liver than administration of a ten-fold higher dose of DT120. Intravenous injection of the same number of the two strains resulted in similar CFU and neutrophil counts in spleen and bone marrow, demonstrating that the stronger pathogenicity of SL1344 is caused by higher epithelial invasiveness rather than a stronger ability to cause systemic infection.

F.19. Host Imprints on Bacterial Genomes: Evolution in Individual Patients Coincides with Variations in Innate Immunity

Jenny Grönberg Hernandez[1], Jaroslaw Zdziarski[2], Catharina Svanborg[1], Elzbieta Brzuszkiewicz[7], Björn Wullt[3], Heiko Liesegang[7], Dvora Biran[4], Birgit Voigt[5], Bryndís Ragnarsdóttir[1], Michael Hecker[5], Eliora Ron[4], Rolf Daniel[7], Gerard Gottschalk[7], Jörg Hacker[6], Ulrich Dobrindt[2]. [1]Institute of Laboratory Medicine, Lund, Sweden; [2]Julius-Maximilians-University Würzburg, Würzburg, Germany; [3]Lund University, Lund, Sweden; [4]Tel Aviv University, Tel Aviv, Israel; [5]Ernst-Moritz-Arndt-University Greifswald, Greifswald, Germany; [6]German Academy of Sciences Leopoldina, Halle/Saale, Germany; [7]Institute for Microbiology and Genetics, Göttingen, Germany

The part commensals play in evolution, health and disease remains poorly understood. While properties that distinguish commensal from virulent bacteria have been characterized, the extent to which the host provides selection pressure for bacterial adaptation is less well understood. Here we provide the first, genome-wide example of a single bacterial strain's evolution in different human hosts. By first obtaining the complete genome sequence of the prototype asymptomatic bacteriuria strain E. coli 83972 and then re-sequencing its descendants isolated after therapeutic bladder colonization of different patients, we identified adaptations at the genomic, transcriptomic and proteomic levels. Interestingly, the different hosts appeared to personalize their microflora as re-isolates from each patient showed a distinct but reproducible pattern of genetic alterations. Our results suggest that in addition to stochastic events, the host drives adaptive bacterial evolution. Ongoing loss of gene function suggested that evolution towards commensalism rather than virulence is favored. To examine how the mucosal host-response drives bacterial evolution, we analyzed how polymorphisms in innate immune response genes affect urine proteomes in patients with asymptomatic E. coli 83972 carriage. The secreted cytokines, chemokines and receptor antagonists varied significantly in patients. Based on these findings, we will discuss how the individual host personalizes its microbial communities, and the possible role of mucosal immunity.

F.20. Plant Originated Food Antigens in Modulation of Host Immune Response via their Specific and Interrelated Impact on Gut Commensal Microbiota

Nadiya Boyko, Mariia Mudryk. Uzhhorod National University, Uzhhorod, Ukraine

Food ags are direct or mediate stimuli in regulation of host immune balance via maintaining of the commensal gut microbiota. Our activities are aimed at characterising microbiologically of selected traditional foods of plant origin in order to : 1) detect the contamination with the microorganisms of groups A ("beneficial", both for food processes and for the human health), B1 (environmental contaminants, "epiphytic", derived from air/soil/water) and B2 (human-relevant contaminants: food-borne and opportunistic pathogens); 2) clarify their influence on human commensal bacteria representatives *in vitro* and *in vivo*. The "beneficial" bacteria - mostly lactobacilli strains - were poorly presented in all plant samples and rarely accompanied with bifidobacteria, enterococci and bacteroides. Category B1 included erwinia herbicola (pantoea agglomerans), xantomonas, bacillus, corinebacterium, pseudomonas, and micrococcus genera, while B2 group was presented by different enterobacteria species: E. coli, or E. hermanii, or E. vulneris, klebsiella pneumoniae/oxytoca, enterobacter agglomerans/cloacae, proteus, citrobacter, serratia others. Food ags of plant origin demonstrated high selectivity in their pro- (stimulating) and anti- (inhibiting) microbial properties against chosen species of commensal bacteria and their compositions *in vitro*; the changes of gut microbial associations were specific to each separately tested plant originated food ag defined *in vivo* on mice model (BALB/c vs. SCID).

F.21. IL-6 Induction in Males, Not in Females, is Necessary for Recovery from DSS-induced Colitis by MIP2-mediated Neutrophil Recruitment

Di Meng[1], Nanda Nanthakumar[2]. [1]Massachusetts General Hospital for Children, Charlestown, MA; [2]Harvard Medical School, Boston, MA

Conventionally raised mice completely recover from Dextran Sulfate Sodium (DSS)-induced mucosal injury via neutrophil and macrophage recruitment by inducing mucosal inflammatory protein-2 (MIP2) and IL-6. The IL-6 knockout-mice (IL-6-/-) were used to determine if induction of IL-6 is necessary for MIP2-mediated recovery and neutrophil recruitment. All female IL-6-/- mice were able to recover from DSS-induced colitis, but male IL-6-/- mice failed to recover because of its inability to induce MIP2 and hence recruit neutrophils. Upon mucosa injury, both male and female IL-6-/- mice contained abundant F4/80+ macrophages, whereas GR1+ neutrophil were absent. Expression of epithelial and endothelial JAM1 is necessary for neutrophil transmigration. JAM1 is expressed in female IL-6-/- mice but absent in male IL-6-/- mice. When recombinant MIP2 was administered, 60% of male IL-6-/- mice recovered from DSS-induced colitis with neutrophil infiltration with JAM1 expression, by day 10. The upstream IL-6 induction is necessary for MIP2-mediated neutrophil recruitment allowing the mice to recover from colitis and preventing mortality associated with systemic sepsis. However, the mechanism by which only female but not male mice were able to recover is unclear. Additional experiments are being carried out to determine if differential adhesion molecule expression explains the gender difference in mucosal recovery.

F.22. Estradiol Inhibits Inflammation through Attenuation of MAPK Activation

Amy Jensen[2], Paul Guyre[1], Patricia Pioli[1]. [1]Dartmouth Medical School, Lebanon, NH; [2]National Cancer Institute, Bethesda, MD

The anti-inflammatory properties of the sex steroid hormone 17β estradiol (estradiol) are becoming widely appreciated. In this regard, numerous studies show that estradiol enhances survival in burn-injured and septic animals and is thought to underlie the enhanced survival of women in human sepsis. We recently reported that pretreatment of primary human macrophages with estradiol attenuates pro-inflammatory cytokine production in response to bacterial lipopolysaccharide (LPS), an effect that likely contributes to the beneficial effects of estradiol noted above. However, the molecular mechanisms by which physiological concentrations of estradiol inhibit inflammation in human macrophages remain to be determined. Based on highly significant microarray and real time PCR preliminary studies, we propose a novel mechanism of immune regulation whereby estradiol "prepares" macrophages for subsequent immune stimulation by upregulating DUSP16. DUSP16 is a dual specificity phosphatase that is a key regulator of the c-Jun N-terminal kinase (JNK) mitogen- activated protein kinase (MAPK) signaling pathway. We have also shown that estradiol pretreatment inhibits JNK phosphorylation. We therefore hypothesize that the net result of enhanced DUSP16 expression is diminished phosphorylation of JNK MAPK, leading to attenuated production of pro-inflammatory cytokines.

F.23. NKp46+ Cells in GALTs of Sheep

Line Olsen[1], Preben Boysen[1], Arild Espenes[1], Caroline Piercey Åkesson[1], Timothy Connelley[2], Anne Storset[1]. [1]Norwegian School of Veterinary Science, Oslo, Norway; [2]University of Edinburgh, Edinburgh, United Kingdom

NK cells are important effector cells in the innate immunity, but they also contribute to the adaptive immunity by producing cytokines and by direct interactions with antigen presenting cells. Recently, there has been an increased interest in NK cells in gut-associated lymphoid tissues (GALTs). In the intestine, a subset of NK cells appear to have significance for normal structure and function of lymphatic tissue in the intestine and also for the gut epithelium. In sheep, little is known about this cell population, but the expression of the natural cytotoxicity receptor NKp46 has recently been demonstrated on ovine NK cells. We have used the newly generated monoclonal antibody against ovine NKp46 to examine the NKp46+ cells in the tonsils, jejunal Peyer's patches (PPs), ileal PP, colonic PPs and the rectal lymphoid tissues of lambs with both multicolour immunofluorescent and flow cytometric procedures. The distribution of NKp46+ cells was studied in four different compartments, i.e. lamina propria (LP), lymphoid follicles, domes and interfollicular areas. NKp46+ cells were abundant in T cell areas of the interfollicular areas and the domes of the PPs, and less frequently

observed in LP and almost absent in lymphoid follicles. We could also observe occasional NKp46+ cells in an intra-epithelial location.

F.25. Cytokine and TLR Expression in Mouse Respiratory Tract After Adjuvant Intratracheal Immunization

Nikolay Evdokimov[1], Peter Zanvit[1], Olga Novotna[1], Martina Havlickova[2], Jiri Hrdy[1], Ludmila Prokesova[1]. [1]Charles University in Prague, Prague, Czech Republic; [2]National Institute of Public Health, Prague, Czech Republic

Mice were immunized intratracheally with inactivated influenza virus type A together with bacterial adjuvant, delipidated Bacillus firmus (DBF). The positive effect of DBF on protective anti-influenza immunity was proved earlier (Zanvit et al., IMLET 115:144, 2008). The mechanism of adjuvant effect was followed on basis of cytokine and TLR expression in lungs and NALT using qPCR. The results are expressed by relative quantification (RQ) and compared using principal component analysis (PCA). DBF increased TLR2 and inflammatory cytokine (IL-1β, IL-6, IL-33) expression both in NALT and lungs. In NALT, the early support of Th1 cytokine response was evident (significant increase of IL-12α and IFN-γ and significant decrease of IL-13 expression). In lungs, the early tendency to Th2 cytokine response was quickly replaced by increasing trend of IL-12α and IFN-γ expression. It is possible to conclude that pro-inflammatory and pro-Th1 activity of DBF explains its beneficial effect in the development of anti-infection immunity in mice.

F.26. Differential Regulation of Cytokine Expression through Vitamin D Receptor Variants in RSV-infected Epithelial Cells

Arie Stoppelenburg, Ron Huis in 't Veld, Louis Bont, Marianne Boes. University Medical Center Utrecht, Utrecht, Netherlands

Epithelial cells are central players in the regulation of immune activation during airway inflammation. The vitamin D receptor (VDR) is a known mediator of cytokine transcription that is highly expressed in lung tissue. VDR is required for the induction of experimental asthma and polymorphisms in this gene correlate to asthma and severe RSV bronchiolitis development in humans. The VDR FokI polymorphism is an alternative start codon variant that strongly correlates to severe RSV bronchiolitis in infants, as was shown by a genetic association study. The biological cause for the increased susceptibility of VDR FokI individuals to RSV infection is incompletely understood, although a role for the ligand 1,25-dihydroxy-Vitamin D3 on RSV-infected epithelial cells was previously reported. We hypothesize that VDR polymorphisms are instrumental in the appropriate polarization of immune reactivity triggered by the RSV infection to raise optimal anti-viral immune responsiveness. To this end, we investigate the role of VDR and its FokI variants in RSV infection. Approaches we use include RSV infection of epithelial cells, qPCR, and multiplex immuno array analyses for measurement of RSV replication efficiency and the expression of various cytokines and chemokines. We believe this work will clarify the role of lung epithelial cells in the RSV bronchiolitis in susceptible individuals.

F.27. Development of T Lymphocytes in the Lung

Angel Balam-May, Maria Ramirez-Estudillo, Gloria Lazo-Vazquez, Marco Vega-Lopez. Centro de Investigacion y de Estudios Avanzados del Instituto Politecnico Nacional CINVESTAV IPN, Mexico DF, Mexico

In spite of the importance of respiratory diseases, especially in children and elderly people, scarce studies on the immune development of the lung are available. Since practical and ethical limitations hamper human studies, we have developed a pig experimental model to carry out these studies. Using monoclonal antibodies to pig CD3, CD4 and CD8, we stained lung cells for FACS analysis from four groups of healthy pot bellied vietnamese minipigs (n=5/group): Two days old (NB), 5 week-old (weaned), 3 month-old and 4 years old (adult) animals. These ages nearly match human newborn, early infancy, teen age, and mature age stages. Our results showed low proportion and numbers of CD3+ CD4-CD8- (double negative, DN), CD3+CD4+, CD3+CD8+ and CD3+CD4+CD8+ (double positive, DP) cells at birth. Only DN+ cells increased dramatically at weaning, and all other populations slowly increased by 3 months-old. However, adult animals showed a dramatic reduction in CD4+ and DP cell numbers. These results may help to explain the increased susceptibility to disease found in the young and the elderly. Acknowledgments: DVM. Manuel Flores-Cano, and DVM Daniel Cortes-Casarrubias (UPEAL-CINVESTAV) for technical support. This work was supported by CONACyT (Project 60941) and the Mexico City's Science & Technology Institute (ICyTDF).

F.28. The Adjuvant Effect of Subcellular Fractions of Bacillus Firmus in Experimental Influenza Infection of Mice

Dana Cechova, Michaela Novakova, Olga Novotna, Peter Zanvit, Jaroslav Julak, Ludmila Prokesova. Charles University, Prague, Czech Republic

Bacillus firmus (BF) is a Gram positive non-pathogenic sporulating bacterium with proven immunomodulatory properties - such as macrophage stimulation, anti-infection, anti-tumour and adjuvant activity. In the present work, we have followed up an adjuvant activity of three subcellular fractions of BF- bacterial cell walls, cytoplasmic membranes and ribosomes- in experimental influenza infection in mice. Every one fraction was previously tested both *in vitro* and *in vivo* experiments and each exerted a stimulatory effect (on the blast transformation, immunoglobulin and IFNγ formation in cultures; and on specific - antibody levels in sera, bronchoalveolar and nasal lavages after intratracheal immunization by influenza virus + adjuvant). Here, we immunized BALB/c mice via respiratory tract with two different subtypes (H1N1 and H3N2) of inactivated A type influenza virus. 10 days after the booster dose mice were exposed to A/H1N1 live influenza A infection. Specific antibody levels, lung histology, weight loss and mortality during infection were evaluated. Results showed that all fractions used as adjuvants stimulated the production of specific anti-influenza antibodies and improved mouse surviving during experimental influenza infection with homologous strain, but bacterial cell walls led even to cross-protection against infection by the heterologous strain.

F.29. Cathelicidin and Calprotectin in Human Airways After Endotoxin Exposure

Marit Hansson, Margaretha Smith, Ingemar Qvarfordt, Pernilla Glader, Anders Lindén. Sahlgrenska Academy at Gothenburg University, Gothenburg, Sweden

Rationale: Antimicrobial peptides (AMP's) are endogenous antibiotics under evaluation for therapeutic use in pulmonary disease. The AMPs cathelicidin (LL-37) and calprotectin are increased in the airways of patients with chronic obstructive pulmonary disease and cystic fibrosis, disease entities characterized by colonisaton of gram-negative bacteria. Here, we investigated whether endotoxin from gram-negative bacteria increase LL-37 and calprotectin in healthy human airways. Methods: Healthy volunteers undervent endotoxin exposure in one bronchial segment and phosphate-buffered saline in the contra-lateral segment. Bronchoalveolar lavage (BAL) samples were collected from both segments after 12 or 24 h and intra-individual comparisons between endotoxin and saline exposure were made. The BAL concentrations of LL-37 and calprotectin were measured by ELISA. Results: After endotoxin exposure, the BAL concentrations of LL-37 were clearly increased in 6/6 subjects after 12h ($p<0.05$) and in 5/6 subjects after 24h. For calprotectin, BAL concentrations were modestly increased in 5/6 subjects at 12h and 5/5 at 24h after endotoxin exposure. Conclusions: The AMP LL-37 is present and increased after endotoxin exposure in healthy human airways. The AMP calprotectin is also present and increased, but to a less pronounced degree. This provides support for AMP involvement in pulmonary host defense against gram-negative bacteria in humans.

F.30. Crucial Role of Lung IL-17 Secreting Neutrophils in the Clearance of Pulmonary Anthrax

Kévin Garraud, Aurélie Cleret, Daniel Fiole, Yves Gauthier, Jacques Mathieu, Anne Quesnel-Hellmann, Jean-Nicolas Tournier. IRBA-CRSSA, La Tronche, France

Pulmonary anthrax resulting from inhalation of bacillus anthracis spores is a highly virulent disease that can occasionally kill humans through inhalational route. Pulmonary infections are opposed by lung innate immune system. So far, the role of IL-17 and neutrophils in the lung has been described as two intricate but independent players, IL-17 triggering downstream recruitment of neutrophils. In this study we have shown that IL-17 is rapidly secreted in the alveolar space following intranasal infection with this pathogen. Not Th17 as usual, but neutrophils are identified as the primary IL-17-secreting subset. Using IL-17RA-/- mice, we confirm that IL-17 signaling is instrumental in the self-recruitment of this population. Moreover, we show that IL-17 axis is critical for survival to pulmonary infection as IL-17RA-/- mice become susceptible to intranasal infection by anthrax sterne spores when the wild type mice are resistant. By depleting neutrophils in wild type and IL-17RA-/- mice, we demonstrate the crucial role of IL-17-secreting neutrophils for mouse survival after anthrax infection in the sterne strain model. In accordance, following infection with a fully virulent strain, neutrophils prove to be critical for survival. This work demonstrates the very intricate role of both IL-17 and neutrophils in the survival to pulmonary anthrax.

F.31. Dynamic Analysis of Respiratory Immune System Provides New Insights on Pulmonary Anthrax

Daniel Fiole, Anne Quesnel-Hellmann, Aurélie Cleret, Kévin Garraud, Jacques Mathieu, Jean-Nicolas Tournier. French Army Biomedical Research Institute, La Tronche, France

Lung efficiency as gas exchanger organ is based on the delicate balance of its associated mucosal immune system between inflammation and sterility. Exploration of lung immune system dynamic under infection has not been performed in detail so far, mainly because ventilation mechanic and chest movement impair microscope focus. We addressed this issue by synchronizing ventilation and imaging, allowing the collection of important information about the early stages of pulmonary anthrax athophysiology, a severe disease due to inhalation of Bacillus anthracis spores. Mice are anesthetized and placed under a ventilator linked to the LSM 710 Zeiss™ microscope allowing two-photon imaging. Alexa chemically labelled Sterne's strain spores are instilled in CX3CR1+/gfp mice, which express green fluorescent protein (GFP) mainly in dendritic cells (DCs) and monocytes. Our study shows for the first time the infection dynamic in the lungs after inhalation of B. anthracis spores, demonstrating that subepithelia DCs take up intraluminal spores through trans-epithelial extensions. To our knowledge, the use of this novel *in vivo* lung imaging protocol led to the first visualization of pathogen uptake by immune cells in the lungs, demonstrating the key role of dendritic cells acting as Trojan horses to penetrate the host defense system.

F.32. Innate Lymphoid Cells Mediate Tissue Repair in the Lung

Laurel Monticelli, Greg Sonnenberg, Michael Abt, Theresa Alenghat, E. John Wherry, David Artis. University of Pennsylvania, Philadelphia, PA

Mucosal tissues such as the intestine and lung provide a protective barrier against environmental stimuli, commensal bacteria and invading pathogens. Recent work has identified a diverse group of mucosal-associated innate lymphoid cells (ILCs) located in the intestinal tract and secondary lymphoid tissues that play critical roles in intestinal immunity and inflammation. However, whether ILC-like cells exist in the lung and whether they influence immune responses or tissue homeostasis has not been examined. Here we identify a lung-resident ILC population that lacks expression of mature cell lineage markers and is defined by expression of Thy1.2, IL-2Rα, IL-7Rα, c-kit, and Sca-1. Lung ILCs also expressed high levels of the transcription factors RORγt and Id2, similar to ILC populations found in the intestine. However, ILCs in the lung lacked expression of CD4 or NKp46, thus distinguishing them from lymphoid tissue inducer cells and intestinal NKp46+ ILCs. Strikingly, depletion of ILCs in RAG-deficient mice during respiratory infection resulted in impaired airway remodeling and compromised epithelial barrier integrity. Collectively, these data identify a previously unrecognized population of lung-resident ILCs that can mediate respiratory tissue repair.

F.33. Engagement of Sodium-dependent Glucose Transporter-1 by the Synthetic Glucose Analogue BLF501 for the Treatment of Asthma
Giuseppina Dusio[1], Andrea Balsari[1], Barbara la Ferla[1], Diego Cardani[1], Fabrizio Marcucci[2], Francesco Nicotra[1], Cristiano Rumio[1]. [1]Università Degli Studi di Milano, Milan, Italy; [2]Istituto Superiore di Sanità, Rome, Italy

We have recently shown that SGLT-1, present at the intestinal lumen surface, holds a novel role, by orchestrating a local and systemic anti-inflammatory and cytoprotective response, in response to an inflammatory stimulus, such as LPS. Activation of SGLT-1 in gut epithelial cells involves activation of the PKB/Akt pathway and consequent inhibition of NF-kB translocation; at a systemic level, PKB/Akt activation increase systemic IL-10 levels (J Immunol. 2008,181:3126). We show here that the engagement of SGLT-1 by a new C-glucoside derivative, BLF501, activating SGLT-1 with increased potency and lower dosage (25 µg/Kg) compared to D-glucose, induces anti-inflammatory effects in lung of mice exposed to aerosolized lipopolysaccharide (LPS) or to ovalbumin (OVA), as assessed by analysis of serum, brochoalveolar lavage and lung morphology in both experimental models. We have demonstrated that BLF501 resolves either LPS-induced lung inflammation or allergic asthma. The synthetic molecule appears very potent and allows a very low dose administration. We observed that SGLT-1 activation in epithelial cells provoked an important release of HSP27, and this heat shock protein stimulated IL-10 release by monocyte cells. We have identified SGLT-1 as a new pharmacological target for compounds that may be useful for treatment of inflammatory diseases of the lung.

F.34. Intranasal Administration of gGamma-PGA Induces Anti-influenza Immunity
Eun Ha Kim[1], Sun-Woo Yoon[2], Jung-Ki Kim[1], Moon-Hee Sung[3], Haryoung Poo[1]. [1]Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea; [2]St. Jude Children's Research Hospital, Memphis, TN; [3]Kookmin University, Seoul, Republic of Korea

Influenza A virus remains an important pathogen that cause respiratory diseases in humans and animals. Four antiviral preparations are recommended by World Health Organization (WHO) for the treatment of influenza. However, a few harmful effects and the emergence of resistant viral strains have been reported previously. Thus, there is a need to develop strategies for the prevention and control of current and next pandemic influenza. We previously reported that γ-PGA would be a novel immunomodulator targeting toll-like receptor 4 which is applicable as a potential candidate of therapeutic agent for cancer and other diseases. Poly-gamma glutamate (γ-PGA) is a safe and edible biomaterial that is naturally synthesized by Bacillus subtilis, isolated from “chungkookjang,” a traditional Korean fermented soybean food. Here, we investigated the protective effect of γ-PGA against infection of H1N1 influenza A viruses (A/Puerto Rico/8/1934 and A/California/04/2009) in mouse model. Intranasal administration of γ-PGA (a single dose of 100µg per day) for 5 days post-infection leads to the improved survival after influenza virus challenge. Analysis of immune responses was performed to understand the antiviral mechanism. The intranasal administration of γ-PGA enhanced the production of cytokines such as interferon β and IL-12 and the activity of natural killer cells. Furthermore, we observed the significant increase of the antigen-specific cytotoxic T lymphocytes activity by counting IFN-γ-producing CD8+ T cells in mouse lung tissue. Collectively, these results suggest that intranasal administration of γ-PGA achieved the protection against both seasonal and pandemic H1N1 influenza A viruses by enhancing antiviral immune responses in mice.

F.35. Role of IL-17 in Modulating B Cell Response During Influenza Infection in Mice
Liwei Lu. University of Hong Kong, Hong Kong

As a proimflammatory cytokine, IL-17 is critically involved in mucosal host defense. In this study, we examined the function of IL-17 in modulating immune responses during influenza infection. We found significantly reduced survival rate of IL-17-deficient mice upon H5N1 infection as compared with wild type controls. Upon viral challenge, IL-17 knockout mice showed severe body weight loss with increased morbidity after H5N1 infection. Compared with wild type controls, the levels of TNFalpha and IL-1beta were markedly increased whereas IL-6 production was greatly reduced in lung homogenates of IL-17 deficient mice. Histological examination of lung sections revealed significantly reduced number of CD19+ B cells with a moderate reduction of neutrophils in H5N1-infected IL-17-deficient mice. Although flow cytometric analysis detected no significant differences in both frequency and total number of splenic B and T cell subsets between IL-17-/- and wild type mice prior to viral challenge, reduced chemotactic migration of B cells from IL-17 deficient mice was observed. Furthermore, adoptive transfer of B cells from wild type mice significantly rescued infected with H5N1-infected IL-17-deficient mice. Together, our results suggest a critical role of IL-17 in antiviral immune response against influenza infection via modulating B cell migration and function.

F.36. Association Between Colonization of Neontal Airways with Streptococcus Pneumoniae and Later Development of Asthma
Marie Kragh[1], Peter Ahrens[2], Katrin Kuhn[2], Klaus Bønnelykke[3], Susanne Brix[1], Anne Mølgaard[3], Jørgen Skov Jensen[2], Hans Bisgaard[3]. [1]Technical University of Denmark, Lyngby, Denmark; [2]Statens Serum Institut, Copenhagen, Denmark; [3]University of Copenhagen, Gentofte, Denmark

In a cohort study of 411 children of asthmatic mothers (the Copenhagen Prospective Study on Asthma in Childhood (COPSAC)), we previously reported, that the presence of opportunistic pathogenic bacteria, as identified by culture from hypopharyngeal aspirates of 4-week-old children, was associated with increased risk of childhood asthma. In the present study, we sought to confirm this finding by analyzing 234 available nasopharyngeal swabs obtained at the same time as the aspirates with a quantitative PCR for S. pneumoniae. Of the 234 neonates, 70 (30%) were positive for pneumococci by PCR from nasopharyngeal swabs compared to 22 (9%) by culture from aspirates ($p<0.0001$). Of 70 children positive for S. pneumoniae at 4-weeks of age, 15 developed childhood asthma, as compared to 17 of 164 children negative for S. pneumoniae at 4-weeks of

age (p=0.03). Odds ratio 2.4 (95% CI 1.1-5.0). Thus, in neonates colonized with S. pneumoniae at 4-weeks-of-age, the prevalence of asthma at 5 years was doubled compared with those not colonized. The use of culture-independent methods confirms our previous findings of an association between colonization of the airways with pathogenic bacteria in neonates and later development of asthma.

F.37. IL-15 Regulates Both the Innate and Adaptive Immune Responses to Influenza Infection via Recruitment of Lymphocytes

Kim Klonowski, Katherine Verbist, Charles Cole, Mary Field. University of Georgia, Athens, GA

Influenza viruses specifically target the epithelial cells lining the lung airways, resulting in the infiltration and accumulation of lymphocytes in the respiratory tract. Productive infection results initially in the recruitment of natural killer (NK) cells to the lung airways to limit infection, and subsequently in the recruitment of influenza specific CD8 T cells to mediate complete viral clearance.We have previously demonstrated that intranasally administered IL-15/IL-15R alpha complexes recruit influenza-specific effector CD8 T cells to the lung airways, augmenting a resultant memory cell pool which is sufficient to provide heterosubtypic immunity. Current studies show that influenza-induced expression of IL-15 in the lung airways induces the migration of both NK cells and influenza-specific effector CD8 T cells to the site of viral infection, demonstrating a role for endogenous IL-15 in promoting the trafficking of lymphocytes of the innate and adaptive immune systems into mucosal sites. These studies also explore the potential efficacy of exogenous IL-15/IL-15R alpha complexes as an immunotherapy at different timepoints following influenza infection. Taken together, these data imply that IL-15 may be a prime candidate for immune intervention in both the innate and adaptive phases of controlling influenza infection.

F.38. Cytokine Production and Antigen Recognition by Human Mucosal Homing Conjunctival Effector Memory CD8+ T Cells

Geraint Williams, Annette Pachino, Heather Long, Emma Yates, Saaeha Rauz, S. Curnow. University of Birmingham, Birmingham, United Kingdom

TCRαβ+CD3+CD8+ cells dominate the conjunctiva. To more precisely identify the phenotype of these cells and their potential role in localised mucosal immunity we studied cells isolated using ocular surface impression cytology (OSIC). Following OSIC from healthy individuals of defined HLA type and CMV/EBV status, conjunctival cells were recovered by gentle agitation. Matched peripheral blood samples were also collected. Cells were analysed by flow cytometry for cell-surface markers, cytokine production and CMV/EBV immuno-dominant epitope recognition using MHC class I-peptide tetramers. Analysis of conjunctival CD8+ cells showed a median of 88%[range 62-100] were CD45RA-CCR7- effector memory (EM) cells and 99%[92-100] expressed αEβ7. This compared to 43%[17.5-63] and 1.8%[0.7-2.3] in peripheral blood, respectively. Of the conjunctival EM cells, 47[24-78]% secreted IFN-γ, with <1% secreting IL-10, IL-17 or IL-22. 0.4%[0-7.9] of conjunctival CD8+ cells recognised CMV antigens and 0.6%[0-1.9] EBV antigens, reflecting peripheral blood frequencies; 1%[0.3-6.7]% and 2%[0-4] respectively. Our data demonstrates that the majority of conjunctival epithelial CD8+ T cells are mucosal homing αEβ7+EM T cells, capable of secreting IFN-γ. The presence of mucosal homing CMV- and EBV-specific cells suggests that though there is no preferential recruitment, the conjunctival CD8+ T cell pool might provide protection against a broad range of viruses.

F.39. Comparison of the Quantitative Recovery of Cytokines from Tears Extracted with Four Different Ophthalmic PVC Sponges

Andrea Nussbaumer, Margarethe Merio, Gerhard Zlabinger, Jacqueline Montanaro, Sophie Biffl, Talin Barisani-Asenbauer. Medical University of Vienna, Vienna, Austria

Quantitative measurements of signaling molecules of the immune system are crucial for the assessment of local immune response at the mucosal surface. For our field studies we aim to find a tear collection method less cumbersome than the collection with capillaries and applicable for adults and children. We tested four different PVC-sponges, 1-Merocel points, 2-Pro-ophta lancet, 3-Keraspear, 4-Weck-Cel using Multiplex Cytokine Panels and the LUMINEX xMAP™ platform to quantify the cytokine recovery from the sponges. For the elution of 25 Cytokines different buffers were tested by titrating the salt and detergent concentration. As expected different extraction buffers had a significant impact on the protein recovery from all sponge matrices. In addition, we found that different cytokines showed diverse recovery percentage to the same buffer used with a given sponge. Not the sponge type but the extraction buffer is the key factor meeting two demands. On one hand an appropriate salt concentration for protein stability and on the other hand an adequate concentration of detergent to detach the proteins from the sponge matrix. After fine-tuning of these two components for every single protein, cytokines with similar requirements can than be combined to panels for measurement in the LUMINEX machine.

F.40. Transgenic Soybean Seeds as a Protein Expression System for Orally Administered Therapeutics

Kenneth Bost, Laura Hudson, Kenneth Piller. University of North Carolina-Charlotte, Charlotte, NC

Soybean seeds expressing transgenic proteins represent a novel, sustainable platform technology which overcomes some of the current limitations for producing proteins useful as mucosal therapeutics. Advantages include low cost of production, greenhouse containment, highest protein/biomass ratio, marketable formulations, safety, accurate dosing, low cost of purification, low-tech sustainability, reduced risk of contamination, scalability, minimal, as well as being a green technology. We have successfully expressed a variety of antigens and immunomodulatory proteins, and are evaluating their usefulness as mucosal vaccines and therapeutics. We have found that purification of transgenic proteins from soy formulations is not required prior to oral administration. This is true for subunit vaccines and immunomodulatory proteins, since no detectable immune responses to soy

protein occur in animals whose diet contains soy. Furthermore, the flexibility of long-term storage of transgenic seeds or powder without degradation allows protein expression to occur years in advance of its therapeutic use. Transgenic protein expression levels of approximately 13 grams per liter of soy powder and 22 kilograms per greenhouse acre represent the highest protein/biomass ratio for the industry, and contribute to low production costs. As protein expression technologies expand and evolve, transgenic soybean seeds represent an emerging technology with significant advantages.

F.41. CCR9-expressing CD14+HLA-DRhi Blood Monocytes Promote Intestinal Inflammation in IBD

Ludvig Linton[1], Mats Karlsson[2], Jeanette Grundström[1], Annelie Lindberg[2], Per Karlén[2], Ola Winqvist[1], Michael Eberhardson[2]. [1]Karolinska University Hospital, Stockholm, Sweden; [2]Södersjukhuset, Stockholm, Sweden

Background: Circulating monocytes have been demonstrated to relocate to the intestinal mucosa during intestinal inflammation, but the underlying mechanisms remain poorly understood. Here, we have investigated a subpopulation of blood monocytes expressing high levels of HLA-DR, CCR9 and CCR7 in patients with Inflammatory bowel disease (IBD). Methods: 51 patients with mild-to-severe ulcerative colitis (UC) or Crohn's disease (CD) were included together with 14 controls. The frequency of CD14+HLA-DRhi monocytes was monitored weekly in peripheral blood using flow cytometry. The surface phenotype and cytokine profile of these monocytes were established using flow cytometry and real-time PCR. Results: The frequency of CD14+HLA-DRhi monocytes was significantly higher in IBD patients with moderate-to-severe disease compared to healthy controls. Furthermore, these monocytes correlated to disease activity in patients with UC and CD. CD14+HLA-DRhi monocytes are defined by their high production of pro-inflammatory cytokines and their surface expression of CCR7 and CCR9. Conclusions: CD14+HLA-DRhi blood monocytes were increased in patients with active Inflammatory bowel disease. These monocytes exhibit a pro-inflammatory gut-homing phenotype with regards to their production of inflammatory mediators and expression of CCR9. Our results suggest that these monocytes are important in mediating intestinal inflammation, and provide potential therapeutic targets in IBD.

F.42. Oral Administration of Probiotic L. Plantarum Expressing Burkholderia Pseudomallei FliC Induces an Antigen-specific Humoral and Cellular Immune Response

Beatriz Del Rio, Luciana Meirelles Richer, Maria Gomes-Solecki. University of Tennessee Health Science Center, Memphis, TN

We have previously reported a platform technology to develop oral vaccines against infectious diseases by providing examples for lyme disease and plague. We used this technology to develop an oral vaccine applicable against two pathogens of biodefense interest, burkholderia mallei and B. pseudomallei. B. mallei has been used as a bioweapon in previous world wars. We generated a recombinant probiotic (lactobacillus plantarum) expressing the flagellar filament structural protein FliC of burkholderia pseudomallei 1026b, fused on the N-terminus to the leader peptide of borrelia burgdoferi OspA (LpssF). We found that oral administration of LpssF induced a significant FliC-specific IgG humoral response (systemic immune response) and a high FliC-specific IgA response (mucosal immune response) in the gut and in distal mucosa sites such as the lung. We also found that *in vitro* stimulation of murine bone marrow derived dendritic cells with LpssF induced the production of pro-inflammatory cytokines such as TNFα and IL-12 (but not IFNγ) that suggests Th1 mediated cellular immune response. LpssF induced IL-10 as well suggesting Th2 involvement. We have designed a promising mucosal vaccine candidate for the prevention of B. mallei and B. pseudomallei infection.

F.43. Activation of PI3 Kinase but Not NFAT or JAK/STAT Signaling by C. Albicans in Oral Epithelial Cells

David Moyes, Celia Murciano, Chengguo Shen, Julian Naglik. King's College, London, London, United Kingdom

Mucosal epithelium is important in host defense and surveillance, being the cell layer that initially encounters most microorganisms. Candida albicans is the most common fungal pathogen of humans and is commonly found at mucosal surfaces. Although immune cell responses to this pathogen are well documented, epithelial cell (EC) responses are poorly described. Gene expression profiling of organotypic oral epithelium after early (6 h) and late (24 h) infection by C. albicans demonstrated enrichment in several ontology groups associated with infection, immunity and signal transduction. Functional analysis of signal pathway activation showed that MAPK, NF-κB and PI3 kinase pathways were activated but not STAT1, STAT3, IRF3 or NFAT pathways. Inhibition of PI3 kinase signaling resulted in increased IL-1α and decreased GM-CSF secretion but had no effect on either MAPK (MKP1 and c-Fos) or NF-κB (IκBα) pathway activation. This data indicates that as well as MAPK and NF-κB signaling, C. albicans infection of ECs leads to AKT signaling activation. However, this pathway plays little role in regulating either cytokine responses or MAPK/NF-κB signaling, suggesting only a minor role in EC responses to infection.

F.44. Anti-tumor Effects of Human Papillomavirus Type 16 E6- and E7-expressing Lactobaccilus Casei Orally Administered in Mice

Sang-Mu Shim[1], Taeyoung Lee[1], Il-Han Lee[2], Moon-Hee Sung[3], Haryoung Poo[1]. [1]Korea Research Institute of Bioscience and Biotechnology, Deajeon, Republic of Korea; [2]BioLeaders Corporation, Daejeon, Republic of Korea; [3]Kookmin University, Seoul, Republic of Korea

HPV E6 and E7 are two major viral oncogenic proteins consistently expressed in HPV infections and HPV-associated malignancies. We express HPV16 E6 or E7 antigen on the surface of Lactobacillus by a novel display system using gamma-PGA synthetase, a surface anchoring protein originated from Bacillus subtilis, chungkookjang. Previously, we reported that oral administration of either E6 or E7 expressing L. casei confers marked antitumor effects against TC-1 (E6 and E7 expressing tumor) in C57BL/6 (published in International Journal of Cancer, 2006 and Cancer of

Immunology Immunotherapy, 2010). We examined whether combinations of E6 and E7-expressing L. casei could induced synergistic antitumor effects against TC-1. Mice were orally administered with the L. casei-PgsA, L. casei-PgsA-E6, L. casei-PgsA-E7 or L. casei-PgsA-E6 and L. casei-PgsA-E7. E6 and E7 specific IgG production was observed in E6 and E7- expressing L. casei immunized mice and significant increase of antibody production was observed after boosting. In order to check cell-mediated immunity, the lymphoproliferation and INF-γ ELISPOT assays were performed. L. casei expressing E6 and E7 immunized group showed higher lymphoproliferation and INF-γ release than L. casei immunized control group. The antitumor effect of orally administered E6 and E7 displayed on Lactobacillus against TC-1 was also checked in C57BL/6. In E6 and E7-expressing L. casei immunized mice group, growth of tumor and tumor size was similar to E6- or E7- L. casei immunized mice group. However, the L. casei expressed E6 and E7 immunized mice lived longer than E6 or E7 alone group. Our data suggest that it may be desirable to combine targeting E6 and E7 to develop better therapeutic antitumor effects against TC-1.

F.45. Aminopeptidase N is a Target for a Very Rapid IgA Response upon a Primary Oral Immunization

Eric Cox, Philippe Bellot, Vesna Melkebeek. University Gent, Merelbele, Belgium

Enteroxigenic Escherichia coli (ETEC) are a major cause of diarrhoea in man and animals. In piglets, ETEC, which produce F4 fimbriae, are responsible for severe diarrhoea and presence or absence of receptors for F4 (F4R) determines sensitivity for infection. The F4R is needed for the protective immune response seen after oral administration of the fimbriae. We identified porcine aminopeptidase N (pAPN) as an F4R involved in clathrin-mediated endocytosis of F4 by comperative proteomic analysis of brush border proteins of receptor positive and receptor negative animals and using pAPN-transfected cells. Binding to pAPN depended on sialic acid containing carbohydrate structures. Endocytosis could also be induced *in vitro* as well as *in vivo* using APN-specific rabbit antibodies. Oral immunisation of pigs with the antibodies combined with choleratoxin rapidly resulted in a rabbit antibody-specific IgA response already reaching its plateau 7 days (mean titer of 240) after the immunisation, whereas pigs immunised with irrelevant rabbit antibodies showed a peak titer of 80 one week after a secondary immunisation. In conclusion, APN was identified as interesting target for oral immunisations.

F.46. Matrix Metalloproteinase 8 (MMP-8) Gene Polymorphisms and Periodontitis Risk

Lydie Izakovicova Holla, Barbara Hrdlickova, Jan Vokurka, Antonin Fassmann. Masaryk University and St. Anne University Hospital Brno, Brno, Czech Republic

Background: Previous studies have suggested that polymorphisms in the matrix metalloproteinase (MMPs) genes are associated with the risk of periodontitis. However, to date, no study has investigated MMP-8 gene variants in relation to chronic periodontitis (CP). The aim of this study was to analyze polymorphisms in the MMP-8 gene and their associations with microbial composition and clinical manifestation of CP. Methods: A total of 619 Czech subjects were included in the present study. Two polymorphisms (-799C/T (rs11225395) and +17C/G (rs2155052)) in the MMP-8 gene were studied in 341 patients with CP and 278 controls. Both polymorphisms were detected using the PCR-RFLP methods. Bacterial colonization was investigated by the VariOr®Dento test in selected subjects. Results: Our results showed no differences in the allele and genotype frequencies of the MMP-8 polymorphisms between patients with CP and controls ($p \leq 0.05$). Nevertheless, the haplotype T (-799)/C(+17) was significantly more frequent in patients with CP than in controls (43.7% vs. 37.6%, $p=0.038$, OR=1.273 (95%CI:1.013-1.601)). No significant relationships between periodontal pathogens, MMP-8 polymorphisms and CP were found ($p \geq 0.05$). Conclusions: Although none of the investigated MMP-8 SNPs was individually associated with periodontitis, specific haplotype showed association with susceptibility to CP. Supported by projects: 1M0528, IGA NT11405-6 and NPVII 2B06060.

F.47. Sublingual Immunization Induces Oral and Vaginal Protective Immunity Against Candida Albicans

Stephen Challacombe, Durdana Rahman, Mukesh Mistry, David Moyes, Julian Naglik. King's College London, London, United Kingdom

A murine model of oral and vaginal colonisation of Candida albicans (Rahman et al 2007) allows the effects of immunisation to be determined. Sublingual immunisation with secreted aspartyl proteinase (SAP2) resulted in inhibition of candida colonisation both orally and vaginally ($p<001$). The aim of this study was to determine mucosal cytokines and antibodies following sublingual (S/L) immunisation with SAP2 compared with intra-nasal. Mice were immunised at weeks 0,1 and 2 and then challenged with Candida albicans at week 3. Samples were taken from oral and vaginal sites at weekly intervals. Cytokines were assayed using the Luminex system, and antibodies by ELISA. Both serum and mucosal (salivary and vaginal) IgA antibodies are induced by S/L immunisation. Serum IgG antibodies were also induced, both maximal at week 7. In vaginal washes, IL-17, TNF-a were detectable soon after immunisation, but at lower levels than found with intra-nasal immunisation. Raised levels of IL-2 and IL-12 were also apparent. In saliva IL-2 was raised in comparison with controls, but not TNF-a or IL-17. The results suggest that decreased levels of candida after S/L immunisation are associated with both mucosal antibodies and with changes in the cytokine profile suggesting Th1 and Th17 involvement.

F.48. Oral Immunization Against Myostatin Enhances Muscle Mass and Body Weight

Jong-Soo Lee[1], Long-Chen Xu[1], Moon-Hee Sung[2], Chul-Joong Kim[1]. [1]Chungnam National University, Daejeon, Republic of Korea; [2]BioLeaders Corporation, Daejeon, Republic of Korea

Myostatin, a member of the TGF superfamily, is a potent negative regulator of skeletal muscle growth. Mice and cattle with mutation in myostatin

gene show a marked increase in muscle mass and body weight. So, inhibition of the myostatin is predicted to increase muscle mass and improve muscle related disorders. The objective of this study was to examine the effect of oral immunization against myostatin on the growth and skeletal muscle mass in mice and chicken. For this study, we create L. casei expressing mature domain of chicken myostatin (Cmyo) on its cell surface. Oral inoculation of mice with L. casei expressing Cmyo led to antibody responses for CMyo and the body weight was increased. We also investigated the effect of L. casei expressing Cmyo inoculated through the feed in chicken. In results, serum IgG antibodies against Cmyo were induced and also the body weight of chick was significantly heavier than that of the control group at 5 weeks post feed. These results suggest that immunization against myostatin is a potential means to improve muscle growth and body weight of chick by myostatin blockade. This work was supported by the 2010 research fund of Chungnam National University in Republic of Korea.

F.49. Evaluation of Anti-LPS O6 Antibody Levels in Colostrum from Healthy Puerperae of Full-term and Pre-term Newborns

Gabriela Comenale, Silvia Massironi, Magda Carneiro-Sampaio, Patricia Palmeira, Gerlandia Pontes. Universidade de São Paulo, São Paulo, Brazil

A high prevalence of systemic infections caused by enterobacteria such as Escherichia coli is observed during the neonatal period. Lipopolysaccharide (LPS) is one of the major factors responsible for septic shock caused by these Gram-negative bacteria. In the present study, we analyzed anti-LPS O6 antibody isotypes in maternal colostrum of healthy mothers, who gave uneventful birth to full-term (n = 15) and preterm (n = 10) neonate infants, by enzyme-linked immunosorbent assay. The main isotype found in colostrum samples from mothers of full-term infants was secretory IgA (range between 28.02 and 4,100 mg/l), followed by IgM (3.09 to 274.18 mg/l) and IgG (0.004 to 2.01 mg/l). Similar findings were found in colostrum from preterm mothers (68.67 to 6,700 mg/l, 2.81 to 786.25 mg/l and 0.007 to 27.13 mg/l, respectively). The anti-LPS O6 antibody levels were significantly higher in colostrum from mothers of preterm neonates when compared with colostrum from mothers of full-term neonates (Mann-Whitney test, $p<0.0001$). The anti-LPS O6 secretory IgA antibody levels in colostrum of mothers of preterm neonates were significantly higher than in the mothers of full-term neonates, demonstrating immunological adaptation in preterm neonate breast-feeding. Financial Support: FAPESP.

F.50. Immune Mechanisms in the Pathogenesis of Periodintitis

Jirina Bartova[1], Kazi Svobodova[1], Stepan Podzimek[1], Lydie Izakovičová-Hollá[2]. [1]Institute of Dental Research, Prague, Czech Republic; [2]Masaryk University, Brno, Czech Republic

Background: The aim of our presented study was to assess the risk factors of periodontitis by studying single nucleotide polymorphisms (SNPs) in genes for cytokines and SNP relationship to imunity regulation of periodontitis patients. Method: Cytokines production were studied after *in vitro* stimulation isolated peripheral blood mononuclear cells (PBMC) by mitogens, dental plaque bacterium (Actinobacillus actinomycetemcomitans, Escherichia coli, Tannerrella forsythensis and HSP 60 and probiotics. Production of cytokines was detected - multiplex analysis by Luminex Results:. We found that mononuclear cells isolated from peripheral blood of patients with proved SNP IL-1β in position 3953 produced significantly decreased amount of IL-1β, IL-4, IFN- γ, IL-6 and IL-10 (after stimulation by A.a., E.coli, T.f., and HSP) and significantly increased amount of IL-5 (after stimulation by A.a.) and IL-8 (after stimulation by P.g.). All proved SNP IL-4 (IL-4 C/T in position -589, C/T in position -33, IL-4 intron 3 in position -254 or -184) indicate higher production of IL-4 after the stimulation by all bacteria and HSP while significantly higher production of INF-γ was confirmed after the stimulation with E.coli. Conclusion: The single nucleotide polymorphisms for 1 cytokine influence the function of mononuclear cells to production another cytokines. Grant NR9775-4.

F.51. Genetic Variations in NOD2/CARD15 in Patients with Orofacial Granulomatosis and Pediatric Liver Recipients with Orofacial Granulomatosis-like Conditions

Gita Gale[1], Robert Saalman[2], Bengt Hasseus[1], Mats Jontell[1]. [1]Institute of Odontology, Göteborg, Sweden; [2]Sahlgrenska Academy, Göteborg, Sweden

Objectives: The goal of our study was to examine if mutations are observed in patients with orofacial granulomatosis (OFG) and in pediatric liver recipients (LTx.). Methods: DNA was isolated from buccal swabs with isohelix DDK in all patients. Allele and genotype variations in exon 4, 8 and 11 were screened after DNA-sequencing. Results: Genetic variations of CARD15 were found in 7/15 patients with OFG and 3/8 patients with LTx. Although the patient material is small it indicates that OFG and OML-patients may have the same extent of genetic variations in CARD15 as seen in CD patients. Conclusions: NOD2/CARD15 may be associated with OFG and OML in LTx, No association between variations in NOD2/CARD15 and the severity of symptoms was found. If the observed variations may give rise to susceptibility to OFG and OML is not known, although they may result in a disturbance in the normal immunological unresponsiveness of the mucosal immune system to components of the commensal intestinal microbiota. Relevance: If there is a similar genetic variation in NOD2/CARD15 in patients with CD, OFG and OML, it could lead to an identification of an etiological denominator of these diseases.

F.52. Immunologic Mechanisms in Reccurent Apthae

Jirina Bartova, Eva Kurova, Kazi Svobodova, Stepan Podzimek. Institute of Dental Research First Faculty of Medicine UK and General University Hospital, Prague, Czech Republic

In the pilot study we examined selected etiological factors in a group of patients with recurrent apthous stomatitis and healthy person. Methods:

Serum concentrations of folate, vitamin B12, IgE, plasma homocysteine and antibodies against gliadin and the production of cytokines after stimulation of peripheral blood mononuclear cells (PBMC) by mitogens, Escherichia coli, HSP 60 and gliadin was examined. Results: In controls, serum concentrations of folate and vitamin B12, IgE and plasma homocysteine were within the range of physiological values, whereas 42% of RAS patients showed increased plasma homocysteine and 54% of RAS patients had increased concentration of IgE and upper level of the physiological border IgG and IgA antibodies reacting with gliadin. PBMC of patients produced significantly increased production of IFN gamma after stimulation by gliadin and. significantly decreased production of IL-5, IL-10 and IL-17 after stimulation with LPS. The relationships between cytokines produced after PBMC stimulation found in the patients was differed from the controls. Conclusion: The most frequent difference was in production of IL-17 to other cytokines in patients. The elevated levels of serum antibodies reacting with gliadin and increased production of IFNgamma after stimulation of PBMC by gliadin suggest that oral mucous barrier may be disturbed. Grant NR9775-4.

F.53. Oral L. Monocytogenes Infection Preferentially Imprints Memory Precursor CD8 T Cells for Gut Retention

Brian Sheridan, Quynh-Mai Pham, Young-Tae Lee, Linda Cauley, Leo Lefrancois. University of Connecticut Health Center, Farmington, CT

The intestinal mucosa contains unique sites designed for antigen presentation and promotion of primary and memory CD8 T cell responses. Thus, generation of adaptive immunity within distinct tissues likely results in T cell responses with specific cellular outcomes. Here, we utilized an oral Listeria monocytogenes (LM) infection model in which internalin A is modified to promote invasion of murine intestinal epithelial cells. Oral infection induced a robust CD8 T cell response. In peripheral nonmucosal tissues, the majority of LM-specific CD8 T cells are short-lived effector cells (CD127- KLRG1+). Surprisingly, the majority of effector CD8 T cells in the intestinal mucosa are memory precursor effector cells (MPEC; CD127+ KLRG1-) early following oral infection. CD103 (integrin αE), which is thought to be vital for retention of intestinal epithelial lymphocytes within the epithelium, was almost exclusively expressed by MPECs. The generation of distinct CD8 T cell subsets uniquely tailored to respond to intestinal pathogens may be a hallmark of oral challenge. Indeed, CD8 T cells that infiltrate the intestinal mucosa following intranasal influenza virus infection are predominately early effector cells (CD127- KLRG1-), but failed to be maintained long-term, demonstrating the importance of intestinal priming for generating robust mucosal memory CD8 T cells.

F.54. IL-10 Producing CD11b+Gr1+ Cells Accumulate in Peyer's Patch During Oral Tolerance Induction

Aya Shiokawa, Satoshi Hachimura. University of Tokyo, Tokyo, Japan

Oral tolerance is specific suppression of immune responses to an antigen by means of prior administration of oral route, and prevents hypersensitivity reactions to food proteins. It has been revealed that CD11b plays an important role in oral tolerance; however, the precise role remains unclear. Therefore, we aimed to elucidate the characteristics of CD11b+ cells in the induction of oral tolerance, focusing on interaction with antigen specific T cells. We have revealed that IL-10 producing CD11b+ DC increased through interaction with antigen specific T cells in Peyer's patch (PP), and CD11b+ DC could induce IL-10 production in T cells *in vivo*. Besides CD11b+ DC, we found that CD11b+ CD11c- cells also increased in PP during oral tolerance induction. *in vivo* BrdU labeling suggested the expansion of CD11b+ cells was not caused by their proliferation. On the other hand, adoptive transfer examination suggested that migration or differentiation of CD11b+ cells was promoted by oral antigen administration. CD11b+CD11c- cells could be divided into mainly two subsets, CD11b+Gr1hi cells and CD11b+Gr1low cells. Similarly to CD11b+ DC, these CD11b+CD11c- subsets prominently expressed IL-10 mRNA compared with CD11b- DC.These results suggest that CD11b+Gr1+ cells could act as an inducer of oral tolerance through IL-10.

F.55. Cooperation of Lymph Nodes and Intestinal Lamina Propria in Foxp3+ Regulatory T Cell Mediated Intestinal Tolerance

Usriansyah Hadis, Oliver Pabst. Hannover Medical School, Hannover, Germany

Oral tolerance induction requires the antigen transport by gut-derived dendritic cells and the generation of FoxP3+ Treg (Treg) in the gut draining mesenteric lymph nodes (MLN). Here we use several mouse models to analyze the cooperation of Treg generation and gut homing in shaping oral tolerance. Oral tolerance was assessed in primed mice by classical delayed type hypersensitivity reactions and a novel approach scoring the severity of diarrhea induced by oral antigen challenge. Depletion of Treg in transgenic mice carrying the diphtheria toxin receptor under control of the FoxP3-promotor was able to break established oral tolerance. Interestingly, in the lamina propria but not in MLN the frequency of antigen-specific Treg increased over time, indicating that the lamina propria itself might be involved in Treg expansion and/or homeostasis. Consistently, oral tolerance was entirely abolished in MadCAM-1 and β7-integrin-deficient mice that fail to direct Treg cells into the gut, despite of normal numbers of Treg generated in the MLN. Proliferation of Treg in the intestine and production of interleukin-10 by gut resident macrophages was blunted in CX3CR1 deficient mice. In addition, oral tolerance is impaired in such mice. This indicates that gut macrophages need Cx3cr1 expression to drive further expansion of Treg locally by IL-10 production. Thus we propose oral tolerance as multistep process that commences in the MLN with generation of Treg. Still oral tolerance requires homing and subsequently expansion of Treg by gut macrophages.

F.56. Tolerogenic Dendritic Cells can Modulate Experimental Arthritis in Mice

Rodolfo Thomé, Marcela Mineiro, Luis Fernandes, Patricia Simioni, Paulo Joazeiro, Wirla Tamashiro. State University of Campinas, Campinas, Brazil

We have recently shown that the consumption of ovalbumin (OVA) mitigates the type II collagen/ovalbumin-induced arthritis (CIA) in BALB/c mice (XXXV Congress of SBI, 2010). Here, we evaluated whether adoptive transfer of tolerogenic DCs (tDCs) could interfere with the course of inflammatory immune response in arthritic mice. BALB/c female mice were fed with OVA (4mg/mL) for seven days, for tolerance induction. Dendritic cells were isolated from spleen cells of tolerant mice and adoptively transferred to naïve BALB/c male mice before subcutaneous immunization with chicken CII+OVA emulsified in CFA, for CIA induction. Clinical and immunological parameters of disease were assessed. We observed that transfer of tDCs prevented the development of clinical signs of arthritis, raised specific antibody production, but significantly reduced specific T cell proliferation. While the frequency of CD25+Foxp3+ was higher, the proportion of IFN-γ/IL-17 was lower in cultures of spleen cells of mice adoptively transferred with naïve(n) DCs and tDCs; culture supernatants of these cells showed the lowest levels of IFN-γ, IL-6 and TNF-α and the highest levels of IL-4 and IL-10. Together, these results indicate that oral tolerance to non-directly related protein modifies the course of experimental arthritis, and the tolerogenic DCs are involved in this alteration.

F.57. The Vagus Nerve as Modulator of Intestinal Immune Homeostasis

Gianluca Matteoli, Martina Di Giovangiulio, Sri Ramulu Elluru, Pedro Gomez Pinilla, Guy Boeckxstaens. Catholic University Leuven, Leuven, Belgium

The central nervous system interacts dynamically with the immune system to modulate inflammation through humoral and neural pathways. Recently, the parasympathetic nervous system, via the vagus nerve (VN), has been proposed to play a crucial role in the regulation of the immune response through the cholinergic anti-inflammatory pathway (CAIP). Stimulation of the VN induces the release of acetylcholine that dampens the activation of immune cells by interacting with the alpha-7 nicotinic acetylcholine receptors. Interestingly, we found that VN stimulation potently reduces intestinal inflammation triggered by abdominal surgery suggesting vagally-mediated modulation of the intestinal immune system. In line with this preliminary findings, selective vagal denervation of the gut results in iteration of the intestinal immune homeostasis. In vagotomized mice we found a significant reduction of CD4+CD25+FoxP3+ Tregs and simultaneous expansion of CD4+IL17a+ T helper cells (Th17) and CD4+IFNγ+ T helper cells (Th1) in the lamina propria. Intriguingly, vagotomized mice also failed to develop systemic oral tolerance to ovalbumin when this antigen was orally gavaged. Our results provide new insights in the cross-talk between the nervous and immune system suggesting that the VN plays an important role in the regulation of the intestinal immune homeostasis.

F.58. Study of Mice Strains Selected for Susceptibility and Resistance to Oral Tolerance Infected with Leishmania Amazonensis

Daniel Tavares[1], Aurelizia Xavier[3], Maria Bellio[4], Maria de Fártima Sarro-Silva[1], Arnaldo Andrade[2], Antonio Carlos da Silva[1]. [1]Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil; [2]Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil; [3]Universidade Federal Fluminense, Niteroi, Brazil; [4]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Two mice strains with extreme phenotypes of Oral Tolerance susceptibility (TS) or resistance (TR) were used in the infection study with Leishmania amazonensis. The profile non-tolerogenic of the TR strain develops large inflammatory lesion accompanied by a small parasite load, unlike the TS strain, that the regulatory activity, prevent the inflammatory lesion, also inhibits the elimination of microorganisms. TR strain produces low levels of IFN-g and IL-10 compared to the TS strain. We suggest that the levels of IFN-g were not been associated with the reduction of parasite load, due to the ambiguous role of IFN-g, that is necessary to kill promastigotes, but can stimulates the replication of amastigotes. TS strain produce a regulatory environment, since IL-10, IFN-g and IL-27 are required for induction of oral tolerance, inducing peripheral tolerance, by suppression of Th17 inflammatory response. In contrast, TR produces an inflammatory environment with production of IL-12 and possibly IL-17. Moreover, when infected, both strains produce high levels of TGF-b, that together with IL-10 stimulate the Treg cells in TS strain, as well as the inflammatory Th17 cells in TR strain, in the presence of IL-6. The study of oral tolerance mechanisms could be a useful approach to treatments of parasite infections.

F.59. Induction of Oral Tolerance in Mice by Whole Eggs and Partially Hydrolyzed Eggs: Role of Lymphocytes in Small Intestine and Mesenteric Lymph Nodes

Feriel Hacini-Rachinel, Elise Heuvelin, Audrey Demont, Annick Mercenier, Guenolee Prioult. Nestlé Research Center, Lausanne, Switzerland

Background and aims: The establishment of oral tolerance by intact dietary proteins prevents digestive disorders such as food allergies. Peptides obtained by hydrolysis of bovine milk proteins were reported to induce oral tolerance in rodents. Here we investigated whether hydrolyzed egg proteins can induce oral tolerance *in vivo* and by which cellular mechanisms. Methods: Mice were fed ovalbumin (OVA), whole eggs or partially-hydrolyzed eggs (HA-eggs) to induce oral tolerance. Tolerance was assessed by ear swelling upon OVA challenge and seric antigen specific antibodies. Lymphocytes were isolated from mesenteric lymph nodes (MLN) and the lamina propria of the small intestine to check for apoptosis, activation and regulatory markers. Results: OVA, whole eggs and HA-eggs were found to induce oral tolerance to OVA in mice. Furthermore, frequency of apoptotic CD4+ T cell and CD4+ T cells with regulatory phenotype were increased in MLN at the end of the 5 day-feeding with whole eggs and OVA. These cells appeared to be activated at later time points in the small intestine. Conclusions: These data illustrate that oral tolerance

to OVA is induced by a 5-day treatment with whole eggs and partially hydrolyzed eggs, likely through T cell involvement.

F.60. Oral Tolerance Can be Induced in IL-10 Deficient Mice with Established Enterocolitis

Ana Cristina Gomes-Santos[1], Thaís Moreira[1], Archimedes Castro Junior[1], Bernardo Horta[1], Luisa Lemos[1], Denise Cara[1], Donna-Marie McCafferty[2], Ana Faria[1]. [1]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; [2]University of Calgary, Calgary, AB, Canada

Introduction: Orally administered proteins induce systemic hyporesponsiveness to the fed protein. The mechanism underlying such 'oral tolerance' depends on the amount of antigen fed with higher doses inducing deletion and anergy, and lower doses inducing regulatory cells. IL-10 is an immunoregulatory cytokine that can modulate immune processes. The importance of this cytokine in shaping mucosal immune responses has been demonstrated by the spontaneous onset of inflammation in the IL-10-deficient (IL-10-/-) mouse. Objetives: To investigate whether IL-10-deficient mice with established enterocolites could be rendered tolerant to ovalbumin (OVA) by either continuous feeding or gavage. Results: Continuous feeding, but not gavage, of OVA was able to induce oral tolerance in 129 Sv/Ev IL-10-/- mice. Moreover, the intraepithelial lymphocytes subset involved in oral tolerance and intestinal homeostasis TCRγδ+ were lower in IL-10-/-. These mice had reduced frequency of CD4+CD25+Foxp3+ T cells and increased activated lymphocytes in the colon since the post-weaning period. Coliltis progression culminates with the reduction of frequency of CD4+LAP+ regulatory T cells in the small and large intestines. The pro-inflammatory cytokine IL-17A was higher in the duodenum but the TGF-β enhanced in the proximal jejunum of IL-10-/- with enterocolitis. Conclusions: Despite the alterations in several regulatory elements of gut mucosa, IL-10-deficient mice with enterocolitis were still able to induced oral tolerance but only by an optimal regimen of feeding.

F.61. Can Diet Selection be Influenced by Genes of the Immune System?

Isabelle Guimarães[1], Mauricio Vericimo[1], Alberto Nóbrega[2], Gerlinde Teixeira[1]. [1]Universidade Federal Fluminense, Niterói, Brazil; [2]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Diet selection is a complex problem that animals in wildlife have to deal with daily. In their natural ecosystem they are beyond a great variety of foods some of which they are able and prepared to eat, yet, not all is eaten. Most animal choose, a few foodstuffs, from what is available to compose their diets. Taste and smell are important factors involved in diet selection. These are sensorial systems that integrate the animal to its environment and ensure that only good food will be eaten, and that inadequate foods will be rejected. Wild mice in urban areas prefer cereals such as oats, rice, corn etc. Their second choice is to foods containing high levels of proteins and fat such as meats, and as a last choice they select foods containing high levels of sugar. To determine whether there is any association between diet selection and MHC we used animals of different H2 haplotypes and their F1 hybrids. Our results show that animals of different H2 haplotypes behave in different manners for example Balb/c Dba-2 and CBA mice prefer Cashew nuts to peanuts, while C57Bl/6 mice prefer peanuts to cashew nuts and all refute mouse chow. Food preference and immunogenetic markers.

Strain	DBA-2	Balb/c	CBA	C3H	C57Bl/6
Preference				nd	
MHC haplotype	d	d	k	k	b
H-2K	d	d	k	k	b
I-A	d	d	k	k	b
I-E	d	d	k	k	-
H2-D	d	d	k	k	b
H-2L	d	d	k	k	b
Igh-C haplotype	c	a	j	j	b
Igh-6 (IgM)	a	a	a	a	b
Igh-5 (IgD)	a	a	a	a	b
Igh-4 (IgG1)	a	a	a	a	b
Igh-3 (IgG2b)	a	a	a	a	b
Igh-1 (IgG2a)	c	a	j	j	b
Igh-7 (IgE)		a	a	-	b
Igh-2 (IgA)	c	a	a	-	b

Peanuts Cashew nuts Mouse chow

F.62. Treatment with B Lymphocytes Pulsed with Antigen Coupled to Cholera Toxin B Subunit Expands Antigen-specific Foxp3 Regulatory T Cells and Protects Against Experimental Autoimmune Encephalomyelitis

Jia-Bin Sun[1], Cecil Czerkinsky[2], Jan Holmgren[1]. [1]University of Gothenburg, Gothenburg, Sweden; [2]International Vaccine Institute, Seoul, Republic of Korea

We recently described the importance of B lymphocytes in induction of antigen (Ag)-specific oral tolerance and the associated increase in CD4+Foxp3+ regulatory T cells (Tregs) after mucosal administration of Ag conjugated to cholera toxin B subunit (CTB) (Sun et al. J Immunol. 2008;181:8278). We have now assessed the effects of B cells pulsed *in vitro* with Ag/CTB conjugate on the induction of Ag-specific Tregs both *in vitro* and after adoptive transfer *in vivo*. We found a strong increase in Tregs when naive T cells were co-cultured *in vitro* with B cells pulsed with OVA/CTB, and the generated Tregs could effectively suppress CD25-CD4+ effector T cells (Teffs) in secondary *in vitro* cultures. A strong increase in Tregs was also seen *in vivo* in recipients after adoptive transfer of Ag/CTB pulsed B cells. Further, adoptive transfer of B cells pulsed with myelin oligodendrocyte glycoprotein (MOG) peptide35-55 conjugated to CTB efficiently (i) suppressed MOG-specific T cell proliferation and IL-17 and IFN-γ production, (ii) increased Foxp3+CD4+ Tregs in draining lymph nodes and (iii) protected against the development of experimental autoimmune encephalomyelitis (EAE); similar effects were seen also when the B cell treatment was given "therapeutically" to mice with earlier on-going EAE. Our results show that B cells pulsed *in vitro* with relevant Ag/CTB conjugates followed by reinfusion of the treated B cells can be used to induce Ag-specific suppression of autoimmune disease. The molecules mechanism through which Ag/CTB renders B cells tolerogenic will be discussed.

F.63. Oral Tolerance Correlates with High Levels of Lymphocyte Activity

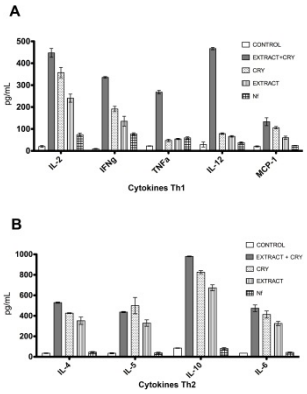
Archimedes Castro Junior, Bernardo Horta, Ana Cristina Gomes-Santos, Andre da Cunha, Ana Faria, Nelson Vaz. Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Defined as a reduction in specific immune responsiveness to proteins previously ingested as food and mimicking self-tolerance, oral tolerance is understood as a decrease of lymphocyte activity. Paradoxically, as shown in our work, oral tolerance involves significant increase in lymphocyte activation phenotypes in the spleen and numbers of immunoglobulin-secreting cells in spleen, comparable to those found in immunized, non-tolerant mice. Orally-tolerant and immunized mice had equivalent numbers of activated and regulatory T cells in spleen; more strikingly, orally-tolerant mice had higher numbers of IgM- and IgA-secreting cells in the spleen whereas immune mice had only IgG secreting cells. Orally tolerant mice had an expansion of activated (CD4+CD44+) and regulatory (CD4+ CD25+ Foxp3+) T cells in the spleen as soon as 24 h after primary immunization. Thus, in spite of inhibiting specific antibody formation and other forms of specific responsiveness, orally tolerant mice display an early and widespread mobilization of T lymphocytes with activation and/or regulatory phenotypes. This is compatible with an alternative view that describes oral tolerance as a stabilizing, rather than recessive, immunological phenomenon.

F.64. Effects of Immunization with pCry1Ac and Total Extract of N. Fowleri on NALT Lymphocytes

Marco Aurelio Rodriguez-Monroy[1], Oscar de Jesus Nieto-Yañez[2], Maria Margarita Canales-Marinez[1], Maria Concepción Peña-Juarez[2], Lucio Rivera-Santiago[2], Saul Rojas-Hernandez[2], Maricela Carrasco-Yepez[2], Xochitl Liliana Olivares-Lopez[2], Arturo Contis-Montes de Oca[2]. [1]Unam Fes Iztacala, Tlalnepantla, Mexico; [2]ESM-IPN, Mexico City, Mexico

Cry1Ac protoxin (pCry1Ac), is a potent mucosal and systemic adjuvant effects on antibody production. In a previous study using the model of primary amoebic meningoencephalitis (PAM) in mice, we demonstrated that pCry1Ac protects against infection by N. fowleri, however it is not characterized the mechanisms which are triggered to confer protection. The MAP model represents a good opportunity to explain the immunoprotective effects of the protoxin, especially in the NALT, because it is through the upper airway, which is given to the amoebae enter the body to produce the disease. Balb/c males of 4-6 weeks were used. Mice were immunized with three different treatments (pCry1Ac, total extract of N. fowleri, and pCry1Ac plus total extract of N. fowleri), after 4 immunizations, mice were challenged with 500 000 trophozoites of N. fowleri, obtaining serum samples and nasal secretions. Cells from NALT and Nasal Passges were obtained. By flow cytometry we analyzed the effect of immunization on lymphocytes populations of NALT and NP and the expression of activation markers. pCry1Ac protoxin is capable of inducing the production of Th2 mainly and Th1 cytokines. Immunization with total extract of N. fowleri and pCry1Ac, causes changes in lymphocytes populations in both tissues.



F.65. Primary and Memory Th2 Response against Helminth Infection in Aged Mice

Chizuru Muto, Chinatsu Saito, Yasuhiro Sugawara, Motoko Morimoto. Miyagi University, Sendai, Japan

This study aims to understand primary and memory Th2 immune responses against nematode and alternative macrophage activation in aged mice. The primary response against Heligmosomoides polygyrus (Hp) is associated with chronic infection, but subsequent reinfection results in a rapid and strong memory response and worm expulsion. 18-months (18M) and 3-months (3M) old Balb/c mice were inoculated with infective third-stage Hp larvae and studied at day 4 and 8 after primary (Hp1°) and challenge infection (Hp2°). Tissues were taken from small intestine for cytokine gene expression analysis and immunofluorescent staining. IL-4 and IL-13 gene expression was up-regulated after infection and the peak was day8 in 3M-Hp1°, but it was significantly low in 18M_Hp1°. Immunofluorescent staining revealed the number of alternatively activated macrophages (AAMacs) was declined in 18M-Hp1°. Meanwhile, Th2 cytokine gene expression and the number of AAMacs in 18M_Hp2° were similar with young group at day 8 after challenge. However, elevations in Th2 cytokines were lower at day4 in 18M_Hp2°. These results suggested that 1) age-associated changes against nematode were markedly developed in primary infection and 2) since a rapid memory response is critical for worm expulsion, the delayed elevation in Th2 cytokines might affect gut function in aged mice.

F.66. Human Cellular Immune Responses Against Giardia Lamblia 5 Years After Acute Infection

Kurt Hanevik[1], Staffan Svard[2], Einar Kristoffersen[3], Steinar Sornes[1], Oystein Bruserud[1], Nina Langeland[1]. [1]University of Bergen, Bergen, Norway; [2]Uppsala University, Uppsala, Sweden; [3]Haukeland University Hospital, Bergen, Norway

Background: Long term cellular immunity against the intestinal protozoan Giardia lamblia is poorly characterized in humans. Method: 19 adults (mean age 39.3 years, 73.6% females) with confirmed Giardia assemblage B infection 5 years previously and 10 controls without previous known Giardia infection were recruited in a non-endemic area. PBMC were cultured for 6 days with Giardia-antigens obtained by sonication of Giardia assemblage A and B reference strains. Cell proliferation was analyzed by 3H-incorporation and T cell subsets by flow cytometry. Results: In the proliferation assays, a mean stimulation index (mSI) of 16.6 was found for both assemblage A and assemblage B in the group with previously confirmed giardiasis. This was significantly higher than the control group (p=0.006 for assemblage A and p=0.001 for assemblage B). By flow

cytometry both groups showed stronger responses in the CD4 T cell compartments (mSI 12.2 for assemblage A and mSI 5.3 for assemblage B) compared to the CD8 compartment (mSI 3.9 and mSI 2.7 respectively). Conclusion: In this patient population, human cellular immunity against Giardia persisted for at least 5 years. CD4 T cells reacted more strongly than CD8 T cells. The cellular immune responses did not differentiate well between assemblage A and B, indicating cross-reacting antigens.

F.67. Signaling Pathways Leading to the Hyperplasia of the Gastric Mucosa During Parasitic Nematode Infections

Peter Geldhof, Belgacem Mihi, Manuela Rinaldi, Prisca Hoorens, Bruno Goddeeris, Edwin Claerebout, Jozef Vercruysse. Ghent University, Merelbeke, Belgium

Infections with gastric pathogens are generally characterized by marked alterations in the gastric physiology, such as the inhibition of acid secretion, hypergastrinemia and cell hyperplasia. The aim of our research is to investigate the cellular fate and proliferation of the different gastric mucosal cell lineages during gastric nematode infections and unravel the cellular signaling pathways involved in the onset and the maintenance of this gastric hyperplasia. Our research on the gastric nematode Ostertagia ostertagi in cattle has shown that during infection the upregulation of several pro-inflammatory molecules in the gastric mucosa, such as IL1beta, IL6, IL8 and COX-2, precedes the over-expression of gastrin and the onset of the cell hyperplasia, which seems to be controlled by the Wnt- and FGF-signaling pathways. The hyperplasia coincides with a significant decrease in the number of parietal cells, explaining the increased gastric pH measured after infection, and changes in the gastric mucus composition, with the upregulation of molecules such as TFF3 and galectin 15. Our current work is focused on further unravelling the signaling pathways leading to the gastric hyperplasia and whether gastric nematode infections in other host species, such as rabbits, mice and pigs, cause similar patho-physiological alterations.

F.68. The Role of p53 Tumor Suppressor Gene in Differentiation Processes of B Lymphocytes

Israel Zan-Bar, Irit Altboum. Tel Aviv University, Tel Aviv, Israel

In efforts to elucidate the role of p53 suppressor gene in B cell response we found that the upstream p53 signal transduction cascade components are identical to those of the immune response signaling cascades invoke by anti-Ig, LPS or LTA stimulation. Specific suppression of PTKs, PI3k, ATM, or ATR suppressed both the activation of the p53 cascade and the immunoglobulin secretion. This work was carried out working with murine B cell lymphoma cell lines which, upon stimulation with various agents can differen- tiate to Ig secreting cells and to Ig isotope switching. We demonstrated that various sublines of the origin IgM bearing cell lines are expressing endogenously wild type or mutant p53.Ig Isotype switching to IgA secreting cells happened only in the WT p53 bearing cells, while mutant p53 bearing cell could be “pushed” only to IgM secretion. Transfection of the cells with Shp53plasmids induces reduction of 50-80% in the expression of both the wt and m p53. LPS stimulation of the wt p53 plasmid transfected cells induces significant reduction in the IgA secretion with high rate of mortality (in necrosis) when treated with cytotoxic agents. The mutant p53 plasmid transfected cell lines revealed reduced resistant to the cytotoxic reagents and dramatic blockage in the IgM secretion upon LPS stimulation. Thus p53 has major role in the regulation of the differentiation and Ig secretion of B lymphocytes. Both wild type and mutant p53 has a role in the Ig switching and non switching Ig secretion processes.

F.69. Retinoic Acid Increases Human IgA Isotype Switching

Goo-young Seo, Ha-Na Yoo, Jini Kim, Jongseon Choe, Pyeung Hyeun Kim. Kangwon National University, Chuncheon-si, Republic of Korea

Retinoic acid (RA) is known to have several activities which lead to potent mucosal IgA response. Nevertheless, its exact role in human IgA synthesis was not delineated yet. Therein, we asked if RA can specifically induce IgA isotype switching in human B cells. RA increased IgA secretion by human tonsil B cells activated with anti-CD40 Ab, anti-IgM Ab, and IL-4, while concurrently decreasing IgM, IgG secretion. Likewise, RA markedly enhanced the expression of germ-line IgA1 and IgA2 transcripts (GLTα1 and GLTα2) which are indicatives of IgA class switching recombination, but not other isotypes such as GLTγ1 and GLTε. Based on limiting dilution analysis, RA increased the frequency of IgA secreting B cell clones by 2.4-fold. This was not accompanied by increased numbers of IgA secreting cells/clone. In addition, RA-induced GLα transcription and IgA secretion were virtually disappeared by LE540, an antagonist of RA receptor (RAR). Taken together, these results indicate that RA has activity as an exogenous IgA switch factor in human.

F.70. PolyI: C-induced Human Polymeric Immunoglobulin Receptor Expression is Inhibited by IL-10

Nobuko Takenouchi-Ohkubo, Masatake Asano, Daisuke Omagari, Kazuo Komiyama. Nihon University School of Dentistry, Tokyo, Japan

Polymeric immunoglobulin receptor (pIgR) is expressed by secretory epithelium cells such as intestinal epithelial cells and transfers the dimeric IgA made by submucosal plasma cells. IL-10 is an anti-inflammatory cytokine and its production is augmented in intestinal bowel diseases. Up to date, the influence of IL-10 on the pIgR expression has never been elucidated. We recently identified that polyI:C-induced expression of pIgR was significantly reduced by the pre-incubation of HT-29 with IL-10. As polyI:C signal was reported to be sensed by toll like receptor 3 and transduced by NF-κB, we attempted to examine whether IL-10 can inhibit pIgR expression through NF-κB inactivation. For these purposes, we performed luciferase assay using plasmid DNA that NF-κB biding sites are tamdemly repeated to the upstream of luciferase gene, and transfected to HT-29 cells, and then, tested luciferase activity. Luciferase activity was increased by stimulation of polyI:C. However, incubation with IL-10 before polyI:C incubation

did not inhibit luciferase activity. Our data suggest that inhibition of polyI:C-induced human pIgR upregulation by IL-10 is not involved in NF-κB pathway.

F.71. Glycosylation Pathways of O-glycans in the Hinge Region of Monomeric and Polymeric Circulatory IgA1

Jan Novak[1], Hitoshi Suzuki[2], Milan Raska[6], Kazuo Takahashi[1], Koshi Yamada[1], Zina Moldoveanu[1], Milada Horynova[6], Jana Novakova[6], Bruce Julian[1], Lea Novak[1], Knud Poulsen[3], Mogens Kilian[3], Andrew Herr[5], Ali Gharavi[4], Matthew Renfrow[1], Jiri Mestecky[1]. [1]University of Alabama at Birmingham, Birmingham, AL; [2]Juntendo University, Tokyo, Japan; [3]Aarhus University, Aarhus, Denmark; [4]Columbia University, New York, NY; [5]University of Cincinnati College of Medicine, Cincinnati, OH; [6]Palacky University in Olomouc, Olomouc, Czech Republic

In addition to 2 Fc N-glycans, the heavy chain of human serum IgA1 has 3-6 hinge-region O-glycans consisting of N-acetylgalactosamine (GalNAc) and galactose (Gal); one or both residues may be sialylated. Whereas control of IgA1 N-glycan synthesis has been described, regulation of IgA1 O-glycosylation remains to be elucidated. To study IgA1 O-glycans and their biosynthesis, we generated IgA1-secreting cell lines from peripheral blood of healthy individuals (HC cells) and patients with IgA nephropathy (IgAN cells). IgA1 O-glycans were analyzed by lectin binding and high-resolution mass spectrometry. Expression of specific glycosyltransferases was measured by RealTime RT-PCR. IgAN cells secreted IgA1 with more sialic acid and more O-glycans that were deficient in Gal, compared to IgA1 from HC cells. This aberrancy affected predominantly the polymeric form of IgA1 and was related to the lower expression/activity of Gal-transferase and elevated expression/activity of a sialyl-transferase. Confocal microscopy studies localized this Gal-deficient oversialylated IgA1 throughout the Golgi apparatus in IgA1-producing cells. Additional studies indicated that this glycosylation phenotype was determined genetically and also affected by specific cytokines. In summary, O-glycans of polymeric, but not monomeric, IgA1 can be aberrantly glycosylated through a biosynthetic process involving abnormal expression of specific glycosyltransferases.

F.72. Production of Enzymatically Active GalNAc-T2 and Studies of Aberrant O-glycosylation of IgA1 in IgA Nephropathy

Milada Horynová[1], Kazuo Takahashi[2], Matthew Renfrow[2], Evzen Weigl[1], Jiri Mestecky[2], Jan Novak[2], Milan Raska[1]. [1]Palacky University in Olomouc, Olomouc, Czech Republic; [2]University of Alabama at Birmingham, Birmingham, AL

In IgA nephropathy, some IgA1 hinge-region O-glycans are galactose deficient, and contain terminal N-acetylgalactosamine (GalNAc). Polymeric IgA1 with terminal GalNAc is recognized by naturally occurring antibodies, leading to the formation of IgA1-containing immune complexes that, after deposition in the glomerular mesangium, induce mesangial proliferation and glomerular injury. O-glycosylation of IgA1 is initiated by GalNAc-transferases (GalNAcT), namely GalNAcT2. As GalNAcT2 abnormalities in IgA1-producing cells can lead to production of galactose-deficient IgA1, it is important to understand the characteristics of this enzyme. Here we describe insect expression of soluble GalNAcT2 fused with murine immunoglobulin-kappa secretory signal peptide, which allowed GalNAcT2 secretion from insect cells infected with recombinant baculovirus. GalNAcT2 was isolated from cell-culture supernatant by affinity NiNTA chromatography. Amino-acid sequence of the secreted GalNAcT2 was confirmed by mass spectrometry. Protein stability was tested under various conditions, i.e., temperatures and buffers. Enzymatic activity of GalNAcT2 was measured as addition of GalNAc residues to synthetic IgA1 hinge-region peptides by lectin Western blot and verified by mass spectrometry. In summary, our data showed that signal-mediated peptide secretion in a baculovirus-based system resulted in the production of enzymatically active recombinant GalNAcT2. Supported by GAP302/10/1055, NT11060, LH11046, MSM6198959223 CR and DK078244, DK082753, DK080301, DK077279, DK083663 NIH.

F.73. A Diet Containing High Cocoa Flavonoid Content Modulates Intestinal IgA Response in Lewis and Brown Norway Rats

Maria del Mar Abril Gil, Malén Massot-Cladera, Patricia Nevot, Sara Ramos-Romero, Cristina Castellote, Francisco Perez-Cano, Angels Franch, Margarida Castell. University of Barcelona, Barcelona, Spain

Previous studies showed that a cocoa diet, a rich source of flavonoids, modified intestinal immune response function in rodents, attenuating the mucosal IgA response. The aim of the present work was focused to demonstrate the effect of a flavonoid-enriched cocoa diet with high polyphenol content (HCP diet) in comparison to a standard cocoa diet (CP diet) on intestinal s-IgA. To achieve this purpose two rat strains were used. Eigth week-old Lewis rats and four week-old Brown Norway rats were randomized into four dietary groups each: two groups received the HCP diet at two different doses (0.4% and 0.8% polyphenols, w/w), one group received the CP diet (containing 0.2% polyphenols, w/w) and a reference group was fed with standard diet. Diets lasted three weeks. Faecal samples were collected along the study and s-IgA was quantified by ELISA. In all strains and diets, faecal s-IgA decreased. However, CP group showed higher effect than HCP groups although the HCP diet had higher content of cocoa flavonoids. Therefore the cocoa attenuating effects on IgA might not be only attributed to its flavonoid content but also to other compounds present in cocoa.

F.74. Regulation Exert by a Probiotic Fermented Milk in a Respiratory Experimental Model of Allergy

Carolina Maldonado Galdeano[1], Eva María del Mar Vélez[2], María Bibas Bonet[2], Oscar Mesón[2], Esteban Carmuega[3], Ricardo Weill[4], Gabriela Perdigon[1]. [1]Cerela Conicet, San Miguel de Tucumán, Argentina; [2]Universidad Nacional de Tucumán, San Miguel de Tucumán, Argentina; [3]Centro de Estudios Nutricionales Infantiles, Buenos Aires, Argentina; [4]Danone, Buenos Aires, Argentina

Mechanisms involved in the beneficial effects of functional food are not well known. We studied the immune mechanisms mediated by probiotic

fermented milk (PFM) in an allergy mice model. Experimental groups: normal-control (NC), Basal (B-5days-PFM); OVA-Sensitization-control (SC), Previous (P)(5d-PFM+OVA+H2O) and Continuous (C)(5d-PFM+OVA+PFM) treatment. At 7 and 15 days post-sensitization (dps) we analyzed: specific-IgE, specific-IgG and IL10 in serum. Non-specific-IgA, IL10 and IFNγ in intestinal fluid (IF), and IL2+,IL4+ and IL10+ cells in the small intestine (SI). IgA+ cells in SI and bronchus and changes in the intestinal microbiota. At 7dps, specific-IgE decreased and IL10 increased in P and C groups compared with SC. IL10+ cells were increased for 7 and 15dps, however IL10 and IFNγ release was not enhanced. The number of IL2+ cells was similar to the NC. IL4+ cells decreased for 15dps in treated groups but not for SC. For 7 and 15dps total s-IgA levels were higher than NC. IgA+ cells from SI and bronchus were increased in C group. Changes in the intestinal microbiota were observed with diminution in enterobacteria, and increases of bifidobacteria populations in the treated groups. PFM was able to regulate IgE levels, by a decrease in the number of IL4+ cells in SI and increases of IL-10. No variations in total s-IgA were found. The immune regulation observed for PFM in this allergy model could be mediated by the immune regulatory capacity exerted by bifidobacteria.

F.75. Improved Response of Intestinal Epithelial Cells Against Salmonella Enterica Serovar Typhimurium Infection in Mice Fed with Lactobacillus Casei CRL431

Natalia Castillo, Carolina Maldonado Galdeano, Alejandra de Moreno de LeBlanc, Gabriela Perdigon. CerelaA-Conicet, San Miguel Tucumán, Argentina

Lactobacillus casei CRL431 (Lc) protected against Salmonella enterica serovar Typhimurium (ST) infection modulating the immune cells in a mouse model. Intestinal epithelial cells (IECs) participate in the early response against ST. We evaluate the IEC response to IL-6 and MPC-1in mice received Lc and were infected with ST. Lc7d-group received Lc during 7days (d), was challenged with ST, Lc administration continued until 10d post-infection (PI). IECs were isolated from treated, untreated (UC) and infected (IC) controls mice. IECs from UC were cultured with ST or Lc for *in vitro* tests. In the *ex vivo* assay IL-6 and MCP-1 release increased for Lc7d-group compared to UC and to IC after 24h PI. IC increased IL-6 levels for 7 and 10d in PI. *In vitro*, IECs cultured with Lc released higher levels of IL-6 than IECs cultured with ST. These results show the IEC as another important source of IL-6, for B-cell clonal expansion and specific-anti-ST-IgA-s production, involved in the protection against ST exerted by Lc. The results obtained for MCP-1showed the participation of macrophages in the protective effect of Lc. ST Infection increased intestinal neutrophils influx and mieloperoxidase activity, which diminished in Lc7d-group. Lc administration induced an earlier response of the IECs with IL-6 and MCP-1 secretion, increasing the protective barrier of the IECs, first step of this infection.

F.76. Effect of Precolonization with the Mixture of Lactobacillus Casei and Lactobacillus Paracasei Strains on the Development of Experimental Birch Pollen Allergy in Mice

Hana Kozakova, Martin Schwarzer, Dagmar Srutkova. Academy of Sciences of the Czech Republic, Novy Hradek, Czech Republic

Disbalanced bacterial colonization in early life affects allergies development later on. We studied the effect of precolonization with the mixture of L. casei and L. paracasei on the development of allergic sensitization to the main allergic component of birch pollen Bet v 1 in mice. Originally germ-free (GF) mice were colonized with mixture of L. casei 900, L. casei 908, L. paracasei 919 strains and after 20 days colonized mice and age-matched GF controls were repeatedly immunized with Bet v 1. Allergen-specific antibody levels and transforming growth factor (TGF)-beta were determined in sera. Th1/Th2 cytokines were evaluated after 48 h cultivation of spleen and mesenteric lymph node cell cultures. Mice colonized with lactobacilli mixture showed significantly lower value of Bet v 1-specific IgG1, IgG2a and IgE and stimulated levels of total IgA in sera and intestinal lavages accompanied by increase of TGF-beta compared to the GF sensitized group. In colonized mice, proallergic IL-5 was reduced markedly and regulatory TGF-beta was found stimulated. Colonization with lactobacilli mixture inhibited the development of allergic immune responses and increased the production of regulatory cytokine TGF-beta and can be thus exploited for prevention of allergic sensitization. Supported by grants IAA500200710 and CZ.3.22/2.1.00/09.01574.

F.77. A Multi-strain Probiotic Reduces Inflammation via the Mitogen Activated Protein Kinase Pathway in HT-29 Cells Challenged with a Double-stranded RNA Viral Mimic

Julie Audy, Olivier Mathieu, Thomas Tompkins. Institut Rosell-Lallemand, Montreal, QC, Canada

Our focus was to determine the anti-inflammatory properties of a bacterial mixture, ProbioKid (PK), made with Bifidobacterium infantis R0033, B. bifidum R0071 and Lactobacillus helveticus R0052. Intestinal epithelial cells (HT-29) were challenged 3 h with the double-stranded RNA viral mimic, polyinosinic acid-poly cytidylic acid [Poly (I:C); 10 μg mL-1]. PK was added 1 h before (-1 h), simultaneously (0 h) or 1 h after (+1 h) stimulation with Poly (I:C). An expression Immune Array was used to screen innate pathways involved in HT-29 cell response to the co-challenge. Gene expression was confirmed by Q-RT-PCR and IL-8 secretion was quantified by ELISA after 3h or 6 h stimulation respectively. Results were compared to the impact of PK additions (-1 h, 0 h & +1 h) on stimulated cells response. PK reduced a number of genes in stimulated cells from MAPK signalling pathway leading to the down-regulation of the transcriptional factor AP-1 (JUN & FOS), but not from the NFκB transcriptional factor. Indeed, PK had an anti-inflammatory effect; IL-8 expression and secretion were significantly reduced in stimulated cells as were IL28B, IL29, CXCL10 and TNF-α genes. We concluded down-regulation of the MAPK signaling pathway was the most important in response to the co-challenge.

F.78. Modulation of NF-**κB Su**b-units by the Probiotic Lactobacillus Helveticus R389

Jean-Francois Mallet[2], Jairo Duarte[1], Chantal Matar[1]. [1]University of Ottawa, Ottawa, ON, Canada; [2]Université de Moncton, Moncton, NB, Canada

The use of probiotics is a promising approach in the treatment of inflammatory disease like Crohn's disease and ulcerative colitis. We have demonstrated before that Lactobacillus helveticus R389 modulate the immune response of intestinal epithelial cells (Clin Diagn Lab Immunol 2005,12:1075). In this experiment, we verified the translocation of NF-κB *in vivo* in Balb/c mice fed for 2, 5 and 7 days with milk fermented with L. helveticus R389. No significant changes were noted independently of our previous work on the effect of L. helveticus on IL-6 production by IEC. To determine how the probiotic L. helveticus modulates the IEC response, cells extracted from the small intestine of mice fed for 7 days with the fermented milk were exposed to E. coli MM294 and LPS in an *ex vivo* cell culture. L. helveticus successfully prevented the translocation of the p65 and p50 sub unit of NF-κB returning them to control level. A diminution of the inflammatory signal IL-6 and CCL3 in the supernatant was also observed. These results demonstrate that the consumption of the probiotic L. helveticus R389 can protect against inflammation caused by E. coli and modulate pro-inflammatory mediators that have a direct impact on the mucosal immune system.

F.79. Lactobacillus Brevis Culture Supernatant Induces Cytoprotective Small Heat Shock Protein HSP27 and Increase Intestinal Barrier Function by Activating the p38 MAPK Pathway

Shuichi Segawa[1], Mikihiro Fujiya[2], Nobuhiro Ueno[2], Hiroaki Konishi[2], Naoyuki Kobayashi[1], Tatsuro Shigyo[1], Yutaka Kohgo[2]. [1]Sapporo Breweries Ltd., Yaizu, Japan; [2]Asahikawa Medical University, Asahikawa, Japan

Background: Probiotics exhibit beneficial effects on human health, particularly in the maintenance of intestinal homeostasis. However, the effective molecules produced by such probiotics are infrequently identified. We investigated the cytoprotective effect of a culture supernatant obtained from the probiotic Lactobacillus brevis by measuring the levels of inducible heat shock proteins (HSPs), which play an important role by protecting cells against oxidative stress and intestinal inflammation. Results: The L. brevis culture supernatant significantly induced HSP27 in Caco2/bbe cells. HSP27-inducible substance was separated by anmonium sulfate precipitation, DEAE anion exchange chromatography and gel filatration. The HSP27-inducible substance was finally obtained. It activated p38 MAPK, but did not activate ERK, JNK and Akt. Furthermore, its ability to induce HSP27 was abolished by the p38 inhibitor SB203580. It also inhibited increased mucosal permeability in small intestinal loops caused by exposure to an oxidant (NH_2Cl 0.3 mM) at the concentration of 1-10 μg/mL and its protective action against oxidant stress was inhibited by p38 MAPK inhibitor. This indicated that its protective effect was mediated by the activation of p38 MAPK. Conclusion: Our results revealed that the HSP27-inducible substance identified from the supernatant of L. brevis induced HSP27 and increased intestinal barrier function by activating the p38 MAPK pathway.

F.80. Effect of Probiotic on Maturation of Dendritric Cells

Beatriz Martínez-Abad[1], José Garrote[2], Sara Vallejo-Díez[1], Enrique Montalvillo[1], Celia Escudero-Hernández[1], David Bernardo Ordiz[3], Enrique Váquez[4], Ricardo Rueda[4], Eduardo Arranz[1]. [1]University of Valladolid/IBGM-CSIC, Valladolid, Spain; [2]Hospital Clínico Universitario-IECSCYL, Valladolid, Spain; [3]Imperial College London, St. Mark's and Northwick Park Hospital, London, United Kingdom; [4]Abbott Nutrition R&D, Granada, Spain

While some bacteria may cause disease, other, like probiotic, have a beneficial effect on the health host. Probiotics reinforce innate immunity in the gut, but also have a modulatory effect on adaptive immunity. We are focus on their effect on dendritic cells (DCs), as a linkage between innate and adaptive immunity. We are characterizing how a group of putative probiotics modify DC cytokine expression profile and surface maturation markers. Our screening included: 4 strains belong to the genus Lactobacillus (A, B, C, D) and 2 belong to genus bifidobacterium (A and B). We use Escherichia coli O111 CECT 727 and Clostridium perfringens CECT 376 as controls. Our data shows that bifidobacterium A induces maturation of DCs by increasing the expression of CD86. On the other hand, only E. coli and C. perfringens induce CD83 expression. We also found that the two strains of bifidobacteria increased IL6 and IL10 expression. Further studies are needed to analyze the involvement of IL6 in both, pro-inflammatory and anti-inflammatory events.

F.81. Probiotics Modulate Colitis-associated Gene Expression Profiles in the Colon and Protect Against the Development of Intestinal Inflammation

Rob Mariman[1], Bas Kremer[1], Marjan van Erk[1], Frits Koning[2], Lex Nagelkerken[1]. [1]TNO, Leiden, Netherlands; [2]Leiden University Medical Centre, Leiden, Netherlands

Host-microbiota interactions in the intestinal mucosa play a key role in immune regulation in the gut. In this study, we evaluated immunomodulatory effects of probiotics in a model of recurrent colitis in BALB/c mice. Oral administration of probiotic L.plantarum or VSL#3 was initiated two weeks before colitis induction and continued throughout the study. This prolonged administration of probiotics reduced intestinal inflammation in response to repeated TNBS challenges. In addition to normalized colon weight/length ratio, this was evident from less influx of innate ($CD11b^+$) and adaptive ($CD4^+/CD8^+$) immune cells in the intestinal mucosa and decreased pro-inflammatory serum cytokines in probiotic-treated mice. Gene expression profiling in colonic tissue at various time-points revealed that probiotic treatment protected mice against colitis by modulating inflammation and immune related processes. These effects of probiotics on colonic gene expression were most abundant during active inflammation, and in particular evident from a diminished expression of genes related to mast cells or encoding anti-microbial peptides. Preliminary data show that modulation of

gene expression by probiotics in the small intestine is even more abundant, indicating that probiotics may mediate their protective effects by modulating intestinal homeostasis at different levels of the GI tract.

F.82. Microbial Diversity and Intestinal Immunity of Weanling Piglets Modulated by Probiotics

Martin Lessard[1], Jean-Philippe Brousseau[2], Jean-François Daudelin[3], Frédéric Beaudoin[1], Éric Nadeau[3], Denis Roy[2], John Fairbrother[3]. [1]Dairy and Swine R & D Centre, Sherbrooke, QC, Canada; [2]Université Laval, Québec, QC, Canada; [3]Université de Montréal, St-Hyacinthe, QC, Canada

The potential of two probiotics, Pediococcus acidilactici (Pa) and Saccharomyces cerevisiae boulardii (Scb), to modulate bacterial populations and immune response of piglets was evaluated. Forty litters of 12 piglets were distributed among following treatments: control, Pa, Scb, Pa+Scb or weanling diet containing antibiotics (ATB). Probiotics were administered daily to piglets from one day of age. Two weeks post-weaning, two pigs per litter were euthanized to characterize ileal microbiota by T-RFLP. Microbial diversity indices were lower in ATB and Pa groups than in the control group (P<0,05). Seven days post-weaning, one pig per litter was orally inoculated with an enterotoxigenic E. coli and a necropsy was performed 24h later. Percentages of $CD4^+CD8^-$ cells were higher in mesenteric lymph nodes (MLN) of infected animals as compared with non infected pigs (P<0,01), particularly in the Pa and Scb groups. On the other hand, circulating $CD4^+CD8^-$ cells were lower in ATB, Pa and Scb than in control after infection (P<0,05). In infected pigs treated with Pa or Scb, percentages of MLN $CD21^+MHCII^+$ cells were lower compared with ATB and control (P<0,05). Finally, IL-6 expression was up-regulated in Pa and Pa+Scb compared with control (P<0,05). In conclusion, both Pa and Scb modulated cell populations in the blood and MLN whereas only Pa influenced bacterial diversity and expression of inflammatory cytokine IL-6 in the ileum.

F.83. Opposite Modulating Effects on Dendritic Cell and T Cell Responses by Low Dose versus High Dose Lactobacilli in Gnotobiotic Pigs

Lijuan Yuan, Ke Wen, Guohua Li, Tammy Bui, Fangning Liu, Yanru Li, Jacob Kocher, Shujing Rao, Lin Lin, Xingdong Yang. Virginia Polytechnic Institute and State University, Blacksburg, VA

Dose effects of Lactobacillus acidophilus NCFM (LA) on dendritic cell (DC) and T cell immune responses after rotavirus vaccination were evaluated in gnotobiotic pigs vaccinated with a human rotavirus oral vaccine in conjunction with low dose (5 feedings; up to 10^6 CFU/dose) or high dose (14 feedings; up to 10^9 CFU/dose) LA or without LA feeding. Low dose LA significantly increased the frequencies of splenic cDC with significantly decreased MHC II expression, and significantly decreased IL-10 expression in blood pDC compared to the vaccine-only group; whereas high dose LA significantly lowered frequencies of splenic cDC with significantly higher MHC II expression and significantly increased IL-10 expression in blood pDC. Low dose LA significantly enhanced frequencies of CD4+IFN-γ+ and CD8+IFN-γ+ T cells in intestinal and systemic lymphoid tissues and reduced frequencies of adaptive regulatory T (Treg) cells and TGF-β expression in those cells; whereas high dose LA reduced frequencies of CD4+IFN-γ+ T cells and increased frequencies of adaptive Treg cells and IL-10 expression in those cells in ileum. Thus, the same LA strain at different doses exerted opposing modulating effects on DC and adaptive T cell responses. These findings have significant implications in the use of probiotic lactobacilli as immune-stimulatory versus immune-regulatory agents.

F.84. Probiotic Bacillus Subtilis CU1 Normalize the Mucosal Immune Responses and Microbiota Balance in a Murine Model of Dysbiosis

Silvia Racedo[1], Claudine Jacquot[1], Iryna Pinchuk[2], Maria Urdaci[1]. [1]Université Bordeaux, Gradignan, France; [2] University of Texas Medical Branch at Galveston, Galveston, TX

Background & aims: While general beneficial effect of the Bacillus probiotics for human health was noted, their positive effects in dysbiosis induced by antibiotic treatments remains unclear. An antibiotic (ATB) induced dysbiosis mouse model was used to establish the effects of the probiotic Bacillus subtilis CU1 on intestinal mucosal immune system and microbial balance. Methods: Swiss albino mice were treated with CU1 spores (3x109spores/day/mouse) before and during the ATB treatments (CU+ATB). Control (C), antibiotics (ATB) and probiotic (CU) treated mice were also studied. Presence of the professional immune cells in Peyer's patches (PP) and mesenteric lymphoid node (MLN) and intestinal goblet cells and IgA+cells were studied using immunofluorescent microscopy and FACS analysis. Results: Treatment with the CU1 strain decrease the ATB induced intestinal inflammation (H&E analysis) and coincides with of the significant reduction of the intestinal goblet cells and IgA+cells. Normalization in the B220+MHCII+ B cells in MLNs and F4/80+ macrophages in PPs was observed in the ATB group treated with probiotic. Moreover, the probiotic treatment reduced ABT induced alterations in the gut microbiota (DGGE analysis). Conclusion: Bacillus probiotics may contribute to the reduction of antibiotic induced inflammation via normalization of: (1) mucosal immune responses and (2) intestinal microbiota.

F.85. Lactobacillus Casei and Lactobacillus Plantarum Strains Downregulate Proinflammatory Genes in Human Colonic Mucosa *ex vivo*

Marta Llopis Pages[1], Christine Baüerl[2], María Antolín Maté[1], Vicente Monedero[2], Manuel Mata[3], Manuel Zuñiga[2], Francisco Guarner[1], Gaspar Perez Martinez[2]. [1]University Hospital Vall d'Hebron, Barcelona, Spain; [2]Instituto de Agroquímica y Technología de Alimentos, CSIC, Valencia, Spain; [3]Hospital General Universitario, University of Valencia, Valencia, Spain

The potential of certain probiotic strains to attenuate inflammation has been demonstrated in several studies. However, their molecular mechanism in humans is still unknown. To this aim, we studied the response of stimulated human colonic mucosa to three probiotic bacteria using whole genome microarrays. Methods: Macroscopically normal colonic tissue was surgically obtained from 3 patients. Mucosal samples (25-35mg), previously decontaminated and stimulated with phorbol-12-myristate-13-acetate (PMA)/ionomycin (IO) (3 hours) were cultured with Lactobacillus casei BL23

(BL23), Lactobacillus plantarum 299v (LP) or Lactobacillus plantarum 299v(A-) (LP(A-)) (10^6 cfu/ml) for 4 hours. Control samples without PMA/IO or probiotic bacteria were included. After RNA extraction, microarray analysis was performed with GeneChip (Affymetrix). Results: Analysis revealed 199 differentially expressed genes between Control, PMA/IO stimulated, and the 3 bacterial treatments. More importantly, a number of PMA/IO induced genes related to immune response such as IL-2, IFN-γ, IL-17A and proinflammatory chemokines CXCL9 and CXCL11 were down- regulated by BL23, LP and LP(A-). The action of the Lactobacillus strains was quite similar, although certain strain-dependent effects were observed. Conclusion: The global transcriptional profile triggered by strains BL23, LP and LP(A-) in stimulated tissue indicated an homeostasis restoring effect, including a decrease of the signals produced by activated T cells.

F.86. Prebiotic and Immunologic Effects of Inulin from Agave Tequilana Weber

Amelia Minjarez-Ibañez[1], Jorge Segura-Ortega[2], Cecilia Guillen-Vargas[1], Vidal Delgado-Rizo[1], Mary Fafutis-Morris[1]. [1]Universidad de Guadalajara, Guadalajara, Mexico; [2]Antiguo Hospital Civil de Guadalajara Fray Antonio Alcalde, Guadalajara, Mexico

The Agave genere is one of Mexico's most important agro-industrial resources. It contains significant amounts of inulin with interesting nutritional and technological properties. These have been considered equivalents to chicory inulin, a fructan commercially used for its prebiotic and immunologic effects. However, despite its characteristics, few reports on the effects of agave inulin exist to this date. Aim: To evaluate the effect of agave inulin on intestinal microbiota, as well as the expression of TLRs and defensins. Materials & Methods: Thirty male Wistar rats were used in this study, divided in three groups of 10. They received 20gr daily for 4 months of a standard diet (STD), a diet supplemented with 10% of Blue Agave Tequilana Weber inulin (TEQ), or cichorium intybus inulin (CHY). Bifidobacterium, Bacteroides and Clostridium were quantified by rtPCR from DNA obtained from feces. Immunohistochemical stains of paraffined sections of small intestine with anti-TLR1,TLR2,TLR4 and TLR6 antibodies and β-defensin-2 were performed. Results: TEQ stimulates the growth of Bifidobacterium significantly when compared to the STD and the CHY groups. The percentages of Bacteroides and Clostridium were similar in all groups. The percentage of epithelial cells positive to TLR1, TLR2,TLR4 and TLR6 remained the same in all groups, while the expression of β-defensin-2 was significantly higher in TEQ and CHY groups when compared to the STD diet. Conclusions: This data suggests that Agave inulin is capable of stimulating selective proliferation of beneficial bacteria in the intestine, allowing as well to the establishment of a dynamic equilibrium between the intestinal immune system and microbiota.

F.87. Neonatal Monocolonization of Germ-free Mice with Bifidobacterium Longum Ssp. Longum RB25P Strain Suppresses Sensitization in Mouse Model of Birch Pollen Allergy

Martin Schwarzer[1], Dagmar Srutkova[1], Irma Schabussova[2], Ursula Wiedermann[2], Hana Kozakova[1]. [1]Academy of Sciences of the Czech Republic, Novy Hradek, Czech Republic; [2]Medical University of Vienna, Vienna, Austria

Microbial colonization in early life is believed to play a major role for maturation of the immune system. Germ-free BALB/c mice were monocolonized by Bifidobacterium longum ssp. longum RB25P strain, mated and naturally colonized offsprings were used at the age of two months. Monocolonized mice and age-matched germ-free controls were three times subcutaneously immunized with major birch pollen allergen Bet v 1/Alum in two-week intervals and sacrificed one week after the last immunization. Bifidobacterium-colonized mice showed significantly reduced level of allergen-specific IgE, IgG2a, IgG1 antibody as well as total IgE in blood sera, reduced Bet v 1 induced recall proliferation of spleen cells and reduced production of IL-5, IL-4, IL-10, IL-13, IL-17 and INF-gamma by spleen and mesenteric lymph node cells compared to germ-free controls. Furthermore, we have shown that TLR2 but not TLR4 is involved in recognition of B. longum in TLR2 and TLR4 transfected HEK293 cells. Maturation and IL-10 and IL-12p70 cytokine production of bone-marrow derived dendritic cells was abolished in MYD88-/- knockout mice (KO), strongly reduced in TLR2-/- KO and unchanged in TLR4-/- KO. We conclude that bifidobacterial strain RB25P has strong immunomodulatory properties which are connected with TLR2 signaling pathway. Supported by grants IAA500200710 and CZ.3.22/2.1.00/09.01574.

F.88. Proteomic Analysis of Saliva and Rectal Lavage of HIV-1-exposed Uninfected Men who have Sex with Men Reveal Novel Elevated Antiviral Factors

Adam Burgener[1], Klara Hasselrot[3], Stuart Mccorrister[4], Emma Hauch[1], Garrett Westmacott[4], Kristina Broliden[3], Blake Ball[2]. [1]University of Manitoba, Winnipeg, MB, Canada; [2]National Microbiology Lab, Winnipeg, MB, Canada; [3]Karolinska Institute, Stockholm, Sweden; [4]Public Health Agency of Canada, Winnipeg, MB, Canada

Studies of HIV-1 highly exposed seronegative individuals (HESN) seek to understand natural correlates of protection against HIV-1 infection. Recent evidence indicates that unique mucosal responses may be protective against HIV-1 infection in some HESN cohorts. Although there has been considerable effort to describe protective mucosal factors in women, this has not been explored in HIV-1-exposed uninfected men who have sex with men (EU-MSM). Using a comprehensive and unbiased proteomics approach, we show that EU-MSM have differentially expressed proteins both in their saliva and rectal mucosa. Results: Saliva and rectal lavage samples were collected from 24 EU-MSM and 22 non-exposed MSM from discordant couples in Sweden. Equal amounts of protein from each group were digested with trypsin, differentially labeled with iTRAQ, and analyzed in duplicate by 2D-LC-MS/MS. Of the more than 360 unique proteins identified in saliva, EU-MSM had 22 proteins overexpressed and 10 underexpressed compared to controls (>1.5 fold cutoff). This was also true of rectal mucosal samples where ~ 60 proteins were differentially expressed. Many overexpressed proteins in EU-MSM have known antiviral activity against HIV-1. This is the first comprehensive proteomic study of the mucosal layers of HIV-1 exposed men and we believe these factors may be contributing to altered susceptibility to HIV-1 infection.

F.89. TLR2 is Required for the Induction of Sustained Airway Inflammation and Generation of Adaptive Immune Responses Following Chronic Bacterial Infection

Amit Lugade[1], Paul Bogner[1], Timothy Murphy[2], Yasmin Thanavala[1]. [1]Roswell Park Cancer Institute, Buffalo, NY; [2]University at Buffalo, State University of New York, Buffalo, NY

Within the respiratory mucosa a delicate balance must exist to limit infection and inflammation-mediated lung tissue destruction. Ligation of pathogen recognition receptors, such as Toll-like receptors (TLRs), during infection are necessary to initiate the protective inflammatory processes and signaling from these receptors is critical for the generation of adaptive immune responses. Therefore, protective immune responses to respiratory pathogens induce and require inflammation to activate antigen-specific activate lymphocytes. We have found that chronic pulmonary exposure to non-typeable Haemophilus influenzae (NTHI) results in high levels of bronchovascular inflammation and the generation of specific T cells and circulating antibodies. Lungs of wild type mice exposed to NTHI exhibit peribronchial inflammation highlighted by extensive cuffs of immune cells organized into aggregates resembling bronchial-associated lymphoid tissue (BALT). TLR2-deficient mice exhibit a significant reduction in immune cell infiltration and the induction of BALT. Mutant NTHI strains that lack a key, conserved outer membrane lipoprotein, the TLR2 ligand P6, are also incapable of eliciting robust pulmonary inflammation. Cognate interaction between TLR2 and its ligand on the outer membrane of the bacterium are pivotal in promoting lung inflammation and eliciting robust adaptive immune responses following respiratory infection. Strategies to limit bacterial mediated pulmonary inflammation while simultaneously promoting robust immune responses may benefit by targeting the TLR2:P6 signaling axis.

F.90. Effects of Vitamin A in Murine Immune System and Metabolism

Flavia Loli, Samara Medeiros, Andrezza Santiago, Andrea Alves, Ana Cristina Gomes-Santos, Rafael Rezende, Raphaela Oliveira, Rafael Pires, Ana Faria. Federal University of Minas Gerais, Belo Horizonte, Brazil

Introduction: Vitamin A and its analogs have been in focus of research because of their powerful regulatory effect on cell proliferation and epithelial differentiation besides its antioxidant activity. Objectives: To evaluate the effects of dietary deficiency and supplementation with vitamin A in the immune system and metabolism of mice. Methods/Results: We fed C57BL/6 mice with four different diets: normal (4000 IU vitamin A), vitamin A-free and two Vitamin A-supplemented diets (10000 IU or 50000 IU vitamin A). After 8 weeks of diet consumption (starting after weaning), we evaluated frequencies of subsets of T and B cells by flow cytometry. To monitor toxicity related to vitamin A supplementation, we also measured biochemical parameters. No significant disturbance was observed in metabolic parameters with these supplementation doses, even in the liver. Supplemented mice had increased frequency of regulatory T cells in spleen and mesenteric lymph nodes (MLN) and of B1 lymphocytes in spleen. Production of IFN-γ by MLN cells was up-regulated in vitamin A-deficient mice. Serum levels of IgG production were also increased in deficient mice. Conclusion: Vitamin A supplementation modulated the frequency of regulatory T cells in healthy mice and the production of IFN-γ, suggesting that it has anti-inflammatory properties *in vivo*.

F.91. High Activity of Regulatory T Cells in Human NALT Tissues and its Association with Pneumococcal Colonization

Qibo Zhang[1], Samuel Leong[2], Paul McNamara[2]. [1]University of Liverpool, Liverpool, United Kingdom; [2]Liverpool Children's Hospital, Liverpool, United Kingdom

Regulatory T cells (Treg) diminish immune responses to infection, which may contribute to preventing inflammation-related tissue damage but may also contribute to chronicity of infection. Pneumococcal carriage is common in young children and can persist for a long periods in some. It is unknown whether the presence of Treg contributes to the persistence of pneumococcus in children. We have investigated the numbers and activities of Foxp3+Treg in adenoids and their association with pneumococcal carriage in children. Expression of Treg cell-related markers were analysed by flow-cytometry in adenoidal MNC and PBMC from children. Unfractionated MNC or Treg-depleted MNC were stimulated with a pneumococcal whole cell antigen (WCA) and T cellular proliferation was measured. Higher numbers of CD25highFoxp3high Treg expressing CD39 and CTLA4 were found in adenoidal MNC than in PBMC. Children who had positive cultures for pneumococcus had higher proportions of Treg and expressed higher CD39 and CTLA-4 than those who were culture negative. WCA induced higher proportion of adenoidal Treg proliferation in pneumococcal culture + than culture - children. Significant numbers of Treg with an effector/memory phenotype exist in adenoids which possess potent inhibitory effect. High activity of Treg in NALT may contribute to the persistence of pneumococcus in children.

F.92. Factors Mediating the Induction of Intestinal IL-17-producing Tregs in Inflamed Gut Mucosa

Zaruhi Hovhannisyan, Lloyd Mayer. Mount Sinai School of Medicine, New York, NY

IL-17-producing CD4+ helper T (Th17) cells are involved in inflammatory bowel disease (IBD) and mucosal immunity. While there is a great degree of plasticity between Th17 and regulatory T (Treg) cell differentiation events, the interplay between these two developmentally related T cell subsets remains obscure. We have recently shown that the unique inflammatory environment in Crohn's disease (CD) contributes to the generation of a distinct intestinal FoxP3+IL-17-producing Tregs in inflamed CD gut mucosa (Gastroenterology, 2010). To further define the factors that coordinate Treg/Th17 cells, CD4+ lamina propria lymphocytes (LPL) isolated from IBD patients or normal controls were incubated with either IBD or normal-derived supernatants from colonic tissue cultures. Remarkably, the supernatants from CD, but not UC or normal-derived mucosal cultures, effectively induced a FoxP3+IL-17+ population in UC, but not CD or normal CD4+ LPLs. Cytometric bead array-based protein analysis revealed significant

increases in IL-6, IL-8 and IL-17A in CD colonic tissue cultures when compared to those derived from UC or normal controls. This, along with the finding that TGF-β induces the same population in UC but not normal LPLs, supports the fact that plasticity of FoxP3+IL-17+ double positive cells is cytokine driven.

F.93. A Targeted Fusion Protein that Stimulates Mucosal Tolerance to Incorporated Peptides

Charlotta Hansson, Annemarie Hasselberg, Karin Schon, Jan-Olov Andersson, Hilda Bergroth, Nils Lycke. University of Gothenberg, Gothenburg, Sweden

To circumvent toxicity we developed the CTA1-DD adjuvant, which is a non-toxic derivative of cholera toxin. Interestingly, when the ADP-ribosylating function of CTA1-DD was eliminated by a single amino acid replacement the fusion protein conveyed strong T cell tolerance rather than an augmented response. Tolerance was observed to peptides incorporated in the fusion protein after intranasal immunizations. The CTA1R7K-OVA-DD molecule stimulated regulatory T cells that produced IL-10 and suppressed OVA-specific T cell proliferation. To dissect which target population that conveyed tolerance-induction we treated dendritic cells (DC) or B cells *ex vivo* with the fusion protein and upon re-injection of the cells and a challenge immunization with ovalbumin in recipient mice, we observed that only DC , but not B cells, were responsible for tolerance. These findings were further supported by experiments in B cell deficient, JHD, and DO11.10 SCID mice, which both exhibited unimpaired antigen-specific tolerance following intranasal immunization. Thus, DC targeted by CTA1R7K-OVA-DD stimulated mucosal tolerance in the DO11.10 T cells. Replacing the DD element with single chain antibodies directed against known DC target molecules will open new avenues to mucosal tolerance induction as will be discussed.

F.94. Human Regulatory T Cells Migrate to the Small Intestine and Produce IL-10 in Response to Treatment with Anti-human CD3 Monoclonal Antibody

Frank Waldron-Lynch, Octavian Henegariu, Paula Preston-Hurlburt, Richard Flavell, Kevan Herold. Yale University Medical School, New Haven, CT

FcR non-binding anti-CD3 monoclonal antibodies have been effective for treatment of human autoimmune diseases. However, the immunologic mechanisms of action in humans have proven difficult to elucidate. Neonatal NOD/SCID/IL2γnull mice were engrafted with human CD34+ cells to reconstitute human immune systems. These humanized mice were treated with Teplizumab. Drug treatment induced migration of T cells from the peripheral circulation and spleen to the small intestine. *Ex vivo* and *in vivo* studies showed that Teplizumab treatment induced expression of the gut homing receptor, CCR6, on T cells which migrated to the gut in response to increased expression of CCL20. Human CD4+CCR6+ and CD8+CCR6+ cells had increased expression of IL-10 and FoxP3 in the duodenum compared to controls or spleens from the same treated animals. Our studies identified CD4CD25highCD127lowCCR6+ T cells that decline in the peripheral circulation and spleen of treated mice. Similarly, we found a transient decline in these cells in patients who were treated with Teplizumab. These findings indicate that Teplizumab induces migration of human T cells to the gut. At that location, the cells acquire regulatory phenotypes and then reappear in the circulation. This suggests that the gastrointestinal tract plays a role in the mechanism of action of Teplizumab.

F.95. Evaluation of the Incorporation of Influenza Vaccines by Antigen Presenting Cells

Kayoko Sato, Hideki Asanuma, Michiyuki Kasai, Shigeyuki Itamura, Masato Tashiro. National Institute of Infectious Disease, Musashimurayama-shi, Japan

Influenza vaccines induce protective immunity against subsequent infections, and a variety of different vaccine types are currently available. Although type-I IFN produced by pDC was reported to delineate the different immunogenicity of these vaccines, the effects on the antigen presentation remains to be clarified in regard to these differences. In this study, correlations of immune responses after vaccination and antigen uptake by macrophages were investigated using both whole- and split-virion vaccines. Mice immunized parenterally with whole virion- or split influenza vaccine (X-179A: A/H1N1pdm09) were challenged intranasally with A/Narita/01/2009 virus and virus titers in the lungs and serum antibody titers were measured. Although each vaccination reduced the virus titer, the whole-virion vaccine was more effective in the reduction of virus titer than the split-virion vaccine. We investigated a possible difference in the antigen uptake process by macrophages between these vaccines. Mouse macrophage hybridoma clone 39 cells were co-cultured with Alexa-labeled whole- or split-virion vaccine and the localization of the vaccine antigens was analyzed. Both vaccine antigens were shown to be captured by the macrophages and were delivered to the MHC classII compartment. Further quantitative studies are underway to elucidate any difference in the antigen processing between both types of vaccines.

F.96. **Low Dose TNFα Enhances the Suppressive Function of Human Foxp3+ Regulatory T Cells**

Gilles Boschetti[1], Bernard Flourie[2], Stephane Nancey[1], Dominique Kaiserlian[1]. [1]INSERM U 851, LYON, France; [2]Lyon-Sud Hospital, Pierre Benite, France

IBD results from an imbalance between proinflammatory effector T cells (Teff) and regulatory T cells (Treg), which chiefly involves TNFα. We recently documented that a single anti-TNFα mAb infusion rapidly enhanced both the number and function of Foxp3+ Treg. To investigate whether reduced levels of TNFα could activate Treg, we tested the effect of TNFα on the phenotype and suppressive function of circulating Treg from healthy donors. CD4+CD25+CD127-Foxp3+ T cells (Treg), were cultured overnight with a low (0,01 ng/ml) or a high (1000 ng/ml) dose of TNFα,

corresponding to doses found in patients in remission and active disease, respectively or were stimulated with anti-CD3 mAb. FACS analysis showed that TNFRII expression was enhanced by activation with anti-CD3, decreased by TNFα at high dose and unaffected by low dose of TNFα. The suppressive function of Treg was enhanced or decreased by TNFα at low (0,01 ng/ml) and high (1000 ng/ml) concentrations, respectively. These data show that TNFα at low dose enhances the suppressive function of Treg without affecting TNFRII expression, while at dose it downregulated both TNFRII expression and Treg function. Thus, low circulating levels of TNFα might favor the anti-inflammatory properties of Treg in IBD.

F.97. Osteopontin-induced Immunomodulation is Enhanced in the Presence of RSV Infection and is Largely Influenced by NKT Cells

Mahboob Qureshi, Brooks Beal, Nagib Qureshi, Kainath Durre. Touro University Nevada College of Osteopathic Medicine, Henderson, NV

Childhood RSV infection is associated with an increased Th2 response and asthma later in life. Our studies demonstrated decreased osteopontin production and an increased NKT cell response in the RSV-infected neonatal lungs. This was associated with a skewed polarization of DC towards plasmacytoid DCs (pDC). The current study examined the immunomodulatory role of osteopontin in RSV-infected neonatal mice with or without NKT cell (CD1d-/-). Flowcytometry revealed a decreased NKT cell response to RSV infection in the neonatal lungs upon administration of exogenous osteopontin. In addition, the proportion of pDCs in the RSV-infected neonatal mice was considerably reduced following exogenous osteopontin administration and the effect of osteopontin was augmented in the presence of RSV infection. Further, the skewed polarization of DC towards pDC was considerably inhibited in the lungs of RSV-infected CD1d-/- neonates compared to that in the BALB/C neonates. A large number of macrophage recruitment was observed in the absence of NKT cells. This was associated with an enhanced activation of T lymphocytes and increased production of IFN-g in the lungs; suggesting an augmented Th1 response to exogenous osteopontin. Understanding the underlying mechanism of this finding would be instrumental in explaining the post- RSV infection associated susceptibility to asthma.

F.98. Bacteroides Fragilis Polysaccharide A Enhances T Regulatory Cell Population In Human PBMC Culture and Causes Differential Induction of IL-10 in Multiple Sclerosis Patients

Kiel Telesford[1], Javier Ochoa-Repáraz[1], Yan Wang[1], Daniel Mielcarz[1], Sakhina Begum-Haque[1], Dennis Kasper[2], Lloyd Kasper[1]. [1]Dartmouth Medical School, Lebanon, NH; [2]Harvard Medical School, Boston, MA

Polysaccharide A (PSA), a zwitterionic molecule found on the surface of human commensal bacteria Bacteroides fragilis, has been shown to elicit immune cell mediated anti-inflammatory responses in both animal models of multiple sclerosis (EAE) and inflammatory bowel disease (experimental colitis). Previously, we have demonstrated that periodic oral treatment with purified PSA (pPSA) in EAE conditioned mice was associated with an enhanced accumulation of a regulatory T cell (Treg)-inducing population of CD103+ dendritic cells and ameliorated clinical scores. Our current work shows that *in vitro* culture of human peripheral blood mononuclear cells (PBMC) with pPSA results in a pPSA dependent enhanced population of CD4+CD39+/-FoxP3+ Tregs and an increase of secreted IL-10. Interestingly, PBMCs from Relapsing Remitting Multiple sclerosis (RRMS) patients produced on average twice as much IL-10 when compared to cultures from healthy donors. The impact of this work is multifaceted. It provides insight to the unique biology underlying the host-commensal bacteria immunological dynamic. Additionally, given that CD39+ Tregs are diminished and functionally impaired in MS patients, our work has implications in further understanding immune responses under an MS paradigm. Finally, it describes a potentially novel therapeutic agent for multiple sclerosis.

F.99. IL-10 Expression Marks Functionally Distinct FoxP3 Subsets in the Lamina Propria of Mice

Korynn Stoyanoff, Arthur Blum, Long Hang, Tommy Setiawan, John Leung, Joel Weinstock. Tufts University, Boston, MA

Introduction: The gut requires a strict level of immune regulation to prevent undesirable inflammation. In mice, infection with the murine intestinal helminth H. polygyrus abrogates inflammation. The lamina propia (Lp) of mice contains FoxP3+ regulatory T cells. We are investigating how infection with H. polygyrus affects the regulatory capacity of two FoxP3 subsets: FoxP3+IL-10+ and FoxP3+IL-10- cells. Methods: Colon Lp cells were isolated from FoxP3-mRFP, IL-10-EGFP double reporter mice and analyzed via flowcytometry or sorted into FoxP3 subsets and used in proliferation or cytokine inhibition assays as a measure of regulatory capacity. Results: The colon Lp contains both FoxP3 subsets with FoxP3+IL-10+ cells predominating by approximately two fold. Both subsets are increased following H. polygyrus infection. FoxP3+IL-10- cells inhibit proliferation and inflammatory cytokine production by effector cells *in vitro* while FoxP3+IL-10+ cells inhibit cytokine production. Conclusions: Both FoxP3 subsets are important in regulating effector cell function. The predominance of FoxP3+IL-10+ T cells in the colon and their requirement for regulation of inflammation suggests a unique role that may be enhanced following infection with H. polygyrus. We are currently investigating differences in inflammatory proteins between the two subsets by using PCR arrays.

F.100. Natural Killer Cells in Upper Respiratory Tract

Kazunari Okada, Shintaro Sato, Ayuko Sato-Kimura, Il Gyu Kong, Hiroshi Kiyono. University of Tokyo, Minato-ku, Japan

Natural killer (NK) cells are known to play critical roles in host defense, for their anti-viral and anti-tumor function. Although NK cells in systemic immunity are well investigated, characteristics of mucosal NK cells are remained to be elucidated. Here we would like to present details about NK cells in upper respiratory tract, which are involved in regulation of respiratory infections.

F.101. Host-pathogen Interactions with Streptococcus Pneumoniae During Colonization and Infection in an Elderly Mouse Model
Cassandra Krone, Tomasz Zborowski, Elizabeth Sanders, Krzysztof Trzcinski, Debby Bogaert. UMC Utrecht, Utrecht, Netherlands

Introduction: Nasopharyngeal colonization with Streptococcus pneumoniae is usually asymptomatic, though an important prerequisite for disease. Elderly are especially at increased risk for severe infections. Aim: To study potential mechanisms of increased risk of pneumococcal infection in an elderly mouse model. Methods: Female C57Bl/6 mice aged 18-23 months (elderly) and 3-4 months (controls) were intranasally inoculated with serotype 6B pneumococci. We determined density and duration of colonization twice a week for 28 days. In addition, we studied dynamics of immune cells infiltrating into the nasopharynx by Cyto-spin/Diff-quick staining and flow cytometry. Results: Although there was no significant difference in colonization density between elderly and young mice at day 3, elderly were colonized significantly longer. We observed greater immune cell numbers in the nasopharynx of elderly before colonization plus a larger increase in cell influx in the days post-inoculation compared to controls. In controls we observed a significant influx of Gr-1^{int}CD11b^{+}F4/80^{+} monocytes at day 7, strongly correlating with clearance of bacteria. These dynamics were less apparent in elderly mice. Conclusion: Elderly mice were not able to clear pneumococcal colonization as quickly as controls. Whether this process is causally related to the observed dynamics in Gr-1^{int}CD11b^{+}F4/80^{+} monocytes needs further study.

F.102. Investigation of Vaccinia Virus Induced Lung Pathogenesis *in vivo*
Wen Chang[1], Shu-Jung Chang[2], Siti Khadljah Kasani[1], Chung-Tiang Liang[3]. [1]Academia Sinica, Taipei, Taiwan; [2]National Yang-Ming University, Taipei, Taiwan; [3]National Laboratory Animal Center, Taipei, Taiwan

Vaccinia virus is a large DNA virus that infects many cell culture *in vitro* and animal species *in vivo*. Although the virus has been used widely as a vaccine, the cell entry pathways are complex in nature and varied among different virus strains and cell types. Recently, we showed that vaccinia A25/A26 proteins are determinants for virus entry into HeLa cells through endocytosis whereas virus mutants deleting these two proteins entered HeLa cells through plasma membrane fusion. Here we investigated *in vivo* pathogenesis caused by wild type and mutant vaccinia viruses in Balb/c mice infected through intranasal infections. Mice were sacrificed and virus titers were determined in multiple organs. In addition, IHC and viral Ag staining were performed and our preliminary data showed a different pathology in the respiratory organs of mice infected by wild type or mutant viruses.

F.103. The Nose-associated-lymphoid-tissue (NALT): Comparison Between Mouse, Rat and Man
Reinhard Pabst. Medical School Hannover, Hannover, Germany

In rodents there are two bell-shaped accumulations of lymphoid tissues on the dorsal flow of the nose, which develops after birth. Danger signals or infections induce the development of germinal centres. There are high endothelial venules with a unique expression pattern of adhesion molecules. The lymphocyte subset composition differs from that of Peyer's patches, spleen or blood. In man, however, NALT can be found in different parts of the nose and is not restricted to one specific site. Diffusely localised immune cells in the nose should not be called NALT as it is not a tissue. NALT can be used as an entry site for vaccination but the species differences have to be considered, when extrapolations are done. The tonsils should not be included as part of NALT as they are not localised in the nose. Rodents have no tonsils so that NALT might particularly have the role of tonsils in these species.

F.104. The Architecture of Palatine Tonsils of the Buffalo (Bos Bubalus)
Mohamed Zidan[1], Reinhard Pabst[2]. [1]Alexandria University, Edfina, Egypt; [2]Medical School Hannover, Hannover, Germany

The palatine tonsils play a key role in initiating immune responses against ingested and inhaled antigens. They are also replication sites of some pathogens. There is no data available on the structure of the palatine tonsils of the Egyptian water buffalo. Therefore, palatine tonsils of 14 clinically healthy buffalo bulls (2-3 years old) were examined macroscopically and microscopically using light and transmission electron microscopes. The tonsils had an elongated kidney shape with a central invagination (tonsillar fossa) containing a single macroscopic opening leading to a small central cavity (tonsillar sinus). A number of macroscopic crypts originated from this sinus (internal crypts). Besides the tonsillar fossa, also small macroscopic crypts (external crypts) were present. The tonsils were enclosed by a thin connective tissue capsule and septa divided the tonsils into incomplete lobes. Within these encapsulated organs mucous glands were very obvious. Each crypt was highly branched and lined with stratified squamous non-keratinized epithelium. Several lymphoid cells infiltrated in the epithelial cells forming patches of lymphoepithelium. The crypt lumen contained lymphocytes, neutrophils and erythrocytes. Lymph nodules with clear germinal centers extended under the epithelial surface. Diffusely distributed lymphocytes were found in the narrow interfollicular region. High endothelial venules, interdigitating dendritic cells, macrophages and plasma cells were observed among the diffuse lymphocytes. Lymphatics filled with lymphocytes drained the tonsils.

F.105. Dextran Sulfate Sodium Induced Alveolar Bone Loss and Decreased Zonula Occludens-1 Expression in Experimental Mouse Model of Periodontitis
Yun sik Choi, Yong chul Kim, Youngnim Choi. Seoul National University, Seoul, Republic of Korea

Periodontitis is a chronic inflammatory disease caused by the interaction of periodontal pathogens such as Porphyromonas gingivalis and host cells,

which leads to alveolar bone destruction. The pathogenesis of periodontitis involves the invasion of periodontal pathogens into gingival tissue. We hypothesized that damage in physical barrier function of gingival epithelia may contribute to the development of periodontitis. To test it, the effect of dextran sulfate sodium (DSS) on alveolar bone loss and the expression level of a tight junction protein zonula occludens-1 (ZO-1) was examined in the mouse model of experimental periodontitis induced by P. gingivalis. Four groups of mice received PBS application, gingival application of 5% DSS, oral infection with P. gingivalis, or the combination of DSS application and P. gingivalis infection. At 42 days post-treatment, alveolar bones and gingival tissues were evaluated. Compared with PBS group, all three experimental groups increased alveolar bone loss and inflammatory infiltratrates but decreased the level of ZO-1 expression. A synergism between DSS and P. gingivalis was not observed. These data suggest that DSS-induced undermining of physical barrier can induce periodontitis in the absence of periodontal pathogens. In conclusion, intact physical barrier is critical in the maintenance of periodontal health.

F.106. An NF-κB-independent and Erk1/2-dependent Mechanism Controls CXCL8/IL-8 Responses of Airway Epithelial Cells to Cadmium

Estelle Cormet-Boyaka, Jessica Rennolds, Kalyn Jolivette, Fatemat Hassan, Payal Mehta, Susheela Tridandapani, Jeanette Webster-Marketon, Prosper Boyaka. Ohio State University, Columbus, OH

Inhalation of the environmental pollutant cadmium has been linked to the development of lung diseases characterized by chronic inflammation, including chronic obstructive pulmonary disease. To address the role of airway epithelial cells in cadmium-induced lung inflammation, we investigated how cadmium regulates secretion of IL-8 by airway epithelial cells. We found that exposure of human airway epithelial cells to sub-toxic doses of cadmium *in vitro* promotes a characteristic cytokine response consisting of IL-8, but not IL-1β or TNFα. Intranasal delivery of cadmium increases lung levels of the murine IL-8 homologs MIP-2 and KC and results in an influx of polymorphonuclear cells (PMNs) into the lung. Inhibition of the NF-κB pathway had no effect on cadmium-induced IL-8 secretion by human airway epithelial cells, suggesting that IL-8 production was mediated through an NF-κB-independent pathway. Cadmium could activate the main MAPKs (i.e., p38, JNK and Erk1/2) in human airway epithelial cells. However, only pharmacological inhibition of Erk1/2 pathway or knock-down of the expression of Erk1 and 2 using siRNAs suppressed secretion of IL-8 induced by cadmium. Our findings identify cadmium as a potent activator of IL-8 in lung epithelial cells and reveal the role of an NF-κB-independent, but Erk1/2-dependent pathway in cadmium-induced lung inflammation.

F.107. Protection of Pigs Against Genital Chlamydia Trachomatis Challenge by Parenteral or Mucosal DNA Immunization

Daisy Vanrompay, Katelijn Schautteet, Evelien De Clercq, Eric Cox. Ghent University, Ghent, Belgium

We have evaluated protection against a genital Chlamydia trachomatis infection in a pig challenge model. Animals were immunized with a major outer membrane protein (MOMP)-based DNA vaccine carrying five pig-specific CpG motifs incorporated in the plasmid backbone (pWRG7079). Protection was promoted by administrating the porcine granulocyte macrophage-colony stimulating factor (GM-CSF) and the Escherichia coli thermo-labile enterotoxin LT, which is an exceptionally potent mucosa-binding molecule, as adjuvants. Protection against genital C. trachomatis challenge through mucosal immunization (combined vaginal and nasal vaccine administration) was compared to systemic (intradermal) immunization. Mucosal vaccination resulted in significant protection characterized by less severe macroscopic lesions, less vaginal shedding of chlamydia and less bacterial replication in the urogenital tract of immunized pigs. Also, mucosal immunized pigs showed significantly higher proliferative responses of peripheral blood lymphocytes. Furthermore, the combination of nasal and vaginal immunization could induce serum antibody titers upon immunization and early upon challenge with C. trachomatis serovar E. However, the infection could not be eradicated. Systemic immunization was significantly less efficient at eliciting protection, which emphasizes the need for a mucosal vaccine in order to obtain significant protection against genital C. trachomatis infection.

F.108. A Qualified Approach to Measuring T Cell Responses in Parallel Blood and Gut Samples for Monitoring HIV Vaccine Clinical Trials

Hannah Cheeseman, Jakub Kopycinski, Harry Kaltsidis, Ambreen Ashraf, Michelle Cashin-Cox, Philip Bergin, Aggeliki Spentzou, Peter Hayes, Peter Kelleher, Jill Gilmour, Dilbinder Gill. Imperial College London, London, United Kingdom

Background: Measurement of HIV vaccine-induced T cell responses has largely been limited to cells isolated from PBMC. Though readily accessible, these may not represent locally occurring responses at mucosal surfaces where T cells are required to control virus infection. Methods: We describe the optimisation and qualification of polychromatic flow cytometry applied to T cells isolated from the gut of HIV +/- volunteers. Internal quality controls were applied and monitored over time to generate robust and reliable data. Results: Data showed good reproducibility, specificity and concordant inter- and intra-operator CVs. Specificity for HIV-specific CD8+ T cell responses (CD107a, IFN-γ & TNF-α) in HIV- donors varied between PBMC and gut. CD8+ T cell responses to Gag and Nef in HIV+ donors did not correlate between compartments. However, responses to the FEC peptide pool were equivalent between compartments in both HIV +/- donors. Discussion: Gut-derived HIV-specific CD8+ T cell responses may play an important role in the assessment of vaccine immunogenicity. We demonstrate both qualitative and quantitative differences in responses to HIV between PBMC and gut, indicating that complete assessment of vaccine-induced cellular responses requires mucosal sampling. Assessing mucosal T cell responses at baseline and post-vaccination in phase I trials will facilitate the prioritisation of promising vaccine candidates in future trials.

F.109. Nanogel Based Adjuvant-free Pneumococcal Nasal Vaccine Induces Protective Immunity Against Pneumococcus

Ilgyu Kong[1], Yoshikazu Yuki[1], Ayuko Sato-Kimura[1], Tomonori Nochi[1], Haruko Takahashi[2], Shinichi Sawada[2], Mio Mejima[1], Shiho Kurokawa[1], Daisuke Tokuhara[1], Kazunari Okada[1], Shintaro Sato[1], David Briles[3], Kazunari Akiyoshi[2], Hiroshi Kiyono[1]. [1]University of Tokyo, Tokyo, Japan; [2]Tokyo Medical and Dental University, Tokyo, Japan; [3]University of Alabama at Birmingham, Birmingham, AL

To establish safe and effective mucosal vaccine, we developed adjuvant-free nasal vaccine-delivery system with a nanometer-sized hydrogel('nanogel') which consists of a cationic type of cholesteryl group-bearing pullulan(cCHP). To test a potential of nanogel to be used as a nasal carrier of vaccines against respiratory pathogens, nanogel-based nasal pneumococcal vaccination system was established. Pneumococcal surface protein A(PspA) was incorporated in nanogel because PspA is relatively well-conserved antigen throughout all pneumococcal serotypes compared to the polysaccharide. Nanogel-based PspA nasal vaccination induced high levels of antigen-specific serum IgG, and nasal and bronchial secretory IgA (SIgA) antibodies. The antigen-specific immune response induced by nanogel-based PspA nasal vaccination was protective against the lethal challenge with the Streptococcus pneumoniae Xen10. Nanogel-PspA vaccinated group thus had less numbers of pneumoccus on the surface of the bronchial mucosa, and perfectly protected from the pneumococcal invasion of the lung parenchyme. Moreover, nanogel-PspA did not accumulate in CNS or olfactory bulbs. These results demonstrate the effectiveness and safeness of the nanogel nasal vaccine system as an adjuvant free universal mucosal vaccine against the respiratory pathogen.

F.110. Intranasal Immunization with Recombinant Pneumococcal Surface Protein A Fused with Vibrio Vulnificus Flagellin Protein FlaB Enhances Cross-protective Immunity Against Streptococcus Pneumoniae Infection in Mice

Chung Truong Nguyen[1], Soo Young Kim[1], Young Ran Kim[2], Myoung Suk Kim[1], Shee Eun Lee[2], Joon Haeng Rhee[1]. [1]Chonnam National University, Gwangju, Republic of Korea; [2]Dongshin University, Naju, Republic of Korea

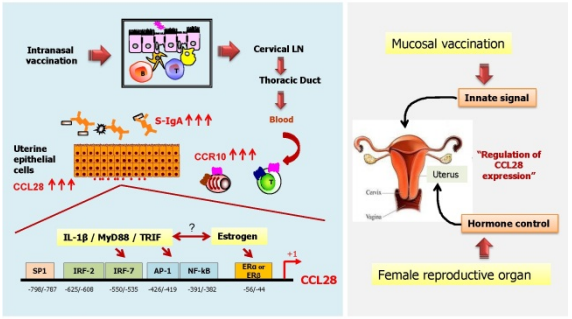
Pneumococcal surface protein A (PspA), a highly immunogenic surface protein produced by all strains of Streptococcus pneumoniae, can elicit protective immunity against fatal pneumococcal infection. We have previously demonstrated that the Vibrio vulnificus FlaB, a bacterial flagellin protein and agonist of TLR5, has strong mucosal adjuvant activity and induces protective immunity. In this study, we have tested whether intranasal immunization with recombinant fusion proteins consisted of PspA and FlaB (PspA-FlaB and FlaB-PspA) are able to elicit more efficient protective mucosal immune responses against pneumococcal infection than immunization with PspA alone or with a mixture of PspA and FlaB. When mice were intranasally immunized with fusion proteins, significantly higher levels of anti-PspA IgG and IgA were induced in serum and mucosal secretions. The mice intranasally immunized with the FlaB-PspA fusion protein were the most protected against a lethal challenge with S. pneumoniae, as compared with the mice immunized with PspA only, a mixture of PspA and FlaB, or the PspA-FlaB fusion protein. FlaB-PspA also induced cross protection against heterologous capsular types. These results suggest that a FlaB-PspA fusion protein alone could be used as an anti-pneumococcal mucosal vaccine or as an effective partner protein for multivalent capsular polysaccharide conjugate vaccines.

F.111. MEC/CCL28 Expression in the Uterus Attracts CCR10+ IgA Plasma Cells Following Mucosal Vaccination via Estrogen Control

Hye-Ran Cha[1], Hyun-Jeong Ko[3], Eun-Do Kim[1], Sun-Young Chang[1], Sang-Uk Seo[1], Nicolas Cuburu[1], Sangryeol Ryu[2], Sunghoon Kim[4], Mi-Na Kweon[1]. [1]International Vaccine Institute, Seoul, Republic of Korea; [2]Seoul National University, Seoul, Republic of Korea; [3]Kangwon National University, Chuncheon, Republic of Korea; [4]Yonsei University College of Medicine, Seoul, Republic of Korea

Previous studies demonstrated the existence of cross-talk between mucosal and reproductive organs during secretory IgA antibody induction. In this study, we aimed to clarify the underlying mechanisms of this cross-talk. We found significantly higher titers of antigen-specific SIgA Ab in the vaginal wash after mucosal vaccination by both the intranasal or intravaginal routes but not by the subcutaneous route. Interestingly, antigen-specific IgA Ab-secreting cells were mainly found in the uterus but not in the cervix and vaginal canal following i.n. vaccination. The fact that the majority of antigen-specific IgA ASCs isolated from the uterus of vaccinated mice migrated toward MEC/CCL28 suggests dominant expression of CCR10 on the IgA ASCs. Further, IgA ASCs in the uterus of vaccinated mice were drastically reduced in mice treated with neutralizing anti-MEC/CCL28 Ab. Most intriguingly, the female sex hormone estrogen directly regulated MEC/CCL28 expression and was augmented by i.n. vaccination with cholera toxin or stimulators for innate immunity. Further, blockage of estrogen function in the uterus by oral administration of Raloxifen significantly inhibited the migration of antigen-specific IgA ASCs following i.n. vaccination with ovalbumin plus CT. Taken together, these data strongly suggest that CCR10+ IgA ASCs induced by mucosal vaccination via the i.n. route migrate into the uterus in a MEC/CCL28-dependent manner and that estrogen might play a crucial role for protection against genital infection by regulating MEC/CCL28 expression in the uterus.

SUMMARY



F.112. Novel Mimotope Peptides as Anti-chlamydial Vaccine Candidates

Judith Whittum-Hudson, Mirabela Hali, Indrajit Sinha, Balaji Bharatwaj, Cynthia Oszust, Alan Hudson, Sandro da Rocha. Wayne State University, Detroit, MI

There currently is no protective anti-chlamydial vaccine. We identified novel genus-specific mimotope peptides by phage display (mAb1 anti-GLXA) and cloning of H-chain CDR from our protective mAb2 anti-idiotype vaccine. Blast searches showed no homology to known chlamydial, mouse, or human entities. Four peptides were tested *in vivo*. Initially BALB/c mice (n=4-5/grp) received three immunizations of 100 µg SC in CFA/IFA. All peptides (Pep) induced specific Ab (titers 1:80-160) prior to vaginal challenge with C trachomatis (UW-31; K). DFA on vaginal swabs indicated 3 peptides reduced shedding. Anti-Pep Ab recognized chlamydial organism, inclusion membranes and matrix, and the aberrant reticulate bodies of persistent infection. IgG isotyping showed both Th1 and Th2 responses. When delivered SC or perorally in PLA microparticles, positive Ab were induced by 10-40 µg Pep. Recognition of C pneumoniae and C trachomatis by anti-Pep antibodies supports the genus-specificity of these vaccine candidates. Importantly, sera from human patients with documented prior chlamydial infection(s) recognized all four peptides in ELISA and support the relevance of these antigens to human chlamydial infection. The transition from a nonphysiologic delivery of peptide in CFA/IFA to encapsulated peptide for SC or oral delivery is further proof of principle that these are viable vaccine candidates.

F.113. Use of a Non-toxic Mucosal Adjuvant to Generate a Protective Response Against Chlamydia

Connor O'Meara[1], Nils Lycke[2], Peter Timms[1], Kenneth Beagley[1]. [1]Queensland University of Technology, Brisbane, QLD, Australia; [2]University of Goteborg, Stockholm, Sweden

C. trachomatis is the most common cause of bacterial STI worldwide and vaccination is recognized to have the greatest potential to impact on rates of infection and disease prevalence. There are a number of experimental vaccines exhibiting promising results in animal models; however, most contain toxic adjuvants considered unsafe for human use. This study compared CTA1-DD, proven safe in primates, to the toxic but protective CT/CpG, by combining either adjuvant with the chlamydial MOMP antigen and delivering via the “needle-free” intranasal, sublingual or transcutaneous routes. CT/CpG consistently induced a significantly greater systemic response, however little difference in cell-mediated or humoral response between the two adjuvants could be measured at the mucosal level. Although intranasal delivery of each adjuvant was overall the superior route for targeting the reproductive tract, transcutaneous and sublingual immunization mounted either comparable systemic antibody or T cell memory in the draining lymph nodes, respectively. Both adjuvants elicited partial protection against a C. muridarum genital tract infection, but also and most importantly minimized the adverse upper reproductive tract pathology associated with ascending infection. CTA1-DD may therefore make a fitting and safe alternate to CT/CpG in a human prophylactic chlamydial vaccine, that could be delivered by needle-free methods.

F.114. Immunogenicity of Swine Rotavirus Protein (VP7) Expressed on the Surface of Lactobacillus Casei

Jong-Soo Lee[2], Eun-Ha Kim[1], Moon-Hee Sung[3], Haryoung Poo[1], Chul-Joong Kim[2]. [1]KRIBB, Daejeon, Republic of Korea; [2]Chungnam National University, Daejeon, Republic of Korea; [3]BioLeaders Corporation, Daejeon, Republic of Korea

Group A rotaviruses are the major cause of acute gastroenteritis in young children and in a wide variety of domestic animals. The outer capsid protein of rotaviruses, VP7, is the major neutralization antigen. For safe and effective delivery of viral antigens to the mucosal immune system, a novel surface antigen display system was developed with lactic acid bacteria; it uses the poly-γ-glutamic acid synthetase A protein (PgsA) of Bacillus subtilis as the anchoring matrix. The recombinant fusion protein comprised PgsA and VP7 and was stably expressed in Lactobacillus casei. Surface localization of the fusion protein was verified by cellular fractionation analyses. Oral and intranasal inoculations of mice with the recombinant L. casei elicited high levels of serum immunoglobulin G (IgG) and mucosal IgA, as demonstrated by enzyme-linked immunosorbent assay (ELISA). A neutralization assay confirmed that the antibodies induced against the VP7 protein prevented rotaviral infection. These results indicate that recombinant L. casei that expresses the rotavirus protein VP7 on its surface can be used to develop vaccines against rotavirus-induced diarrhea in infants. This work was supported by the 2010 research fund of Chungnam National University in Republic of Korea.

F.115. Nasal Dendritic Cell Targeting Flt3 Ligand as a Safe Adjuvant Elicits Effective Protection Against Fatal Pneumococcal Pneumonia

Kosuke Kataoka[1], Keita Oma[3], Yoshiko Fukuyama[2], Susan Hollingshead[2], Shinichi Sekine[3], Shigetada Kawabata[3], David Briles[2], Kazunori Oishi[3], Kohtaro Fujihashi[2]. [1]University of Tokushima,Tokushima, Japan; [2]University of Alabama at Birmingham, Birmingham, AL; [3]Osaka University, Suita, Japan

We have previously showed that pneumococcal surface protein A (PspA)-based vaccine containing DNA plasmid encoding the Flt3 ligand (FL) gene (pFL) as nasal adjuvant prevented nasal carriage of Streptococcus pneumoniae (Fukuyama Y. et al. J. Immunol. 2010). In this study, we further investigated the safety and efficacy of this nasal vaccine for the induction of PspA-specific secretory-IgA (S-IgA) and IgG antibody (Ab) responses against lung infection with S. pneumoniae. In order to examine initially dynamic translocation of pFL, C57BL/6 mice were nasally administered with pFL. pFL was taken-up by nasal dendritic cells and epithelial cells, but not in the central nervous systems including olfactory nerve and epithelium. Of importance, nasal pFL induced FL protein synthesis with minimum levels of inflammatory cytokines in the nasal washes and bronchoalveolar lavage fluids. In addition, one week after mice were nasally immunized with recombinant PspA/Rx1 (rPspA) plus pFL three times at weekly intervals, rPspA-specific Abs induced effectively inhibited bacterial growth in airway secretions and blood of mice challenged nasally with S. pneumoniae WU2.

Our findings show that nasal pFL is a safe and effective mucosal adjuvant for the enhancement of bacterial Ag (rPspA)-specific protective immunity.

F.116. An Efficient Rice-based Expression System Producing Nasally Immunogenic Nontoxic Subunit Fragment of Clostridium Botulinum Type-A Neutrotoxin

Yoshikazu Yuki[1], Mio Mejima[1], Masaharu Kuroda[2], Shiho Kurokawa[1], Tomoko Hiroiwa[1], Il Gyu Kong[1], Yuko Takahashi[1], Yingju Chen[1], Tomonori Nochi[1], Daisuke Tokuhara[1], Tomoko Kohda[3], Shunji Kozaki[3], Hiroshi Kiyono[1]. [1]University of Tokyo, Tokyo, Japan; [2]National Agriculture Research Center, Niigata, Japan; [3]Osaka Prefecture University, Osaka, Japan

Rice-based vaccines (MucoRice) offer a highly practical and cost-effective strategy for mucosal vaccinating large populations against mucosal infections. However, it needs to be overcome low yield of expression of vaccine antigens with high-molecular weight. Here we introduced the RNAi technology to advance the MucoRice system by co-introduction of antisense genes specific for endogenous rice storage proteins to minimize their expression for the expansion of space of inserted vaccine antigen accumulation in rice seeds. When used a combination of RNAi suppression of major rice endogenous storage proteins, prolamin 13 kDa and glutelin A in a T-DNA vector, we could successfully express a 45kDa-C-terminal half domain (BoHc) of botulinum Type A neurotoxin vaccine, an average of 200 microgram per seed. The MucoRice-BoHc was water soluble and expressed in cytoplasm but not in protein body I and II of rice seeds. When immunized with purified MucoRice-BoHc intranasally with or without cholera toxin as a mucosal adjuvant, MucoRice-BoHc induced protective immunity against challenging with botulinum Type A neurotoxin in mice. These findings showed that the advanced MucoRice could use production of mucosal vaccine antigens with high-molecular weight.

F.117. Use of Secretory IgA to Deliver HIV Antigen in the Intestinal Mucosa

Nicolas Rochereau[1], Charlotte Primard[1], Vincent Pavot[1], Bernard Verrier[1], Blaise Corthésy[2], Christian Genin[1], Stéphane Paul[1]. [1]Université de Lyon, St. Etienne, France; [2]CHUV de Lausanne, Lausanne, Switzerland

The secretory IgA are important players in the humoral immune response at the mucosal surface level. The IgA2 have the very interesting feature to be able to cross the intestinal mucosa in both directions (through M cells of Peyer's patches) by transcytosis. We want to use these important properties of IgA2 in a vaccine approach to deliver a vaccine antigen of HIV in the GALT (Gut Associated Lymphoid Tissue) after mucosal administration. In this work, we describe first the important role of glycosylation in the interaction between human secretory IgA and M cells in a specific *in vitro* intestinal human model. Briefly, this model is based on the coculture between Caco2 (human intestinal cells) and Raji (B cells lymphoma).We tried also to identify the domain of IgA2 involved in the reverse transcytosis, and if its transcytose is IgA2 restricted. Then, we have modelised the *in vivo* transport of IgA in the intestinal mucosa in an *in vivo* ligated loop model. Using specific fluorescent vehicles as nanoparticles, we have described an important network of antigen presenting cells which could uptake specific candidate for mucosal vaccination. We think that such type of vaccines could be use as prophylactic approach to deliver Nabs inducing immunogen as the GP41 glycoprotein.

F.118 Evaluation of Shigella Sonnei Live Vaccine Candidates in the Gnotobiotic Piglet Model

Shoshana Barnoy[1], Malabi Venkatesan[1], Saul Tzipori[2], Kwang-il Jeong[2]. [1]Walter Reed Army Institute of Research, Silver Spring, MD; [2]Tufts University Cummings School of Veterinary Medicine, Boston, MA

Gnotobiotic (Gb) piglets provide an important animal model for evaluating Shigella strains. In humans, Shigella is transmitted orally and results in an acute inflammatory disease leading to diarrhea and/or dysentery. Gb piglets administered virulent Shigella orally, also produce diarrhea with significant constitutional and gastrointestinal pathology. Gb piglets were administered S. sonnei strain Moseley, Moseley-2DShET2 and vaccine candidates WRSS1 and WRSs3 which lack VirG(IcsA) and cannot spread intercellularly. WRSs3 further lacks senA, senB, and msbB2. The Gb piglets were monitored daily up to several post inoculation days (PID). Moseley caused severe diarrhea at PID2 to PID4, marked mucosal damage in the gastrointestinal (GI) tissues at PID1 to PID3 and elicited predominantly proinflammatory cytokines IL-8 and IL-6 early at PID1 to PID3, and Th1 cytokine IL-12 at PID3 to PID8. Like Moseley, WRSS1 and WRSs3 robustly colonized the GI tract, however, WRSs3 strains developed significantly milder clinical and histopathological indexes, and moderate cytokine responses. WRSs3 was rarely detected in organs outside the GI tract. Present results support the Gb piglet model to be a sensitive in vivo model for evaluating different S. sonnei strains, enabling identification of potential live vaccine candidates. Funded by NIAID, NIH, Department of HHS, under Contract Number N01-AI-30050.

F.119. Evaluation of Chlamydial Vaccine-induced Immunity and Pathology in Wild Koalas (Phascolarctos Cinereus)

Avinash Kollipara[1], Carmel George[1], Martin Cheung[1], Adam Polkinghorne[1], Jon Hanger[2], Jo Loader[3], Peter Timms[1], Kenneth Beagley[1]. [1]Queensland University of Technology, Brisbane QLD, Australia; [2]Endeavour Veterinary Ecology, Brisbane, QLD, Australia; [3]Australia Zoo, Brisbane, QLD, Australia

Chlamydial infections are a major threat to the Koala with many wild populations on the verge of extinction due partly to this disease. Chlamydial infections in koalas cause keratoconjunctivitis, respiratory tract disease (pneumonia, pharyngitis and bronchitis) and uro-genital infections causing incontinence (wet bottom) and infertility. An effective vaccine is desperately needed to protect koalas. We report here preliminary results of trials of an experimental vaccine, the first of it's kind to be used in naturally-infected wild koalas. Koalas (healthy Chlamydia-free and recovered from chlamydial disease) were immunized with recombinant chlamydial MOMP and NrdB mixed with Immunostimulating Complex (ISC) adjuvant to

answer three basic questions: (1) Does a previous chlamydial infection affect vaccine-induced antibody responses? (2) Does a previous chlamydial infection affect vaccine-induced CMI? and (3) Does vaccination of animals cured of a natural infection by antibiotics result in increased clinical disease?Preliminary results from 22 infected/recovered koalas demonstrated comparable systemic IgG responses to those seen in immunized healthy animals, however there was considerable variation in recall T cell proliferative responses. Most importantly, to date we have seen no signs of worsening of disease in immunized animals, whereas increased inflammatory pathology was observed in some animals in the adjuvant-only placebo group.

F.120. Oral Rice-based Vaccine Induces Active and Passive Immunity Against Enterotoxigenic E. Coli-mediated Diarrhea in Pigs

Natsumi Takeyama[1], Daisuke Tokuhara[2], Kazuki Oroku[1], Mio Mejima[2], Shiho Kurokawa[2], Yuko Takahashi[2], Masaharu Kuroda[3], Tomoko Hiroiwa[2], Yingju Chen[2], Aya Chubachi[2], Tomonori Nochi[2], Takehiro Masumura[4], Kunisuke Tanaka[4], Toshiaki Kodama[1], Shinya Nagai[5], Tetsuo Nunoya[1], Hiroshi Kiyono[2], Yoshikazu Yuki[2]. [1]Nippon Institute for Biological Science, Ome, Japan; [2]University of Tokyo, Tokyo, Japan; [3]National Agriculture Research Center, Niigata, Japan; [4]Kyoto Prefectural University, Kyoto, Japan; [5]Nisseiken Co. Ltd., Ome, Japan

Enterotoxigenic Escherichia coli (ETEC) causes severe diarrhea in both neonatal and weaned pigs. Heat-labile toxin (LT) produced by ETEC induces water outflux in pig small intestine. Because cholera toxin B subunit (CTB) has 78% amino acid homology to LT B-subunit, we selected MucoRice-CTB as a vaccine candidate against ETEC-induced diarrhea. When orally immunized with MucoRice-CTB containing 1mg of CTB for a total of three or four times at 2-week intervals, 2-month-old weaned minipigs or pregnant sows induced antigen-specific mucosal IgA as well as systemic IgG and IgA. Moreover, a higher amount of antigen-specific IgG and IgA were secreted in colostrums of the vaccinated sows compared to those in sera and they were passively transferred to sera of suckling piglets. All antibodies tested also cross-reacted perfectly with LTB. To evaluate protection of diarrhea by MucoRice-CTB, an intestinal loop assay was tested. As a result, the fluid volume accumulated in the loops of minipigs immunized with MucoRice-CTB was significantly lower than that of control minipigs, indicating that MucoRice-CTB can protect pigs from diarrhea caused by ETEC. Our results showed that MucoRice-CTB could be an excellent candidate of oral vaccine to induce both active and passive immunity against ETEC diarrhea in pigs.

F.121. Development of Monoclonal Antibodies Neutralizing IgA Specific of the C-terminal Region of gp41 of HIV

Fahd Benjelloun[1], Reham Dawood[1], Nadine Vincent[1], Zeliha Oruc[2], Michel Cogné[2], Christian Genin[1], Stéphane Paul[1]. [1]Universite de Lyon, St. Etienne, France; [2]University of Limoges, Limoges, France

Background: The C-terminal region of the envelope glycoprotein 41 of HIV represents an important target for the development of HIV vaccine. This region contains three major domains (MPER, TM and Kennedy epitope) involved in the anchoring and the fusion of the virus to its target cells. MPER contains the epitopes of the three broadly neutralizing monoclonal antibodies (NAbs) (2F5, 4E10 and Z13e) used in passive serotherapy approaches. In most animal model, studies indicate that high serum neutralizing concentrations of NAbs are required to provide significant benefit in protection experiments. In this regard, one of the important challenges on the HIV vaccine is to induce broadly NAbs at the mucosal site. Methods/Results: The aim of this work is to construct new conformational tools to identify new potent mucosal-specific epitopes and produce therapeutic mucosal Nabs which could be used as prophylactic or therapeutic molecules. In our study, we have constructed human cell lines expressing the entire gp41 membrane-bound molecule or more specifically the MPER domain. This new tools have been used to screen specific IgA responses in parotid saliva or lactosera of HIV infected patients. Some of the cells lines are also currently used as immunogen for the production of chimeric human IgA1 in a specific transgenic humanized mouse model. We show that mucosal compartments contains IgA wich recognize conformational and not linear epitopes. Conclusions: We think that the use of conformational epitopes as immunogen could improve drastically the efficacy of IgA Nabs.

F.122. Indispensableness of Vaccine-delivery to Peyer's Patch M Cells and Villous M Cells for the Induction of the Antigen-specific Immune Response

Gemilson Soares Pontes[1], Tomonori Nochi[2], Haruyo Nakajima-Adachi[1], Yosuke Kurashima[1], Jun Kunisawa[1], Yoshikazu Yuki[1], Hiroshi Kiyono[1]. [1]University of Tokyo, Tokyo, Japan; [2]University of North Carolina, Chapel Hill, NC

The most benefit of using the mucosal vaccine is due to the fact that antigen-specific immune responses were induced in the mucosal and systemic compartments. In this study, by taking advantage of our previously established monoclonal antibody (NKM 16-2-4) that recognizes specifically murine M cells located in the epithelium of both Peyer's patches (PP M cells) and intestinal villi (villous M cells), via the recognition of M cell specific carbohydrate moiety containing α (1,2) fucose, we orally immunized PP-, MLN-, and PP and MLN double-deficient, and WT mice with Tetanus toxoid (TT) conjugated with NKM 16-2-4 (NKM 16-2-4-TT) or control antibody (Rat IgG-TT). High levels of TT-specific serum IgG antibody responses were induced in all animals by oral administration with NKM 16-2-4-TT but not Rat IgG-TT. However, TT-specific intestinal IgA antibody responses were not induced in the PP- and double- deficient mice. Low levels of antigen-specific response were induced in the MLN-lacking mice compared to WT mice. These results highlight the importance of vaccine-delivery to PP and villous M cells in promoting the induction of antigen-specific mucosal IgA immune responses. Taken all together, the antigen-delivery to both M cells might be important strategy for generation of antigen-specific mucosal immunity.

F.123. Effect of Using Multiple TLR Ligands in a Nanocarrier System for Nasal Immunization

Charlotte Primard, Farnaz Esmaeili, Simon Heuking, Emmanuelle Sublet, Gerrit Borchard. University of Geneva, Geneva, Switzerland

Mucosal vaccination is a real challenge to induce protective immunity against mucosal pathogens: successes (oral Polio vaccine, nasal-spray Flu vaccine) are moderated by failed attempts (HIV, mycobacterium). Synthetic biodegradable particles have been widely used for vaccine application; however their immunostimulator effect on immune cells is often weak. To increase potency and specificity of mucosal immune responses, additional immunostimulatory signals, such as TLR ligands, could be delivered together with the antigens. We developed biodegradable nanocarrier systems loaded with multiple TLR agonists: incorporated (imiquimod for the intracellular TLR7) and/or decorated (Pam3Cys for the cell surface TLR2) on the PLGA particles. These nanoparticles were optimised for loading and release of TLR ligand and protein antigen. Their capacity to stimulate epithelial cells via cytokine production has been tested *in vitro*, as their capacity to induce a cellular and humoral immune response (IR) after their administration both by systemic and mucosal (nasal) routes in mice. The nasal and sub-cutaneous administrations of particles induce a comparable strong systemic IR. As expected, the TLR2 ligand favours a humoral mucosal IR, while the TLR7 ligand provoke a switch of IR towards a cellular response both when administered alone with the antigen protein, or in association with the TLR2 ligand.

F.124. Oral Tolerance of B Cells is Dependent on β7 Integrin

Norbert Wagner[1], Angela Schippers[1], Annika Kochhut[2], Thomas Clahsen[1], Werner Müller[3]. [1]RWTH Aachen University, Aachen, Germany; [2]Helmholtz Center for Infection Research, Braunschweig, Germany; [3]University of Manchester, Manchester, United Kingdom

The specific induction of local and systemic hyporesponsiveness to dietary antigens and luminal bacterial flora in the gastrointestinal tract is necessary to prevent allergies or deleterious immunologic reactions to food or environmental antigens. It is a multifactorial process termed oral tolerance which involves anergy/deletion of effector T cells as well as active suppression by regulatory T cells. The aim of this study was a detailed evaluation of the impact of β7 integrin on oral B cell tolerance. β7 integrin deficient mice displayed reduced numbers of IgA-secreting plasma cells in the lamina propria of the small intestine. They were unable to mount a normal IgA response following oral immunization with either Ovalbumin or Choleratoxin, whereas the IgG response was unchanged in comparison to control mice. Most important, oral tolerance to Ovalbumin did not develop in the absence of β7 integrin. After adoptive transfer of spleen cells from wildtype mice in RAG-2 deficient mice or β7 Integrin/RAG-2 double deficient mice, only RAG-2 deficient mice were able to develop oral tolerance. This observation points to a role of β7 integrin in the process of B cell tolerance, probably by mediating migration of innate immune cells (e.g. dendritic cells, macrophages). (supported by the DFG)

F.125. FcR Targeting of Antioxidant Mutants of Francisella Tularensis: A Novel Approach to Enhance Immune Protection Against Tularemia

Mohammad Rashel[1], J. Melendez[2], Chandra Bakshi[3]. [1]Upstate Medical University, Syracuse, NY; [2]Albany Medical College, Albany, NY; [3]New York Medical College, Valhalla, NY

Tularemia is an acute febrile disease caused by Francisella tularensis (Ft). The severity of the disease depends on the route of infection. Ft has long been considered a potential biological weapon due to its ability to cause severe illness upon inhalation. Previously, we have established that Francisella's antioxidant armature not only provides direct protection from oxidative attack, but also interfere with host cell signaling that regulates protective immunity, and Ft strains defective in antioxidant enzyme activity can serve as effective immunogens. Moreover, the vaccination strategies directed at Fc receptor (FcR) cross linking of immune complexes can also enhance protection against virulent Ft SchuS4. We further investigated if immunogenicity of the antioxidant mutants (AO) of Ft can be enhanced by targeting to FcR via Ft-mAb (AO-Ft-mAb) immune complexes. Infection of macrophages with AO-Ft-mAb immune complexes elicited a robust pro-inflammatory cytokine response compared to wild type Ft. The elevated production of these cytokines was dependent upon NADPH mediated production of reactive oxygen species (ROS). Further, AO-Ft-mAb immune complexes were presented by infected macrophages more effectively to T cells when analyzed in an antigen presentation assay. These results demonstrate that deficiencies in oxidant scavenging capacity of Ft enhance immunogenicity and macrophage function alone or when targeted via FcR in an ROS-dependent manner. Studies defining the role of AO-Ft-mAb as effective immunogens in providing protection against virulent Ft strain in a mouse model are currently underway.

F.126. Systemic and Genital Tract Antibody Responses to Sublingual or Intramuscular Immunization of Healthy Adult Female Volunteers with Human Papillomavirus L1 Protein Virus-like Particles

Zhiming Huo[1], Sara Bissett[2], Simon Beddows[2], Rafaela Giemza[1], David Lewis[1]. [1]St. George's University of London, London, United Kingdom; [2]Health Protection Agency, London, United Kingdom

L1-specific (serotypes HPV6, HPV16 & HPV18) IgG and IgA antibody levels were measured in healthy female volunteers following three immunisations with alum-adjuvanted quadrivalent Human Papillomavirus (HPV) Virus-Like Particle (VLP) vaccine ("Gardasil") administered either intramuscularly (IM, n=6) or sublingually (SL, n=12) . SL immunisations induced very low-level serum IgG responses in comparison with IM. L1-specific IgG and IgA responses were detectable in cervical and vaginal secretions after both SL and IM immunisation, but were consistently more frequent and higher level after IM immunisations. Circulating L1-specific IgG and IgA antibody secreting cells were detectable after both IM and SL

immunisation. L1L2 pseudovirus neutralising activity was detectable in cervical and vaginal secretions after SL and IM immunisation. Overall, topical sublingual application of alum-adjuvanted HPV L1 VLP appears to be weakly immunogenic in humans but may be further optimised by use of mucosal adjuvants or effective delivery systems.

F.127. Maltose-binding Protein (MBP) is a Potential Carrier for Oral Immunizations

Philippe Bellot, Vesna Melkebeek, Eric Cox. Ghent University, Merelbeke, Belgium

In humans and most animal species such as pigs, vaccination via the oral route is a prerequisite for induction of a protective immunity against enteropathogens. Hereto, live attenuated microorganisms are used. However, these microorganisms often are either too attenuated to induce sufficient intestinal immunity or are still too virulent resulting in clinical signs. We previously demonstrated that it is possible to induce immunity against enteropathogens by targeting antigen towards enterocytes. Recombinant proteins are often fused to maltose-binding protein (MPB) to improve their yield and to increase their solubility. In mice, these fusion proteins showed an enhanced immunogenicity following systemic immunization. More recently, this has been attributed to interaction of MBP with TLR4 on dendritic cells (DCs). TLR4 is also expressed in the enterocytes of the gut. Therefore, we examined if oral administration of an MPB-FedF to 4-week-old pigs could be used to induce an immune response against F18+ verotoxigenic E. coli in pigs. Cholera toxin was used as oral adjuvant. Results showed an enhanced systemic and mucosal immune response against FedF and a significant decrease in the faecal excretion, demonstrating the potential of MBP as a carrier for induction of an intestinal mucosal immune response.

F.128. The Adjuvant Effect of Gantrez®AN Nanoparticles on Oral Vaccination of Pigs and Mice with F4 Fimbriae is Strongly Influenced by Polymer Degradation

Vesna Melkebeek, Katrien Vandamme, Chris Vervaet, Eric Cox. Ghent University, Merelbele, Belgium

We analysed the adjuvant effect of Gantrez nanoparticles NP on oral immunisation of pigs and mice with F4 fimbriae. The animals were vaccinated with F4, F4 encapsulated in Gantrez NP, called gF4 NP, or F4 + empty Gantrez NP, called F4 + gNP, and intragastrically infected with F4+ ETEC. In pigs, a clear F4 specific serum IgA and IgG response following vaccination could only be observed in the F4+g NP group. Also in mice, the strongest response could be seen in the F4 + g NP group. In contrast to the results in pigs however, encapsulation of F4 in NP reduced the response. An important difference between mice and pigs is that pigs have an intestinal F4 receptor, whereas mice don't have this receptor. Taken together, in both mice and pigs, the best adjuvant effect was seen by adding empty NP to the fimbriae. These data suggested that functional groups at the surface of the NP are likely to play a significant role in the immunogenicity. To analyze if the adjuvant effect of the empty NP was sufficient to protect suckling pigs, the experiment was repeated 6 months later. However, the response in the F4 + g NP group was not improved compared to the F4 group. To explain the discrepancy between the studies, the polymer was characterized again and a second mice experiment was performed to analyze the influence of storage on the polymer properties.Changes in polymer weight and polymer weight distribution occurred. In addition, the adjuvant effect of empty NP on F4 in mice was lost. Nevertheless, this could be restored by crosslinking the NP more strongly.

F.129. Sublingual Co-administration of OVA and Fve Protein Suppressed the Development of Th2 Immune Responses

Chiung-Hui Huang, Lee Hui Chua, Kaw Yan Chua. National University of Singapore, Singapore

Background: Fve protein from the Flammulina velutipes mushroom can modulate immune responses through IFN-γ mediated mechanisms. IFN-γ was demonstrated to regulate the conversion of $CD4^+CD25^-$ cells to regulatory T cells. We tested if sublingual co-administration of allergen with Fve as adjuvant could enhance the efficacy of sublingual immunotherapy. Methods: Mice were sensitized with ovalbumin (OVA) in alum and then given OVA in the presence or absence of Fve or PBS sublingually and followed by intranasally challenged with OVA. Cytokines profiles were analyzed from the splenocytes and lung draining lymph nodes. The T cell proliferation was assayed by thymidine uptake. OVA specific serum IgE, IgG1, IgG2a antibodies were measured by ELISA. Results: Sublingual co-administration of OVA alone or OVA with Fve resulted in significant suppression in the production of OVA specific IgE. However, mice received OVA with Fve but not OVA alone resulted in significant suppression of antigen specific T cell proliferation. Furthermore co-administration of OVA with Fve showed more effective suppression of IL-4 production than that of OVA alone. Conclusion: Sublingual co-administration of OVA and Fve significantly enhanced the suppression of Th2 immune responses suggesting that Fve could be used as a mucosal adjuvant for sublingual allergen-specific immunotherapy

F.130. HPV16 L1 Virus-like Particle Expressed in Lactobacillus Casei Induces Mucosal and Systemic Immune Responses *in vivo*

Sun-Woo Yoon[4], Doo-Jin Kim[1], Moon-Hee Sung[2], Il-Han Lee[3], Haryoung Poo[1], Yong Taik Lim[5]. [1]Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea; [2]Kookmin University, Seoul, Republic of Korea; [3]BioLeaders Corporation, Daejeon, Republic of Korea; [4]St. Jude Children's Research Hospital, Memphis, TN; [5]Chungnam National University, Daejeon, Republic of Korea

Human papillomaviruses (HPVs) are non-enveloped DNA viruses causing warts on skin or genital track, and malignant cancer as well. Among more than 100 different strains or types, HPV type 16 (HPV16) has been regarded as one of the most causative agents for cervical cancer. L1, the major capsid protein of HPV, self-assembles into virus-like particles (VLPs), and is currently used as a HPV vaccine component because of its strong immunogenicity *in vivo*. In this study, we generated the HPV16 L1 VLPs in Lactobacillus casei (L. casei), which is a potential vaccine vector for the

induction of both mucosal and systemic immune responses. Oral administration of L. casei/HPV16 L1 induced strong systemic IgG and mucosal IgA antibody responses in Balb/c mice, which is comparable to the immune responses of conventional HPV16 L1 VLP-injected group. Also, oral immunization of L. casei/HPV16 L1 resulted in higher neutralizing activity against HPV16 pseudovirus infection to 293TT cells *in vitro*. More importantly, L. casei/HPV16 L1 conferred significant protection against pseudovirus challenge through the genital tract, a major infection route of HPV. Collectively, our results show that L. casei/HPV16 L1 could be an efficacious prophylactic vaccine which induces strong neutralizing immune responses in mucosa with the safety and ease of administration.

F.131. Application of M Cell Targeting Receptor to Improve the Systemic and Mucosal Immune Response Induction Oral Vaccination

Sae-Hae Kim[1], Dae-Im Jung[1], In-Young Yang[1], Ki-Weon Seo[1], Kyung-Yeol Lee[1], Tomonori Nochi[2], Hiroshi Kiyono[2], Yong-Suk Jang[1]. [1]Chonbuk National University, Jeonju, Republic of Korea; [2]University of Tokyo, Tokyo, Japan

In mucosal immune system, M cells are known as specialized epithelial cells to uptake the luminal antigens and microorganisms. However, both the receptors expressed on the apical surface of M cells and mechanisms of antigen transcytosis are less well-understood. In this study, we report the expression of complement fragment C5a anaphylatoxin receptor (C5aR, CD88) on apical surface of mouse M cells and human M-like cells and its possible use as target receptor for mucosal vaccine delivery. *In vitro* human M-like cells, level of C5ar mRNA expression was about 6-fold higher than mono-cultured Caco-2 cells. In addition, C5aR expression was detected together with glycoprotein 2 (GP2) M cell-specific protein on apical surface of the M-like cells and mouse Peyer's patch M cells. After oral administration of Y. enterocolitica, which is known to interact with C5aR, not only dense clustering of C5aR but also phosphorylation of the clustered C5aR were detected on mouse Peyer's patch M cells. More interestingly, Y. enterocolitica OmpH β1α1-mediated antigen targeting to C5aR leads the effective induction of fused antigen-specific immune responses in systemic and mucosal immune compartments. This study was supported by the Korea Institute of Planning and Evaluation for Technology of Food, Agriculture, Forestry and Fisheries.

F.132. An Oral Vaccine Containing Nontypeable Haemophilus Influenza (NTHi) Reduces Exacerbations by Augmenting Natural Presentation of Aspirated Antigen that Drives a Th_{17} T Cell Response

Robert Clancy[1], Margaret Dunkley[2]. [1]Hunter Area Pathology Services, Newcastle, NSW, Australia; [2]Hunter Immunology Limited, Newcastle, NSW, Australia

Acute exacerbations are a major complication in chronic obstructive pulmonary disease (COPD). We present data from two human trials and animal studies that document a novel 'physiological' route of airways protection based on antigen presentation to Peyer's patches following aspiration of bronchus content, that demonstrate the mechanism depends on T cells (specifically Th_{17} cells) that home to the bronchus mucosa where they mobilise and activate phagocytes which reduce bacterial colonisation/prevent inhaled bacteria accessing small airways, and prevent moderate/severe exacerbations in COPD. In each human study 6×10^{11} inactivated NTHi are given in three monthly cycles. In severe COPD (38 subjects) reduction of exacerbations requiring corticosteroids was 63% ($P=0.05$) and admission into hospital was 90% ($P=0.04$). In smokers (64 subjects) a seasonal increase in specific T cells in the placebo group ($P<0.05$), was enhanced following oral NTHi, and serum IgG antibody correlating with exposure to NTHi was only detected in the placebo group ($P<0.05$). Lysozyme, a marker of inflammation in secretions, was reduced in the active group ($P<0.05$). Studies in a rat model of airways clearance complemented the human studies, demonstrating the predominant T cell population in nodes and lung following oral NTHi as Th_{17}.

F.133. Reactivity of Serum and Secretory IgA Antibodies from Healthy Brazilian Parturients to Vaccine Antigens

Patricia Palmeira[1], Camila Quinello[1], Fernanda Ampessan[2], Milene Tino-De-Franco[2], Solange Carbonare[2], Magda Carneiro-Sampaio[1]. [1]University of São Paulo, São Paulo, Brazil; [2]Butantan Institute, São Paulo, Brazil

Secretory IgA (SIgA) antibodies are crucial for the immune response in mucosal sites through a process known as immune exclusion. There is evidence that serum monomeric IgA fulfills an anti-inflammatory role in serum, down-regulating IgG-mediated response. The study of SIgA specificity associated to the research of serum IgA reactivity with different antigens, is not only an immunological study, but also provides important epidemiologic data from our population. The aim is to evaluate serum and colostrum IgA antibodies' reactivity with tetanus and diphtheria toxoids, rotavirus G9 and whole cell pertussis. Specific IgA levels were evaluated in serum and colostrum samples from 54 parturients by ELISA. It was verified significantly lower specific IgA titers in serum than in colostrum. The lowest IgA titers found in serum and colostrum samples were reactive with rotavirus and the highest were directed to tetanus and diphtheria toxoids. Significant correlation indexes were observed among serum and colostrum IgA directed to tetanus, diphtheria and pertussis antigens. These results demonstrate that serum and colostrum IgA levels reflect the immunological experience of each mother, whether by vaccination or direct stimulation in the mucosal surfaces, which will protect the infant against the prevalent pathogens in its environment. Financial Support: CNPq (475034/2009-0).

F.134. Vaginal Immunization using Virosomal Candida albicans rSap2 Vaccine: Toxicity and Immunogenicity Studies in Animals

Gaëlle Vacher[1], Christel Gremion[2], Mario Amacker[2], Christian Moser[2], Robert Gurny[1], Gerrit Borchard[1]. [1]Université de Genève, Genève, Switzerland; [2]Pevion Biotech Ltd., Ittigen, Switzerland

To prevent the increasing number of recurrent vulvovaginitis in women, a strong vaginal immunity must be required as the first line of defence, meaning both systemic and local immunization, as the vaginal mucosa is the main portal of Candida infection. Virosomes, defined as a reconstituted influenza virus envelopes comprising the viral surface proteins hemagglutinin (HA) and neuraminidase (NA) but lacking the viral genetic material of the genuine pathogen, was able to elicit both humoral and cellular immune response in previous studies. A new promising vaginal formulation for virosomes C. albicans rSap2 (secretory aspartyl proteinase 2) antigen, which is critically required for Candida infections, was developed to deliver rSap2 antigen through vaginal mucosa. Based on preliminary *in vitro* analysis, *in vivo* release, toxicology and immunological assays in female New Zealand rabbits and female Göttingen minipigs were performed under GLP conditions. After intravaginal administration of capsules containing virosomes conjugated with rSap2 in both animal models, local (vaginal secretion) and systemic (serum) antibody responses (anti-rSap2 and anti-HA) were induced. No adverse events related to the medicated capsules application were observed. Based on the promising data, a phase I clinical trial was initiated in 2010.

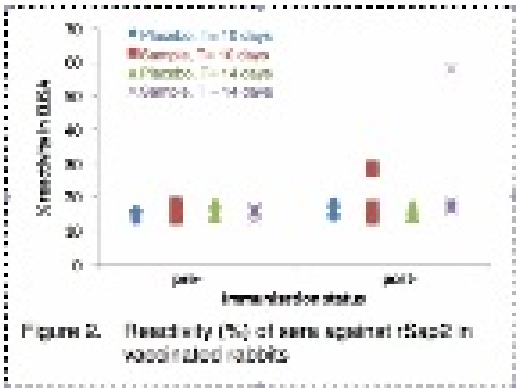


Figure 2. Reactivity (%) of sera against rSap2 in vaccinated rabbits

F.135. Inhibition of the Growth and Morphology of Candida Albicans by Lasioglossins

Alena Kašperová[1], Saima Imran[1], Jaroslav Turanek[2], Evzen Weigl[1], Milan Raška[1]. [1]Palacký University, Olomouc, Czech Republic; [2]Veterinary Research Institute, Brno, Czech Republic

Lasioglossins are antimicrobial peptides from bee venom. Anti-proliferative effect of derivatives LLIII and LLIII-D was analyzed in Candida albicans. *In vitro* anti-proliferative effect of LLIII and LLIII-D was analyzed by eosin-Y staining of unviable cells. Candida growth inhibition of LLIII measured at 16μM was 75% (24 h of incubation) and 88% (48 h). LLIII-D at 16μM exhibited >85% inhibition after 24 h and 82% after 48 h. Subsequently, Balb/C mice with experimentally induced vaginal candidiasis were treated for three weeks by daily application of 800 ng or 1500 ng of LLIII per mouse. The inhibitory effect of LLIII at lower and higher dose was 29.8 % and 34.5% respectively after one week of infection as compared to infected untreated controls. After two weeks, the inhibitory effect was 40.4 % for lower and 51 % for higher LLIII dose. Lasioglossins also have significant effect on inhibition of the morphological transition of Candida blastospores toward virulent hyphal stages. In the presence of LLIII we observed the Candida cells with atypically enlarged nuclei and cells with two or more micronuclei. Lasioglossins significantly affect C. albicans growth making them promising candidates for local therapy of vaginal candidiasis. Supported by MSM6198959223, GAP304/10/1951, Czech Republic.

F.136. Sublingual Immunization of Subunit Influenza Vaccine with LT Mutants Induces Th17 and IgA at the Mucosal Site

Simona Gallorini, Filomena Nardelli, Sushma Kommareddy, Sylvie Bertholet, Manmohan Singh, Derek O'Hagan, Barbara Baudner. Novartis Vaccines and Diagnostics, Siena, Italy

Influenza is a vaccine-preventable disease, but remains a major health problem world-wide. The needle and syringe are still the primary delivery device, injecting liquid vaccine into the muscle. Novel delivery approaches suggest that vaccines could be more effective if they were delivered by the mucosal route. It has been demonstrated that induction of systemic and mucosal innate and adaptive immune responses, including pathogen-neutralizing IgG , CD4 T-helper and Th17 cells, and secretory IgA (sIgA) at the site of pathogen entry, have a critical role for effective protection. E. coli heat-labile enterotoxin (LT) and its mutants induce Th17 cell responses after intranasal immunization against rotavirus, and have already been used in sublingual immunization to induce antigen-specific IgA. The purpose of this study was to evaluate a subunit influenza vaccine adjuvanted with LT mutant for its potential to induce immunogenicity both in the systemic and mucosal compartments after sublingual immunization in the mouse. Sublingual immunization in the presence of LT mutants (but not in absence) induces serum HI antibody and IgA-secreting B cells in cervical lymph nodes. Furthermore, priming of Th17 CD4 T cells was detected after mucosal immunization; whereas systemic immunization did not induce either mucosal IgA nor Th17 cells.

F.137. *In vitro* Transport of Polylactic Acid (PLA) Nanoparticles Carrying HIV Antigens through a Reconstructed Oral Mucosa Model

Vincent Pavot[1], Beste Kinikoglu[2], Nicolas Rochereau[3], Odile Damour[2], Christian Genin[3], Thierry Delaire[1], Stéphane Paul[3], Bernard Verrier[1]. [1]CNRS, Lyon, France; [2]Hôpital Edouard Herriot, Lyon, France; [3]Jacques Lisfranc, Saint Etienne, France

Mucosal vaccination is one of the greatest challenges today, as induction of strong immune responses at portals of entry should be able to prevent invasion of most pathogens. New safe mucosal administration routes are actively needed for elicitation of distal mucosal immune responses. Among innovative routes, oral mucosa administration (sublingual) is very promising as it is known for their capacity to induce S-IgA in blood, stomach, intestine, respiratory and reproductive tract. However, effective and safe mucosal adjuvants that enhance immunity of subunit vaccines are

necessary to initiate immune response against mucosal administered vaccine antigens. Current adjuvants could induce potential side effects which could be detrimental to vaccine acceptance by regulator agency. Alternative adjuvant or delivery system able to bypass oral mucosa are urgently needed. Our lab has already developed a surfactant free vaccine vehicle made of PLA (poly(lactic acid)) nanoparticles (NPs), and able to cross intestinal mucosal barrier. Therefore, we have investigated the capacity of these NPs to cross oral mucosa when carrying viral antigens. To address this question, we tested the capacity of fluorescent PLA nanoparticles carrying HIV p24 gag antigens to cross a reconstructed multilayer oral mucosa by using a recent published model, consisting in a successive coculture of human lamina propria fibroblasts and human oral epithelial cells isolated from the non keratinized region of oral cavity. We showed that fluorescent PLA NPs carrying p24 could cross oral mucosa. Indeed fluo NPs were seen in the dermis, and after different time points, p24 has been detected in the sub-mucosa. These findings suggest that biodegradable PLA nanoparticles could be an innovative tool to deliver vaccine antigens for buccal immunization.

F.138. Fusion of HIV-1 p24 Antigen with Autologous Heat Shock Protein 70 Increase Markedly Immunogenicity in Immunized Mice

Michal Krupka, Katerina Zachova, Lydie Czernekova, Evzen Weigl, Milan Raška. Palacky University, Olomouc, Czech Republic

P24 is the antigen of HIV virus, transmitted predominantly through the mucosal surfaces leading to AIDS associated with fatal outcome and high social and economic impact. This antigen is one of promising candidates for preventative vaccine construction. Heat shock protein 70 (Hsp70) is involved in presentation of antigenic peptides on MHC I and can act as activation signal for antigen presenting cells. In our study, we prepared two recombinant proteins derived from murine Hsp70 and HIV-1 p24 antigen fused in either p24-Hsp70 or Hsp70-p24 orientation. Proteins were expressed in Escherichia coli and purified to remove endotoxin. Balb/c mice were vaccinated twice with fusion proteins and p24 and Hsp70 alone as controls by intredermal route. After second immunization, mice were sacrificed and their spleens and sera were used for evaluation of immune response. P24-Hsp70 and Hsp70-p24 immunizations induced considerably higher titers of p24-specific antibodies (especially IgG2a and IgG2b) then unfused p24. Production of IFN-γ by *in vitro*-p24-stimulated splenocytes was higher in fusion protein-vaccinated groups as well. In conclusion the fusion of p24 antigen with Hsp70 presents promising approach for boosting p24-specific Th1 type response necessary for effective CD8 T cell protection. Supported by LSHP-CT-2006-03720 EU and MSM 6198959223 CR.

F.139. Intranasal Immunization of Chickens with Inactivated Newcastle Disease Virus Adjuvanted with a Low-viscosity Nanoemulsion

Ming-Chung Deng[1], Chwei-Jang Chiou[2], Tong-Rong Jan[3], Hong-Jen Liang[4]. [1]Animal Health Institute, Taipei, Taiwan; [2]Bureau of Animal and Plant Health Inspection and Quarantine, Taipei, Taiwan; [3]National Taiwan University, Taipei, Taiwan; [4]Yuanpei University, Hsinchu, Taiwan

We evaluated the efficacy of a novel oil-in-water type of low-viscosity nanoemulsion (LVNE) stabilized by surfactants as an intranasal adjuvant for mucosal vaccination. Chickens were vaccinated with inactivated Newcastle disease virus vaccine adjuvanted with LVNE (NDV/LVNE) intramuscularly or intranasally using MicroSprayerTM. Our Results showed that intramuscular vaccination induced a moderate increase in the serum IgG response, but no secretory IgA (s-IgA) response was detected. Intranasal vaccination delivered by MicroSprayerTM significantly stimulated the serum antibody response compared to that induced by inactivated NDV alone. In addition, a marked s-IgA response in the lungs and nasal cavity was induced by intranasal vaccination. Both intramuscular and intranasal vaccination resulted in protection against intranasal challenge with a lethal dose of a virulent NDV strain. The present study demonstrated that LVNE could be employed as an effective mucosal adjuvant system for induction of high systemic as well as mucosal antibody responses against Newcastle disease in chicken.

F.140. Evaluation of Influenza Hemagglutinin Subunit Vaccines Reinforced with Chitosan/poly-gamma-glutamic Acid Nanoparticles as an Effective Mucosal Adjuvant

Jong-Soo Lee[1], Ho-Jin Moon[1], Young-Ki Choi[1], Moon-Hee Sung[2], Chul-Joong Kim[1]. [1]Chungnam National University, Daejeon, Republic of Korea; [2]BioLeaders Corporation, Daejeon, Republic of Korea

In present, new generation influenza vaccine candidates have been continually studied and developed for preparation of pandemic outbreak. Especially, it is important factor that complete protection against influenza virus infection is mediated primarily by secretory IgA antibodies in the respiratory tract and virus neutralizing serum IgG induced by direct mucosal immunization. In this study, we assessed the protective potential of influenza hemagglutinin (HA) subunit vaccine which reinforced with nanoparticles (NP) composed of chitosan and ploy-gamma-glutamic acid (gamma-PGA) as a mucosal adjuvant. Intranasal immunization with a mixture of purified globular head domain of HA antigen and PGA-NPs induced high titer of anti-HA protein specific antibodies (serum IgG and local IgA) which included neutralizing antibodies. Also, HA vaccine with PGA-NPs protected mice against challenge with the highly pathogenic avian influenza virus (H5N1). The results suggested that adding PGA-NPs to the HA subunit vaccine enhances effective protection and indicate PGA-NPs are a potent mucosal adjuvant candidate for mucosal influenza vaccine [This research was supported by the National Agenda Project grant from Korea Research Council of Fundamental Science].

F.141. Immunogenicity of Mucosal Delivered Influenza Fusion-active HA2 and M2 Subunit Vaccines with Poly-gamma-Glutamic Acid as an Effective Mucosal Adjuvant

Jong-Soo Lee[1], Mohammed Y. E Chowdhury[1], Su-Kyung Seo[2], Moon-Hee Sung[2], Chul-Joong Kim[1]. [1]Chungnam National University, Daejeon, Republic of Korea; [2]BioLeaders Corporation, Daejeon, Republic of Korea

The complete protection against influenza virus infection may be mediated primarily by secretory IgA antibodies in the respiratory tract and virus-neutralizing serum IgG induced by direct mucosal immunization and also for the broad protection against multiple influenza virus strains, including multiple subtypes may be necessary the universal vaccine. In this study, we assessed the protective potential of conserved extracellular domain of influenza M2 protein (M2e) and fusion-active HA2 of hemagglutinin (HA) protein vaccine which reinforced with nanoparticles (NP) composed of ploy-gamma-glutamic acid (gamma-PGA) as a mucosal adjuvant. Intranasal immunization with a mixture of purified M2e, HA2 antigen and PGA-NPs induced high titer of anti-M2e and anti-HA2-specific antibodies (serum IgG and local IgA). Also, M2e, HA2 antigen with PGA-NPs protected mice against challenge with the highly pathogenic avian influenza virus (H5N1) and influenza virus A/PR/8/34. The results indicate that the universal vaccine candidates (M2e, HA2 antigen) with PGA-NPs as an effective mucosal adjuvant may offer an attractive approach for mucosal influenza vaccine. This research was supported by the National Agenda Project grant from Korea Research Council of Fundamental Science & Technology and the 2010 research fund of Chungnam National University in Republic of Korea.

F.142. Primary T Cell Activation by Vaginal and Nasal Immunization

Fabio Fiorino, Elena Pettini, Annalisa Ciabattini, Gianni Pozzi, Donata Medaglini. University of Siena, Siena, Italy

Generation of primed T cells is crucial for the development of optimal vaccination strategies. The rational design of mucosal vaccine formulations should consider multiple aspects including the selection of the optimal mucosal route, considering the physiological differences between the inductive sites associated with different mucosal surfaces. To this aim, we studied and compared CD4+ and CD8+ T cell priming following vaginal and nasal immunization with ovalbumin (OVA) model antigen and the mucosal adjuvant CpG ODN1826. *in vivo* T cell priming was analysed by using the adoptive transfer model of naïve OVA-specific transgenic CD4+ and CD8+ T cells. The primary T cell clonal expansion and the expression of activation and migration markers were assessed in both draining and distal lymph nodes and in the spleen at different time-points. Both intravaginal and intranasal routes of immunization induced high T cell clonal expansion in the respective draining lymph nodes since the third day after vaccination. Five days after immunization, primed T cells were also detected in distal lymph nodes and in the spleen, with higher percentages observed following nasal immunization. The modulation of CD44, CD45RB and CD62L markers expression indicated that proliferating T cells were activated and acquired migration properties.

F.143. FluGEM: An Intranasal Vaccine in the Protection Against Influenza

Bert Jan Haijema. Mucosis BV, Groningen, Netherlands

Mucosis BV is a Dutch biotechnology company developing innovative mucosal bacterial-like particle (BLP) based vaccines that can be applied needle-free via the nose. BLP is a recently developed vaccine technology consisting of bacterial particles produced by acid treatment of the bacterial cells of the food-grade bacterium Lactococcus lactis. The result is a cell-shaped cell wall matrix. The primary goal is to develop BLPs as a novel matrix for use in vaccines administered via the mucosal route (intranasal) and validate the platform by obtaining proof-of-concept using BLP together with Split Virion Influenza antigen (FluGEM). Here we show in a mouse model that FluGEM induces both a robust and long lasting mucosal and systemic immune response resulting in superior protection against an otherwise lethal influenza challenge. Moreover, we show that the BLP maturates and activates dendritic cells via the TLR2 receptor. Excellent safety and tolerability was demonstrated in both a rodent and non-rodent animal model. In a next step a clinical phase I trial will be started Q2 2011. Clearly, FluGEM is a promising and attractive influenza vaccine candidate: easy to administer and raising a protective immune response, already protecting against influenza at the port of entry.

F.144. Preimmunization with Lactococcus Lactis Expressing the HPV-16-E7 Antigen Improves the Therapeutic Effect of Adenovirus Encoding Calreticulin Fused to E7

Odila Saucedo Cardenas[1], Blanca Rangel-Colmenero[2], Jorge Gomez-Gutierrez[3], Arnulfo Villanueva-Olivo[2], Julio Villatoro-Hernandez[4], Santos Guzman-Lopez[2], Carlos Montes-de-Oca[2], Rodrigo Elizondo-Omaña[2], Roberto Montes-de-Oca-Luna[2]. [1]Instituto Mexicano Del Seguro Social, Monterrey, Mexico; [2]UANL, Monterrey, Mexico; [3]University of Louisville, Louisville, KY; [4]University of Groningen, Groningen, Netherlands

Developing effective vaccines that target HPV-16-E6 and-or E7 has become important, because despite current polyvalent vaccines that prevent the development of cervical cancer, they cannot be used to treat patients that already have the disease. We previously showed the effectiveness of an adenovirus expressing calreticulin fused to the antigen E7 (Ad-CRT-E7) in a cervical cancer animal model, and demonstrated that intranasal immunization of Lactococcus lactis encoding E7 (LI-E7) anchored to its surface, produced a significant E7-specific immune response. In this study we assessed the combination of both approaches in a cervical-cancer animal model. We found that an intranasal preimmunization with LI-E7 improved the antitumor effect of a single dose of Ad-CRT-E7 increasing significantly the survival rate of mice from 20% to 70%. Significant apoptosis and CD8+ cytotoxic T-lymphocyte infiltration was associated with a tumor regression. Our results, suggest that preimmunization with LI-E7 enhances the Ad-CRT-E7-mediated antitumor effect, which provides an advantage over repeated applications of Ad-CRT-E7 by avoiding a systemic toxicity.

F.145. Vitamin A Deficiency Affects Serum Antibody Responses to Rotavirus Vaccination and Challenge in a Gnotobiotic Piglet Model

Kuldeep Chattha, Anastasia Vlasova, Christine Siegismund, Linda Saif. Ohio State University, Wooster, OH

Rotaviruses (RV) are a major cause of gastroenteritis in children in developing countries. Vitamin A deficiency (VAD) contributes to the reduced efficacy of vaccines and higher incidence of diarrheal infections in these children. We established a VAD gnotobiotic piglet model to investigate the effects of vitamin A (VA) levels on immune responses to human RV (HRV) vaccination and challenge. Piglets derived from VAD (n=26) and VA+ normal sows (n=22) were vaccinated orally (attenuated HRV 3X) and/or supplemented with VA and/or challenged with virulent HRV. VAD piglets had significantly lower hepatic VA levels (at euthanasia) which coincided with higher magnitude and longer duration of diarrhea and HRV shedding in vaccinated and control VAD piglets compared to VA+ piglets. A trend for higher serum IgA RV antibodies was detected in VA+ vaccinated piglets post-challenge, suggesting higher anamnestic responses, whereas serum IgG RV antibody responses were higher in VAD vaccinated piglets post-challenge. Total serum IgA and IgG titers did not differ between VAD and VA+ groups. We conclude that VAD piglets are more susceptible to RV diarrhea and have lower serum IgA RV antibodies, suggesting a role for vitamin A in enhancing vaccine efficacy in VAD children in developing countries.

F.146. Perinatal Maternal Administration of Lactobacillus Paracasei NCC 2461 Modulates Immune Responses and Prevents Development of Allergy in Offspring in a Mouse Model of Birch Pollen Allergy

Irma Schabussova[1], Karin Hufnagl[1], Elisabeth Hoflehner[1], Gerhard Loupal[1], Sophie Nutten[2], Adrian Zuercher[2], Annick Mercenier[2], Ursula Wiedermann[1]. [1]Medical University Vienna, Vienna, Austria; [2]Nestlé Research Center, Lausanne, Switzerland

The hygiene hypothesis implies that microbial agents including probiotic bacteria may modulate foetal/neonatal immune programming, although their mechanisms of action are poorly understood. We investigated whether oral administration of Lactobacillus paracasei NCC 2461 to mothers during gestation/lactation can protect against airway inflammation in offspring, and examined the immune mechanisms involved. BALB/c mice were treated daily with L. paracasei in drinking water in the last week of gestation and during lactation. Their offspring were sensitized with recombinant Betv1, followed by aerosol challenge with birch pollen extract. Maternal exposure to L. paracasei prevented the development of airway inflammation in offspring, as demonstrated by attenuation of BAL eosinophil influx; IL-5 levels in BAL, and in lung and lung lymph node cell cultures; peribronchial inflammatory infiltrate and mucus hypersecretion. Offspring of L. paracasei supplemented mothers had significantly reduced Betv1-specific as well as ConA-induced responses in spleen and MLN cell cultures, suggesting that maternal L. paracasei feeding modulates both antigen-specific and bystander immune responses in offspring. These effects were mediated via TLR4 and TLR2 signalling, and were associated with increased FoxP3 mRNA expression in the lung. Our data show that perinatal administration of L. paracasei to pregnant/lactating mothers protects against the development of airway inflammation in offspring by activating T regulatory pathways, likely through TLR2/4 signalling.

F.147. Differential Glycosylation of gp120 Affects the Recognition by Specific Antibodies and HIV-1 Infectivity

Milan Raska[1], Zina Moldoveanu[2], Lydie Czernekova[1], Katerina Zachova[1], Matt Elliott[2], Stacy Hall[2], Jana Vrbkova[1], Michael Hoelscher[3], Leonard Maboko[4], Rhubell Brown[2], Jiri Mestecky[2], Jan Novak[2]. [1]Palacky University in Olomouc, Olomouc, Czech Republic; [2]University of Alabama at Birmingham, Birmingham, AL; [3]Clinic of the University of Munich, Munich, Germany; [4]Mbeya Medical Research Programme, Mbeya, United Republic of Tanzania

Variably glycosylated envelope glycoprotein (gp120/gp41) on the surface of HIV-1 is responsible for interactions between virus and hosT cell. N-glycans represent approximately half of the molecular mass of gp120. Glycans serve as potential epitopes but could also shield virus against immune recognition. We previously reported that N-glycosylation of gp120 is dependent on tissue origin of producer cell lines, including embryonic kidney cells (293T), T cell line (Jurkat), muscle cell line (RD), hepatocyte-derived cell line (HepG2), and Chinese-hamster ovary cells (CHO). Here, we show that the recognition of gp120 by V3-loop-specific and broadly neutralizing glycan-specific monoclonal antibodies and polyclonal antibodies in the sera of patients infected with HIV-1 is affected by cell-specific gp120 glycosylation. Furthermore, the recombinant gp120 preparations inhibited the *in vitro* HIV-1 infection of target reporter cells, but the IC50 concentrations varied depending on the producer cell-type-specific glycosylation of gp120. In summary, the cell system used for the production of gp120 affects gp120 glycosylation and, thus, should be experimentally tested when designing HIV-1 envelope-based vaccines. Supported by MSM6198959223 and KONTAKT II LH11046 CR.

F.148. Neonatal Nasal Vaccination with the Nucleoprotein of the Respiratory Syncytial Virus Elicits Virus-protective but Airway-pathogenic Th2-biased Immunity that can be Modulated by the Choice of Adjuvants

Aude Remot, Xavier Roux, Catherine Dubuquoy, Julie Bernard, Mohammed Moudjou, Jean-François Eléouët, Sabine Riffault, Agnes Petit-Camurdan. Institut National de la Recherche Agronomique, Jouy en Josas, France

There is still no licensed vaccine against human respiratory syncytial virus (RSV) which causes severe bronchiolitis in yound children. The nucleoprotein N, a major component of the RSV nucleocapsid, is remarkably conserved among RSV subtypes and is recognized as a target of protective T cell responses. We reported a method to produce recombinant N assembling in homogenous rings composed of 10-11 N subunits. Intranasal vaccination of adult BALB/c mice with N-rings and detoxified E.coli enterotoxin LT(R192G) as adjuvant (provided by J. Clements, USA), proved protective against an RSV challenge, without causing significant lung inflammatory reactions. In the present study, we evaluated the vaccine

potential of N-rings in 5 to 7 day-old BALB/c pups: a single intranasal administration of N-rings with LT(R192G) provided a significant reduction of the viral load after an RSV challenge at five weeks of age. However, neonatal vaccination also generated an enhanced lung infiltration by eosinophils following the RSV challenge. Analysis of antibody subclasses and cytokines produced after an RSV challenge or a boost administration of the vaccine suggested that neonatal vaccination induced a long lasting Th2 biased local immune memory. This early Th2 bias could be prevented by adding CpG-ODN to the vaccine formulation, but then the protection against virus replication was also reduced. In conclusion, protective vaccination against RSV can be achieve in neonates but requires an appropriate combinations of adjuvant.

F.149. Intranasal Vaccination of Piglets Against Actinobacillus Pleuropneumoniae: Effect of Adjuvant Formulation

Katerina Nechvatalova[1], Sebastien Deville[2], L. Dupuis[2], G. Ionkoff[2], F. Bertrand[2], Mariana Blahutkova[1], Eduard Gopfert[1], Martin Faldyna[1]. [1]Veterinary Research Institute, Brno, Czech Republic; [2]SEPPIC, Paris, France

Development of vaccines for an intranasal application should bring a benefit in prevention of respiratory diseases. In our trials, 4 adjuvants were tested with the aim to know how local inflammatory reaction induced by an adjuvant application correlates with an intensity of immune response. Various adjuvants technologies were compared: polymeric adjuvants (Montanide™ Gel 01 and Essai 948105), Water/Oil/Water (Montanide™ ISA 201 VG) and nanoparticles based formula (Montanide™ IMS 1313 N VG). One group of animals served as control. In the first trial, adjuvants were intranasally administered and local signs of immune system activation were evaluated by histological methods 3 and 7 days after application. All tested formulations demonstrated an absence of general reaction. On the other hand immune system was activated, because higher numbers of lymphocytes were found in respiratory tract mucosae and also in examined lymph nodes. In the second trial, tested adjuvants were applied with a model antigen. Production of antigen-specific antibodies of IgA isotype was detected using ELISA in a nose lavage fluid. It could be concluded from the results that a using of adjuvants could increase immune response to intranasally administered antigen without strong local reaction. Supported by SEPPIC and projects AdmireVet (CZ.1.05/2.1.00/01.0006, ED0006/01/01) and MZe0002716202.

F.150. Mucosal Immunization Generates Protective Immunity to Primary and Recurrent Genital Herpes

Josefine Persson, Karolina Thörn, Ali Harandi. Sahlgrenska Academy, Gothenburg, Sweden

No vaccines are currently available for genital herpes, one of the most prevalent sexually transmitted infections world-wide. Previous attempts centered on parenteral route of immunization have so far failed to elicit protection to genital herpes in humans. We and others have previously documented the ability of mucosal immunization for induction of protective immunity to primary genital herpes in mice. Here, we investigated if nasal immunization with HSV-2 envelope glycoprotein D (gD) alone or together with CpG could elicit protective immunity to primary and recurrent genital herpes in female guinea pigs. The immunized guinea pigs were subjected to immunological readouts or otherwise challenged intravaginally with HSV-2 and examined for outcome of the disease. We found strong antigen-specific systemic and vaginal IgG antibody response as well as T cell proliferative and IFN-γ responses in PBMC, splenocytes and genital lymph node cells of only the gD plus CpG immunized guinea pigs. High HSV-2 neutralizing antibody titer was also detected in this group. The gD plus CpG animals remained largely protected to primary genital herpes and recurrent outbreaks were either absent or at low frequency, and latency establishment of the virus in dorsal root ganglia drastically reduced.

F.151. The Cholera Toxin but not its B Subunit Induces Murine CD4+CD25+Foxp3+ Regulatory T Cell Depletion *in vitro*

Fatou THIAM[1], Claire Cachia[1], John Clements[2], Evelyne Kohli[1], Christelle Basset[1]. [1]Université de Bourgogne, Dijon, France; [2]Tulane University, New Orleans, LA

The cholera toxin (CT), the heat labile enterotoxin of E.coli (LT) and their non toxic mutants are potent mucosal adjuvants enhancing immune responses against coadministered antigens. The B subunit of CT, CTB, has been shown to have low adjuvant effect and rather promote tolerance to heterologous antigens. In a previous work, we showed that LT-R192G, a non toxic mutant of LT which retains adjuvant properties, dramatically decreased CD4+CD25+Foxp3+ regulatory T cells *in vitro* from different murine lymphoid tissues (Thiam et al. Special Issue Enterotoxins, Toxins, 2010, 2(8) 2007-27). Objectives: To compare the effects of CT and CTB on CD4+CD25+Foxp3+ regulatory T cells with those of LT-R192G. Methods: Cells from mesenteric lymph nodes were incubated with LT-R192G, CT or CTB (5ug/mL) or medium only. CD4+CD25+Foxp3+ T cells were analyzed on day 2 and 4 by flow cytometry. Results: CT, as previously shown with LT-R192G, induced *in vitro* CD4+CD25+Foxp3+ T cell depletion. At the opposite, no depletion of CD4+CD25+Foxp3+ T cells was observed with CTB. Conclusion: The unique effects of CTB observed *in vitro* on CD4+CD25+Foxp3+ regulatory T cells, compared with CT and LT-R192G, may be related to its capacity to promote tolerance to heterologous antigens.

F.152. Differences in CD4+CD25+Foxp3+ Induced Regulatory T Cells (Tregs) in Mice Mucosally Immunized with 2/6-rotavirus-like Particles (2/6-VLP) in the Presence of the Cholera Toxin (CT) or the Non-toxic Mutant of the Heat Labile Enterotoxin of E. Coli (LT-R192G)

Fatou THIAM[1], Claire Cachia[1], John Clements[2], Annie Charpilienne[3], Didier Poncet[3], Evelyne Kohli[1], Christelle Basset[1]. [1]Université de Bourgogne, Dijon, France; [2]Tulane University, New Orleans, LA; [3]Université Bourdeaux, Gif/Yvette, France

The cholera toxin, the heat labile enterotoxin of E.coli and their non toxic mutants are potent mucosal adjuvants. Using a murine model of mucosal immunization, we previously showed that LT-R192G, a non toxic mutant of LT, allowed the induction of rotavirus 2/6-VLP specific

CD4+CD25+Foxp3- (helper) and Foxp3+ (Treg) T cells that could be recalled *in vitro*. Moreover, it induced adjuvant specific CD4+CD25+Foxp3+ Tregs (Thiam et al. Special Issue Enterotoxins, Toxins, 2010, 2(8) 2007-27). Objectives: To compare CT with LT-R192G for its capacity to promote the induction of antigen specific CD4+CD25+Foxp3- and Foxp3+ T cells and induce specific CD4+CD25+Foxp3+ Tregs. Methods: Cells from mesenteric lymph nodes from mice intrarectally immunized with 2/6-VLP and LT-R192G or CT or from non-immunized mice were incubated for 2 and 4 days with 2/6-VLP, LT-R192G, CT or medium. CD4+CD25+Foxp3- and Foxp3+ T cells were analyzed by flow cytometry and IL-2 was assessed in supernatants. Results: Both adjuvants promoted the induction of VLP specific CD4+CD25+Foxp3- and Foxp3+ T cells but the response was weaker with CT and correlated with IL-2 production. LT-R192G, but not CT, induced specific CD4+CD25+Foxp3+ Tregs. Conclusion: A different profile of memory CD25+CD4+ T cells, both Foxp3- and Foxp3+, was observed between these adjuvants.

F.153. Stimulation of Toll-like Receptors Blocks the Strong Adjuvant Effect of Aluminum Hydroxide on T Helper-2 Immunity

Luciana Mirotti, Esther Florsheim, Wanessa Scorza, Lucas Faustino, Eliane Gomes, Momtchilo Russo. University of São Paulo, São Paulo, Brazil

The toll-like receptors are an important family of innate immune response that appears to be involved in the protection or susceptibility to asthma. Epidemiological evidence indicates that endotoxin lipopolysaccharide (LPS); that activates immune cells via toll-like receptor 4 (TLR4) can influence the development of asthma. LPS triggers immune responses through TLR4 that in turn activates two major signaling pathways via either MyD88 or TRIF adaptor proteins. Aluminum hydroxide (Alum) is a strong Th2 adjuvant that is used in the murine ovalbumin (OVA) model of asthma. Here we used TLRs agonists that signal via MyD88 or TRIF molecules adsorbed onto Alum to investigate whether TLRs stimulation during allergic sensitization affects the development of Th2 immunity. We found that TLR agonists signaling through Myd88 (TLR2, TLR4, TLR9) but not TRIF (TLR3) blocked all the Th2-type response, such as airway eosinophilia, serum levels of total IgE, specific of IgE and IgG1. Therefore, TLR stimulation might be a potential mechanism for vaccination against allergy.

F.154. Induction of CD4 T Cell Responses Towards Influenza Nucleoprotein Using a Synthetic Nanoparticle-conjugated Vaccine

Stephan Kontos, Melody Swartz, Jeffrey Hubbell, Eleonora Simeoni. Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland

Despite the long history of influenza vaccination in modern medical care, the protective efficacy of standard vaccines remains hindered by the capability of the virus to alter hypervariable regions of its proteome. Towards developing a universal vaccine against influenza, we have created a nanoparticle-based formulation using an engineered variant of nucleoprotein containing strain-conserved regions yet lacking hypervariable domains. The engineered nucleoprotein was covalently conjugated to synthetic polymer-based 35 nm nanoparticles in a reducible disulfide-bonded manner to allow effective processing and presentation by antigen presenting cells. The nanoparticle vaccine was adjuvanted with CpG and intranasally administered and boosted at day 14 and day 28 in BALB/c mice. One week following the first boost, mice administered nanoparticle-conjugated nucleoprotein exhibited enhanced numbers of blood CD8+ T cells specific for the MHC-I displayed immunodominant epitope of nucleoprotein. One week following the last boost administration, CD4+ splenocytes isolated from nanoparticle vaccinated mice exhibited higher TNF-α expression levels upon *ex vivo* restimulation with recombinant wild-type nucleoprotein. Humoral nucleoprotein-specific immunity was also induced in nanoparticle-vaccinated mice, as they exhibited enhanced serum levels of antigen-specific IgG, IgG1, and IgG2a, as well as IgA in the bronchoaveolar lavage. We are currently exploring optimized adjuvants and formulations for improved responses.

F.155. A Novel Targeting Molecule Promotes NLRP3 Activation and Enhanced T Cell Responses to a Particulate Nasal Vaccine

Karen Misstear[1], Edel McNeela[1], John Fox[2], Ed Lavelle[1]. [1]Trinity College Dublin, Dublin, Ireland; [2]Merrion Pharmaceuticals, Dublin, Ireland

Introduction and objectives: Mucosal vaccination is very attractive but is hampered by a lack of safe and effective adjuvants. A novel mimetic of the plant lectin Ulex europaeus agglutinin I (UEA-1m) targets mucosal antigen-sampling M cells, thus its potential to increase the efficacy of particulate mucosal vaccines was investigated. Results and conclusions: Intranasal immunisation of mice with antigen attached to nanoparticles enhanced T cell responses in draining lymph nodes, in comparison to immunisation with antigen alone, indicating the priming of Th1 and Th17-type responses by nanoparticles. Targeting nanoparticles with UEA-1m further enhanced T cell priming, but this effect was lost in NLRP3$^{-/-}$ mice, demonstrating a role for the NLRP3 inflammasome in the adjuvant effect of targeted nanoparticles. Targeted nanoparticles induced cellular responses in the absence of a humoral response, indicating their potential in vaccines requiring Th1/Th17-priming. However, co-immunisation of targeted nanoparticles with other immunostimulatory adjuvants stimulated the production of antigen-specific antibodies locally and systemically, thus broadening the potential of our targeting approach in developing novel mucosal vaccines.

F.156. Mechanisms by which Intestinal Helminth Infection Compromises Vaccine Efficacy

Joanna Wroblewska, Onyinye Iweala, Megan McBee, Austin Kline, Tiffany Patton, Andrew Stefka, Cathryn Nagler. University of Chicago, Chicago, IL

Widespread, endemic infection with gastrointestinal helminths is a challenge to successful vaccination in underdeveloped regions of the world. We have shown that infection with the natural mouse pathogen H. polygyrus significantly reduces serum antibody responses to both parenteral immunization with ovalbumin (OVA) adsorbed to alum, and oral vaccination with an attenuated OVA-expressing Salmonella strain. The proportion of both IL-10 secreting FoxP3+ Tregs and FoxP3- Tr1 cells is significantly increased in helminth-infected mice; this increased Treg response correlates

with marked shifts in the composition of the intestinal bacterial microbiome in infected hosts. The helminth induced polyclonal IgE and IgG1 response also changes the activation status and composition of APC populations in the MLN; ImageStream analysis shows that the distribution of fluorescently labeled vaccine among potential APC populations (DC, B cells, F480+ macrophages and neutrophils) is altered in helminth infected mice. Taken together, these data suggest that H. polygyrus infection compromises vaccine efficacy directly, via its influence on APC function, and indirectly through its effects on immunoregulatory cell function and alterations in the host microbiome.

F.157. Effectiveness of Pre-administered Nasal Adjuvants for the Induction of Influenza-specific IgA Antibody Responses in the Upper Respiratory Tract

Hideki Asanuma[1], Shinichi Sekine[2], Yoshiko Fukuyama[2], Akira Ainai[1], Hideki Hasegawa[1], Masato Tashiro[1], Kohtaro Fujihashi[2]. [1]National Institute of Infectious Diseases, Tokyo, Japan; [2]University of Alabama at Birmingham, Birmingham, AL

Our previous study demonstrated that nasal administration of influenza PR8-Ag plus a combination of CpG ODN and plasmids expressing Flt3 ligand (pFL), induced high number of IgA Ab forming cells (AFC) in the upper respiratory (UR) tract. In this study, we investigate whether pre-administration of a combined nasal adjuvant enhances effectiveness of influenza vaccine since these nasal adjuvants are known to activate dendritic cells. BALB/c mice were nasally administered with CpG ODN and pFL without Ag. Six, 24, 48 hr or immediately after the administration of a combined adjuvant, mice were nasally immunized with PR8 alone. Ten days after the immunization, mice were administrated with secondary PR8 alone and the numbers of influenzas-specific IgA AFCs in the UR tract were determined by ELISPOT assay. Essentially, no IgA AFCs were induced in the UR tract of mice given adjuvant before nasal PR8-Ag inoculation. In contrast, mice nasally immunized with PR8 immediately after CpG ODN and pFL administration resulted in increased numbers of influenza-specific IgA AFCs. Our findings suggest that simultaneous activation of different immune cells involved in the generation of antigen-specific mucosal B cells is essential for induction of protective antibody responses. Supported by NIH grants AG025873 and DE12242.

F.158. Dietary Vitamin A/Retinoic Acid Modulates the Efficacy of an Antileishmanial Oral Vaccine

Carolina DiBarros, Izabella Bezerra, Bartira Rossi-Bergmann. Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Cutaneous leishmaniasis is a vector borne disease caused by intracellular Leishmania parasites. We have previously demonstrated that oral tolerization of mice against pathology-inducing leishmanial antigens (LaAg) leads to peripheral protective immunity. Here, the role of dietary vitamin A and its metabolite retinoic acid (RA) in oral vaccine efficacy was compared in mice fed with Vit A-deprived (VitA-) and VitA-supplemented (VitA+) food. Animals were vaccinated with 2 oral doses of 100 µg LaAg by intragastric gavage. We found that CD4+FoxP3+ T regulatory cells were expanded in the mesenteric lymph nodes of VitA+ but not in VitA- animals. Only VitA+ but not VitA- mice were protected against peripheral challenge with Leishmania amazonensis, producing enhanced amounts of IFN-gama and TGF-beta and reduced of IL-4 and IL-10 in the infected footpads. Treatment of Vit A+ mice with daily oral doses of 300 mg/Kg of the RA inhibitor Citral during vaccination reverted vaccine efficacy. We conclude that dietary vitamin A / RA is required for an effective expansion of CD4+Foxp3+ T regulatory cells in the gut-associated mucosa, that may be critical for vaccine efficacy against cutaneous leishmaniasis.

F.159. HIV-1gag/pol DNA Vaccine Elicits Gut-associated Immune Responses when Combined with Highly Optimized Mucosal Chemokine Molecular Adjuvants

Noshin Kathuria[1], Daniel Choo[2], Kimberly Kraynyak[2], Berje Shammassian[1], Jian Yan[2], Diane Carnathan[2], MIchael Betts[2], Amir Khan[3], Michele Kutzler[1], David Weiner[2]. [1]Drexel University, Philadelphia, PA; [2]University of Pennsylvania School of Medicine, Philadelphia, PA; [3]Inovio Biomedical, Blue Bell, PA

The development of a systemic-delivered vaccine approach driving secretory IgA and mucosal T cell immune responses will be critical for protection against many pathogens that use mucosal surfaces as portal of entry and replication including HIV. We hypothesized that immunization with chemokine molecular adjuvants and HIV-1gag/pol plasmids will augment anti-HIV-1 T and B cell responses at the primary site of HIV entry. Balb/c mice were immunized with pHIV-1gag/pol in combination with CCL25, CCL27 or CCL28 followed by electroporation. Co-delivery of chemokines mediated significant enhancement in the polyfunctionality of HIV-1 specific splenic and gut-associated CD8+ T cell as determined by multicolor flow cytometry when compared with antigen alone group. Elevated HIV-1-specific sera and fecal levels of sIgA following co-immunization with mucosal chemokines were observed. Preliminary data suggests a role for CCR9+ dendritic cells and the retinoic acid pathway in peripheral lymph nodes upon co-immunization with CCL25 in preferential accumulation of effector CD8+ T cells in the gastrointestinal mucosa. These data support the use of highly optimized molecular adjuvants in our HIV-1gag/pol vaccine platform for targeting HIV-1 specific T and B cells to mucosal sites. This work is supported by funding through the NIH/NIAIDS (F32 to MA, HIVRAD to DBW).

F.160. Immunization with CVD 1256 Attenuated Shigella Dysenteriae 1 Reduces Bacterial Shedding Following Wild-type Challenge in Cynomolgus Macaques

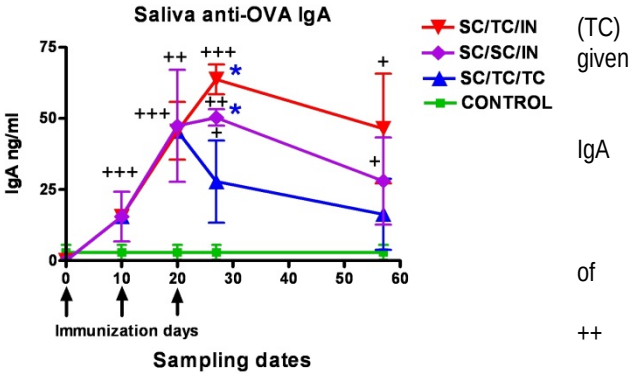
Franklin Toapanta, Abdul Khan, Aruna Panda, Steve Shipley, Louis DeTolla, Haiyan Chen, Eileen Barry, Myron Levine, Marcelo Sztein. University of Maryland, Baltimore, MD

Currently, there are no vaccines against Shigella. Shigella dysenteriae serotype 1(S. dysenteriae 1), which produces shigatoxin is an important public health problem. We evaluated the immunogenicity of the live-attenuated vaccine candidate CVD 1256 in a NHP model of infection. Cynomolgus monkeys received CVD 1256 or mock vaccination . Four weeks later, animals were challenged with wild-type S. dysenteriae 1 (strain 1617) and observed for disease development. Shedding of CVD 1256 and strain 1617 was monitored in stools post-vaccination and -challenge. Anti-shigella antibodies and antibody secreting cells (ASC) were evaluated in blood and cells from various segments of the intestinal tract post-immunization and -challenge. All vaccinated animals developed anti-Shigella antibodies two weeks after vaccination and remained elevated following challenge. None of the vaccinated animals developed clinical signs of shigellosis following challenge. Although only 17% of the mock vaccinated animals had diarrhea after challenge, this group had significantly higher bacterial shedding counts. Anti-LPS, -IpaB, and -VirG ASC were detected in blood two weeks after vaccination and disappeared by day 28. Colon and cecum ASC were detected only after challenge. In conclusion, CVD1256 is a promising live-attenuated candidate vaccine that is immunogenic, produces minimal secondary side effects and reduces bacterial shedding after challenge.

F.161. Combined Immunization Protocols to Induce Systemic and Mucosal Immune Responses

Marco Vega-López[1], Gabriela Alvarado[2], Maria Ramirez-Estudillo[1], Rosa Zenteno[3], Gloria Lazo[1]. [1]CINVESTAV-IPN, México, Mexico; [2]FES-Cuautitlan-UNAM, Cuautitlan Izcalli, Mexico; [3]Universidad Autonoma de Puebla, Puebla, Mexico

Respiratory infections annually account for up to 30% of the total infectious diseases deaths in the world, especially in young and elderly people. Parenteral (IM) immunization has proven ineffective to induce mucosal immunity, and direct mucosal immunization requires high doses of antigen, toxic adjuvants and repeated immunization, rendered not practical for extensive use. Thus, development of effective protocols to induce mucosal immunity is of paramount importance. Using a swine model, we test combined (parenteral/mucosal) protocols of immunization to analyze serum, nasal and saliva specific antibody production to ovoalbumin. Groups of 5 weaned animals were subcutaneously (SC) immunized, followed by either trancutaneous or SC boost 10 days later. Finally, 10 days later an intranasal (IN) boost was and anti-OVA IgG and IgA specific Ab were measured in serum, saliva and nasal secretion at experimental days 0, 10, 20, 27 and 55 by quantitative ELISA. Our results showed IgG and IgA immune responses in serum and a predominantly response in saliva (figure 1) and nasal secretion with the three protocols (SC/SC/IN, SC/TC/TC and SC/TC/IN), demonstrating that using a combined protocol it is feasible to induce mucosal Ab responses. Work partially funded by CONACyT and ICyTDF, Mexico. Figure 1. Anti-OVA IgA concentration in saliva immunized pigs. Each dot represents the mean of 5 animals ± SEM. Symbols represent statistical difference with control (+) and other group (*). + or * P<0.05, P<0.01, +++ P<0.001. SC: Subcutaneous, TC: Transcutaneous, IN: Intranasal.



F.162. *In vivo* Immunomodulatory Mechanisms of a Novel Mucosal Adjuvant Derived from a Type II Heat-labile Enterotoxin (LT-IIa)

Chang Lee[1], Patricia Masso-Welch[1], George Hajishengallis[2], Terry Connell[1]. [1]Univeristy at Buffalo, Buffalo, NY; [2]University of Louisville, Louisville, KY

Many pathogens invade at mucosal surfaces. Thus, development of agents that enhance protective immune responses on mucosal surfaces is a desirable objective. Heat-labile enterotoxins (HLT) are potent mucosal adjuvants. Yet, the mechanisms by which HLT evoke mucosal immune responses are poorly characterized. LT-IIa-B5, the non-toxic B pentameric subunit of a type II HLT (LT-IIa), is a strong mucosal adjuvant. Intranasal immunization of mice with Ag, in the presence of LT-IIa-B5: (1) increased uptake of Ag by dendritic cells (DC) in the nasal-associated lymphoid tissue (NALT); (2) enhanced migration of DC from the NALT to the cervical lymph nodes in a manner that required expression of TLR2 and the C-C chemokine receptor 7 (CCR7); (3) increased expression by NALT-derived CLN DC of the costimulatory molecules CD80, CD86, and CD40; and (4) increased Ag-specific DO11.10 CD4+ T cell proliferation in the CLN of wt, but not in TLR2-deficient mice. LT-IIa-B5 dramatically increased Ag-specific salivary IgA and serum IgG in a strictly TLR2-dependent manner. Thus, the mucosal adjuvant properties of LT-IIa-B5 depend, in part, on its capacity to activate and promote migration of DC in mucosal tissues. This is the first report that links immunomodulatory activity of a heat-labile enterotoxin with TLR signaling.

F.163. Intranasal Administration of an Inactivated Influenza Vaccine Effectively Induces the Neutralizing Secretory IgA Antibodies on the Surface of Nasal Mucosa in Human

Akira Ainai[1], Shin-ichi Tamura[1], Elly Riet[1], Tadaki Suzuki[1], Ryo Ito[1], Hideki Asanuma[1], Takeshi Tanimoto[2], Yasuyuki Gomi[2], Takato Odagiri[1], Masato Tashiro[1], Tetsutaro Sata[1], Takeshi Kurata[1], Hideki Hasegawa[1]. [1]National Institute of Infectious Diseases, Musashimurayama, Japan; [2]Osaka University, Kanonji, Japan

Parenteral vaccination against influenza virus induces primarily hemagglutinin (HA)-specific IgG antibodies in serum, which are highly effective for the prevention of pneumonia induced by the homologous virus infection. On the other hand, intranasal administration of vaccine can mimic the natural infection and induce secretory IgA (S-IgA) antibodies on the surface of nasal mucosa. S-IgA antibody in nasal mucus is superior to IgG antibody in serum for the protection against infection. In this study, the efficacy of intranasal administration of an inactivated whole-virion vaccine was investigated among healthy adult volunteers. The volunteers received the intranasal vaccination of an inactivated whole-virion vaccine [A/Victoria/210/2009 (H3N2)] twice at 3-week intervals, that contains 3-fold concentrated HA molecules (45 μg) per dose. Serum and nasal wash (NW) were collected at each time point, and assayed for neutralizing antibody (NT) titer and hemagglutination inhibition (HI) titer against A/Victoria/210/2009. The high levels of NT and HI titer were detected not only in the serum but also in the NW after the intranasal vaccination for the first time. These titers in sera were correlated with IgG antibody titers, while those in NWs were correlated with S-IgA antibody titers.

F.164. Oral Delivery of Recombinant Norovirus Virus-like Particles Inside Plant Cells is Capable of Producing a Th2-dominant Antibody Response in Mice

Robert Shepherd, Amanda Warmsley. Monash University, Clayton, VIC, Australia

Recombinant antigens produced in plant-based expression systems have been discussed for decades, yet none have moved beyond clinical trials. The end goal of this technology is to cheaply produce large quantities of antigenic proteins that can be delivered via the oral route. Hindering advancement of this strategy is the complexity of delivering intact recombinant antigens to the correct sites of GALT, and minimizing tolerance when co-delivered with dietary antigens. Our research aims to use plant-intrinsic factors including cellular encapsulation and phytometabolites to protect antigen during transit in the GIT and minimize tolerance, respectively. We have recently observed that when delivered in plant cells, Norwalk Virus Capsid Protein virus-like particles (VLPs) generate a strong, Th2-domainant antibody response to an oral vaccination regime, in comparison to VLPs co-delivered with plant material that are unable to generate an antibody response. Also, we observed a variation to the seroconversion and antibody titre of rats in a prime-boost vaccination strategy when tomatoes with higher levels of the saponin-like metabolite α-tomatine are used to delivery VLPs. Ongoing investigations seek to determine the mode of action for these phytometabolites, and our research hopes to find synergies between the goals of strong, long-lasting and protective immune responses and the use of plants as antigen production systems.

F.165. Comparison of Recombinant T. Gondii Antigens and Toxoplasma Lysate Antigen for Vaccination Against Toxoplasma Cyst Formation in Mice

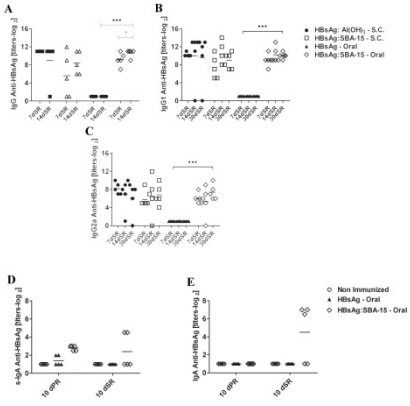
Angelika Wagner[1], Jozef Kur[2], Lucyna Holec[2], Bärbel Ruttkowski[3], Anja Joachim[3], Ursula Wiedermann[1]. [1]Medical University Vienna, Vienna, Austria; [2]Gdansk University of Technology, Gdansk, Poland; [3]University of Veterinary Medicine, Vienna, Austria

Toxoplasmosis is a worldwide prevalent infection, representing a severe disease for immunocompromised patients and pregnant women. Our aim is to develop a preventive vaccine for people at risk. Toxoplasma tachyzoite lysate antigen (TLA) as well as selected recombinant antigens (SAG1, MAG1, GRA7 (SMG)) were applied systemically as well as orally in order to reduce the parasite burden in a mouse model of T. gondii infection. Mice immunized subcutaneously with TLA developed markedly higher antibody levels before and during the course of infection than non-vaccinated infected controls. Furthermore, the subcutaneously TLA vaccinated mice exhibited increased IFN-gamma and IL-17 levels after TLA stimulation of splenocytes and lower levels of IL-10 in serum during the acute phase of infection compared to non-vaccinated infected mice. Importantly, a lower parasite cyst burden in the brain was measured in the subcutaneously TLA vaccinated mice indicating an amelioration of the clinical course of infection by this vaccine approach. Interestingly, we could also detect a lower cyst burden in the brain after subcutaneous vaccination with recombinant SMG antigens. Our data indicate that subcutaneous vaccination with T. gondii antigens may lead to a milder course of infection.

F.166. Hepatitis B Nanovaccine: The Use of SBA-15 Silica as an Oral Adjuvant

Karina Scaramuzzi[1], Denise Oliveira[2], Luciana Carvalho[1], Marisa Rizzi[3], Juliana Mussalem[3], Márcia Fantini[4], Viviane Botosso[2], Osvaldo Sant Anna[1]. [1]Butantan Institute, São Paulo, Brazil; [2]Butantan Institute, São Paulo, Brazil; [3]Cristália Pharmaceutics, São Paulo, Brazil; [4]Physics Institute, University of São Paulo. Brazil, São Paulo, Brazil

SBA-15 is an inorganic material with ordered channels of hexagonal nanostrutured pores able to interact with atoms, ions and molecules. We aim to prove the applicability of SBA-15 as an adjuvant in oral immunizations with HBsAg, the main component of Hepatitis B vaccine. Groups of female 8 week-old BALB/c mice received subcutaneously [s.c.] or orally, 0.5 μg of HBsAg encapsulated/adsorbed on SBA-15 or adsorbed on Al[OH]3 in a final volume of 0.25 mL PBS. Boosters were administered 30 and 110 days after first immunization. Serum and fecal samples were collected for specific antibodies titration.



Analysis of s-IgA showed that mice orally immunized with HBsAg on SBA-15 had increased levels of specific antibodies at day 14 post first immunization [3 log2] and at day 7 after booster [4 log2]. Serum IgA [3.5 log2] and IgG [10 log2] titers in HBsAg:SBA-15 orally immunized mice remained the same after the second dose. Immunizations with Al[OH]3 [s.c.] showed detectable serum IgA titers only 7 days after the third dose [4 log2] and IgG levels were 11 log2. Our results indicate the promising use of SBA-15 as an oral adjuvant due to the physical protection of antigens and the efficient activation of the immune system.

F.167 Toll-like Receptor 4 Promoter Polymorphisms: Common TLR4 Variants May Protect Against Severe Urinary Tract Infection

Bryndís Ragnarsdóttir[1], Klas Jönsson[2], Alexander Urbano[2], Jenny Grönberg-Hernandez[1], Nataliya Lutay[1], Martti Tammi[3], Mattias Gustafsson[1], Ann-Charlotte Lundstedt[1], Irene Leijonhuvfud[1], Diana Karpman[1], Björn Wullt[1], Lennart Truedsson[1], Ulf Jodal[4], Björn Andersson[5], Catharina Svanborg[1]. [1]Lund University, Lund, Sweden; [2]Biomedical Sciences Institutes, Singapore, Singapore; [3]National University of Singapore, Singapore; [4]Queen Silvia Children's Hospital, Gothenburg University, Gothenburg, Sweden; [5]Karolinska Institutet, Stockholm, Sweden

Polymorphisms affecting Toll-like receptor (TLR) structure appear to be rare, as would be expected due to their essential role in innate immunity. Since Tlr4-/- mice develop asymptomatic carrier state, we investigated genetic variation affecting TLR4 expression, rather than structure, as a mechanism to diversify innate immune responses. We sequenced the TLR4 promoter (4,3 kb) in Swedish blood donors, healthy controls and children with mild or severe disease. We performed a case-control study of pediatric patients with asymptomatic bacteriuria (ABU) or those prone to recurrent acute pyelonephritis (APN). Promoter activity of the single SNPs or multiple allelic changes was tested. We then conducted a replication study in an independent cohort of adult patients. Last, *in vivo* effects of the different GPs were examined after therapeutic intravesical inoculation of 19 patients with E. coli 83972. We identified eight TLR4 promoter sequence variants in the Swedish control population, forming 19 haplotypes and 29 GPs, some with effects on promoter activity. Compared to symptomatic patients and healthy controls, ABU patients had fewer genotype patterns, and their promoter sequence variants reduced TLR4 expression in reporter assays in response to infection. The ABU associated GPs also reduced innate immune responses in patients who were subjected to therapeutic urinary E. coli tract inoculation. The results suggest that genetic variation in the TLR4 promoter may be an essential, largely overlooked mechanism to influence TLR4 expression and UTI susceptibility. Reduced TLR4 expression is likely to attenuate the innate mucosal response, thus promoting an asymptomatic carrier state rather than severe disease.

F.168. Utility of Bacterial Toxins in the Induction of Oral Tolerance to Antigens

Nicolae Miron, Ioana Neagoe, Victor Cristea. University of Medicine and Pharmacy, Cluj-Napoca, Romania

Oral tolerance is defined as suppression of immune responses to antigens (Ag) that have been previously administered by oral route. Given the fact that immune over-reactivity to certain peptides plays an important role in the pathogenesis of autoimmune or allergic disorders, inducing immunological tolerance to autoantigens might prove a valuable tool in therapy. The first tolerization attempts consisted in oral administration of the incriminated antigen alone. However, experimental studies on animal models, using oral administration of autoantigens in association with superantigens, have produced promising results. Due to their highly potent polyclonal lymphocyte stimulation, superantigens could be a mean of enhancing the physiological pathways of peripheral immunological tolerance to self proteins targeted by pathological immune responses. This can be achieved by superantigen-induced stimulation of Ag-specific regulatory T cells (Treg), a key cellular subset for immune suppression. Using staphylococcal enterotoxin A (SEA), a member of the superantigen group, we are trying to enhance oral tolerance to myelin basic protein (MBP) in a mouse model of experimental autoimmune encephalomyelitis. We are measuring the levels of Treg in mesenteric lymph nodes and cytokines produced by peripheral blood mononuclear cells when stimulated *ex vivo* with MBP.

F.169 Sublingual Immunization with Adenovirus Encoding H5 and Conserved Protein Induces Broad Cross-protection Across Influenza Subtypes

Hae-Jung Park[1], Alexander Pereboev[2], Jeong S. Hong[2], Gye-Yeong Han[1], Byoung-Shik Shim[1], Man-Ki Song[1], Young-Ho Byun[3], Baik-Lin Seong[3], Cecil Czerkinsky[1], Huan H. Nguyen[1].[1]International Vaccine Institute, Seoul, Korea; [2]University of Alabama at Birmingham, Birmingham, AL; [3]Yonsei University, Seoul, Korea

The usefulness of influenza vaccines designed to inducing antibody (Ab) responses against viral surface antigens (hemagglutinin [HA] and neuraminidase [NA]) is limited by the ability of the virus to mutate these major antigenic glycoproteins. Vaccines that target determinants conserved among influenza A viruses to generate broad protection against infection with different influenza A subtypes; i.e. heterosubtypic immunity, remain elusive. Sublingual immunization has been found to be a safe and effective route for disseminating humoral as well as effector T cell responses to systemic and mucosal compartments, including the respiratory tract. In this study we generated a recombinant adenovirus (Ad) vector co-encoding HA (H5 subtype) and a conserved ectodomain of matrix protein 2 (M2e) (AdH5/M2e). This vaccine construct was evaluated as a potential sublingual vaccine against infections with H5 and other influenza virus subtypes. Mice were immunized sublingually or intranasally with live AdH5/M2e and subsequently challenged with homologous (H5) or heterologous (A/ H1N1) influenza virus subtype. We found that sublingual and intranasal administration of AdH5/M2e induced significant antibody and T cell responses. These responses were associated with protection against infection with H5 virus as well as other influenza A virus subtypes. In addition, sublingual or intranasal immunization with AdH5/M2e was found to activate dendritic cells in the lungs. The results underscore the usefulness of recombinant Ad vectors encoding both H5 and M2e as a potential vaccine against influenza viruses, including pre-pandemic H5N1 and newly emerging subtypes.

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