SOCIETY FOR MUCOSAL IMMUNOLOGY

ICMI2015

July 14th - 18th • Berlin, Germany • Maritim Hotel

17th International Congress of Mucosal Immunology

Establishing Equilibrium at Mucosal Surfaces

Abstract Supplement

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ANTIGEN UPTAKE

OR.61. Specific Expression of Phagocytosis and Membrane Ruffling Associated Molecule Aif1 by M Cells

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Microfold (M) cells are known as antigen uptake intestinal epithelial cells. It has been reported that transcription factor Spi-B acts as a pivotal transcription factor for the development of M cells with inducing M-cell-specific functional protein, Glycoprotein2 (GP2). The DNA microarray analysis of the follicle associated epithelium of Peyer's patches from $Spib^{+/+}$ and $Spib^{-/-}$ mice revealed M-cell specific expression of Aif1 associating with phagocytosis and formation of membrane ruffling in microglia and macrophages. Confocal microscopic analysis confirmed that Aif1 was specifically expressed in GP2-positive matured M cells. Therefore, we next investigated whether newly found Aif1 is involved in M-cell development and/or function. When the presence of M-cell was examined in Aif1-deficient mice, comparable numbers of M cells were found with wild-type (WT) mice suggesting that Aif1 is not involved in M-cell development. Contrary, however, Aif1-deficient mice showed significant lower particle antigen uptake compared with WT mice, suggesting that Aif1 has an essential role in M-cell transcytosis function. We are now investigating cellular and molecular mechanisms which might be involved in the Aif1-regulating antigen uptake by M cells.

OR.63. Antigen Uptake and Presentation by Mononuclear Phagocytes in the Peyer's Patch

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The gut-associated-lymphatic-tissue (GALT) contains mononuclear phagocytes (MPs) including dendritic cells (DCs) and macrophages. An important inductive GALT site is the Peyer's patch (PP). At least two different DC populations populate the PPs, one resides underneath the follicle-associated epithelium (FAE) covering the PP while the second inhabits T cell zones. MPs can sample luminal antigens which traverse microfold (M) cells located within the FAE. These antigens are then presented in the T cell zone. However, it is unclear how M cells and MPs cooperate as they sample antigen. It is also unclear whether the antigen is presented to T cells by the MPs that initially sampled it, or transferred to DCs found in the T cell zone. Using two-photon microscopy in live transgenic mice, we investigated the interactions between M cells, MPs and T cells in the PP. We began to investigate the interaction between MPs and M cells, capturing previously-unobserved behavior of MPs as they migrate within the epithelium, enter into microfolds and interact with M cells. Antigen uptake and T cell activation by MPs was assessed following administration of fluorescent antigens. MPs accumulated and presented particulate antigen *in vitro*, resulting in T cell proliferation. Particles injected to the intestines accumulated in MPs in the PP. Administration of soluble antigen activated T cells, which formed dynamic clusters in the T cell zones and upregulated CD69. These results present our abilities to follow MPs as they interact with M cells and T cells as they are activated within the PP.

W1. Development of Nano/Micro-Size Virus Like Particle (VLP) Based Human Papillomavirus (HPV) Vaccine to Treat Cervical Cancer

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Objective: Cervical is the second most cancer among women all over the world. Two HPV type-specific prophylactic vaccines are used in several countries world-wide. But these vaccines are expensive, require cold chain storage and trained personnel to administer injections. The goal of this project is to develop a particle based HPV vaccine that will be cost effective and can be used in resource poor countries. Method: The particulate vaccine containing VLPs was prepared in a simple one step spray drying process using a Eudragit polymer. The size, shape and surface morphology of the particles was determined by scanning electron microscopy (SEM). Within the particle, the presence of VLP was determined using SDS-PAGE analysis and quantified using Western blot. VLP conformation was ascertained by transmission electron microscopy (TEM). The vaccine was tested in an *in vivo* animal model using Swiss Webster mice and the antibody from serum was analyzed using ELISA. Results: The percent yield of particles after spray drying was 55%. The SEM image showed that the average size of the particles were 5 µm. The presence of intact VLPs was confirmed by TEM images. Western Blot analysis further confirmed the presence of L1 and showed that about 50% of the VLP was encapsulated. The animal study showed the significant ly high titer at week 8. Conclusions: Based on the

advantages of particulate vaccines, we envision that the current formulation would offer mucosal and systemic protection at multiple anatomic sites that are vulnerable to HPV infection and associated disease progression.

W2. Improving Trans-Mucosal Delivery of Peptides and Proteins by Carrier-Peptide Coupling

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Mucosal delivery of macromolecular drugs or antigenic material across the intestinal epithelium still remains a major challenge in drug development. However, efficient mucosal transport is essential for immune modulation in the small intestine. Therefore we investigated the potential of a newly discovered carrier peptide, namely the 13C-peptide. Immunohistochemical analysis of small intestinal tissue after injection of fluorochrome coupled 13C-peptide revealed enhanced peptide uptake by epithelial cells. Furthermore, application of 13C coupled peptide led to an efficient systemic distribution. To investigate the potential of 13C-peptide to enhance the intestinal transcytosis of coupled proteins, the model protein streptavidin was conjugated to 13C or a scrambled control peptide. While 13C-streptavidin was very efficiently taken up by small intestinal epithelial cells, streptavidin conjugated to the scrambled control peptide was not transported across the mucosa. Interestingly, 13C-protein construct was not only detected inside epithelial cells, but could also be visualized in CD11c positive cells in the villi as well as in Peyers Patches, suggesting that dendritic cells play a major role in the transport mechanism. As a next step we plan to conjugate anti-inflammatory cytokines to 13C-peptide to specifically target the inflamed intestinal mucosa. We believe that local treatment with 13C coupled cytokines can be a promising therapeutic tool in the treatment of inflammatory bowel disease (IBD).

W3. A Novel Role for IL-13 in Enteric Infections and Allergy

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IL-13 driven goblet cell (GC) hyperplasia and mucus hyper-secretion are characteristic of intestinal helminth infections and allergic responses. How IL-13 contributes to these processes is largely unknown. Intestinal GCs can form goblet cell associated antigen passages (GAPs) delivering luminal antigens to lamina propria dendritic cells (DCs) to shape gut immune responses. GAP formation occurs spontaneously in the small intestine in response to acetylcholine, but not in the colon where it is inhibited by the microbiota. We observed that IL-13 induced GAPs in the small intestine and colon by mechanisms independent of acetylcholine. IL-13 induced GAPs within 30 min of administration, and the effect lasted for 48h. The prolonged induction of IL-13 induced GAP formation resulted in increased luminal antigen delivery to lamina propria DCs as assessed by their ability to induced T cell proliferation. In contrast to GAP formation in response to acetylcholine, IL-13 induced GAPs were mediated via activation of CD38 and the downstream ADP ribose pathway. Here we describe that a cytokine associated with enteric infections and allergy, promotes trans-epithelial delivery of luminal antigens to induce immune responses. This function of IL-13 could play a central role in responses to helminths and allergic responses.

CELIAC DISEASE

OR.55. Microbiota Modulates Host Response to Dietary Gluten in NOD/DQ8 Mice

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Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically susceptible individuals expressing the HLA-DQ2 or DQ8 genes. Dysbiosis has been described in patients with CD, but it is unknown whether these microbial changes are a CD-promoting factor. We therefore took a gnotobiotic approach to investigate the influence of the microbiota on host responses to gluten in adult NOD/DQ8 mice, a model of gluten-sensitivity. Both conventional specific pathogen free mice, which harbor opportunistic bacteria including Proteobacteria, and germ-free mice developed gluten-induced small

intestinal inflammation, characterized by increased intraepithelial lymphocytes, decreased villous-to-crypt ratios, and gluten specific immune responses. However, mice colonized with a limited, defined microbiota devoid of opportunistic pathogens and Proteobacteria (altered Schaedler flora; ASF) were protected from gluten-induced small intestinal inflammation and gluten-specific immune responses. Protection in ASF-colonized mice was reversed by colonization with Pseudomonas aeruginosa, a member of the Proteobacteria phylum, isolated from the small intestine of an active CD patient. These results provide evidence of modulation of host responses to gluten by intestinal microbiota. The presence or absence of specific opportunistic pathogens may promote or prevent gluten-induced inflammation in a genetically susceptible host.

OR.56. Celiac Disease-Related Inflammation is Marked by Reduction of NKp44/NKp46-Double Positive Natural Killer Cells
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Natural killer (NK) cells are the first line of defense against viruses and down-regulation of NK cell cytotoxic receptors represents one of the strategies by which viruses escape the immune system. NK cells are supposed to contribute to the intestinal epithelial damage in celiac disease (CD), whose onset has been associated with viral infections. However, it remains unclear whether CD-associated inflammation is characterized by abnormal distribution of NK cell receptors recognizing viral-infected cells. Here, we characterized the tissue distribution of NK cells in CD.NK cell markers were analyzed in intraepithelial lymphocytes(IELs)isolated from duodenal biopsies of CD patients(both active and inactive)and healthy controls(HC)and jejunal specimens of patients undergoing gastro-intestinal bypass by flow-cytometry. Cytokines were assessed in cells either freshly isolated or stimulated with IL-15, IL-21 and IFN-α.The percentage of total NK cells and NKT cells did not significantly differ between CD patients and HC and no alteration in the expression of NKG2D, NKG2A and HLA-E was seen in CD. The fractions of NK cells and NKT cells expressingNKp30 were slightly, but not significantly, increased in active CD. Although the percentage of NK cells and NKT cells expressing either NKp44 or NKP46 did not differ between CD and controls, the fraction of NK cells and NKT cells expressing both these cytotoxic receptors was significantly decreased in CD compared to controls.NKp44/NKp46-double positive cells produced granzymeB, but not TNF-α,IL17 or IL-22,and such a production was increased by IL-15,but not IL-21 or IFN-α.Data indicate that NKp44/NKp46 double positive NK cells and NKT cells producing granzymeB, a subset of cells involved in recognition of viral-infected cells, are significantly decreased in active CD.

W4. Cell Death in Small Intestine Mucosa in Active Celiac Disease
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Celiac disease (CD) is an immune-mediated enteropathy that develops in genetically susceptible individuals following gluten ingestion. Loss of epithelia, crypt hyperproliferation and lymphocytic infiltration are characteristics findings in active CD. The major histological change, villus atrophy, is considered a consequence of increased enterocyte apoptosis. Mechanisms including FAS/FASL and MICA/NKG2D interactions, between enterocytes and cytotoxic cells, have been reported in the CD lesion. Though different pathways of cell death have been described, there is no detailed description of the role of these mechanisms in small intestine. Here, we have studied the cellular stress and signaling pathways associated with apoptosis, necroptosis and inflammation in active CD. Studies by quantitative PCR, confocal microscopy, westernblot and flow cytometry were performed in duodenal biopsies from CD patients and control subjects. We observed higher proliferative response in the crypts, and increased cellular stress but not $TUNEL^+$ cells in differentiated epithelium. In addition, caspase 3 and PARP1 showed higher levels in active CD mucosa accompanied by increased caspase 1. RIPK3, a marker of necroptosis, was found in cells in the crypts in untreated CD. Our results suggest that different cell death pathways, not only apoptosis, might operate in the small intestine mucosa in untreated CD and can be also linked to pathways of chronic inflammation.

W6. Changes in Duodenal Innate Lymphocyte Subsets Indicate a Shift Towards a Pro-Inflammatory State in Celiac Disease
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Innate lymphoid cells (ILCs) comprise distinct subsets of immune cells with unique functions. Although alterations in the frequencies of certain ILCs have been reported in inflammatory diseases, including Crohn's disease, their roles in celiac disease (CD) pathogenesis have not been adequately explored. Hence, we investigated the frequency, phenotype and transcription factor and cytokine profiles of ILC populations in patients with newly diagnosed or active celiac disease (ACD) and those on a gluten-free diet (GFD). Intraepithelial lymphocyte fractions isolated from proximal small intestinal (duodenal) biopsies from ACD (n=7) and GFD (n=9) patients and normal controls (n=11), were analyzed. Lineage-negative (Lin-) $CD103^+$ cells comprised two ILC1 populations, $NKp44^+CD56^+$ and $NKP44^-CD56^-$. The percentage and absolute numbers of $NKp44^+CD56^+$ ILCs were significantly reduced in ACD (%: 4.5±2.7, p<0.0001; abs#: 466±198, p<0.001) and GFD (%: 7.2±2.2, p<0.0001; abs#: 2239±1482, p<0.02) compared to controls (%: 49.9±6.3; abs#: 11608±2987). On the contrary, $NKP44^-CD56^-$ ILCs were increased in ACD (%: 63.6±4.0, p<0.0001; abs#: 8360±2485, p=0.08) and GFD (%: 33.8±3.8, p=0.025; abs#: 5877±1875, p=0.29) compared to controls (%: 19.4±4.0; abs#: 4180±941). Upon *in vitro* stimulation with PMA and ionomycin, $NKp44^+CD56^+$ ILCs did not secrete IFN-γ, IL-17, IL-22 or IL-5 and they lacked expression of ILC subset-specifying transcription factors. In contrast, $NKP44^-CD56^-$ILCs expressed T-bet and a significant fraction (34.18± 6.6%) produced IFN-γ. Our findings indicate heterogeneity within the ILC1 subset in the duodenal epithelium. The observed changes in ILC composition, especially an increase in the $NKp44^-CD56^-$ ILC1 population, suggest that ILCs may contribute to the ongoing inflammation in CD.

DENDRITIC CELLS

OR.1. The Lymph Nodes Draining the Small Intestine and Colon are Anatomically Separate and Immunologically Distinct
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Dendritic cells (DCs) in the small intestine (SI) and colon are fundamental to direct intestinal immune responses; they migrate to the mesenteric lymph nodes (MLN) and prime T cells. Using photo-convertible Kaede mice we demonstrate anatomical segregation of lymphatic drainage from the intestine, specifically that DCs from the SI and colon migrate to different nodes within the MLN, here called the SI draining sMLN and colon draining cMLN. As a consequence, the different frequencies of phenotypically and functionally distinct DC subsets observed in the SI and colon are reflected among the DCs in the sMLN and cMLN. Consistent with the SI's function in absorbing food, fed antigen is presented only in the sMLN, but not the cMLN. Furthermore, the levels of expression of CCR9 and $\alpha_4\beta_7$ are increased on T cells in the sMLN compared to the cMLN. DCs from the cMLN and colon are unable to metabolize vitamin A to retinoic acid; thus DCs may contribute to the differential expression of tissue homing markers observed in the sMLN and cMLN. In summary, the sMLN and cMLN and the DCs that migrate to these lymph nodes are anatomically and immunologically separate. By separately analyzing the appropriate nodes of the mesenteric chain, we have identified changes in cell populations that have previously been missed. This non-overlapping lymphatic drainage from the SI and colon to distinct parts of the MLN provides a mechanism by which immune responses in these functionally, anatomically, and immunologically specialized tissues can be independently controlled.

OR.2. What Shapes Intestinal Dendritic Cells into Critical Gatekeepers of TGFß-Dependent T Cell Responses in the Gut
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Induced regulatory T cells (iTregs) and Th17 cells are critical components of intestinal immune responses and breakdown of their balance has been associated with the development of Inflammatory Bowel Disease. Interestingly, development of both iTreg and Th17 cells requires TGF-β, which thus has a critical role in maintenance of gut immune homeostasis. Importantly TGF-β is ubiquitously expressed in an inactive latent form and must be activated before it can signal to T cells. TGF-β signaling to T cells requires activation of latent TGF-β by αvβ8 integrin on DCs. Recently, we have shown that under homeostatic conditions, β8 is

highly restricted to CD103$^+$ DCs from MLN, conferring on these cells their preferential ability to activate TGF-β and generate Tregs. However, the precise mechanisms by which DCs acquire this specialized ability to activate TGF-β and orchestrate T cells responses are unknown. Here we present evidence that signals from the mucosal microenvironment promote induction of β8 expression in DCs, hence its preferential expression in DCs derived from the gut. This is dependent on both immune-, dietary- and microbiota-derived factors, and mice deficient in their downstream signaling present a modified pattern of β8 expression in DCs. Furthermore, we provide evidence that cell lineage participates in establishing the precise expression profile of β8 by modulating the ability of DC subsets to differentially respond to these signals. Together these data show that combination of lineage, environment and immune factors shape intestinal DCs into critical gatekeepers of TGF-β-dependent immune responses through regulation of β8 integrin expression.

OR.3. Lymph Borne CD8α$^+$ DCs Are Uniquely Able to Cross-Prime CD8$^+$ T Cells with Intestinal Epithelial Cell-Derived Antigen

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Cross-presentation of cellular antigen is crucial for priming CD8$^+$ T cells, and generating immunity to intracellular pathogens-particularly viruses. It is unclear which intestinal phagocytes perform this function *in vivo*. We therefore examined dendritic cells (DCs) from intestinal lymph of IFABP-tOVA 232-4 mice, which express ovalbumin in small intestinal epithelial cells (IECs). Among lymph DCs (LDCs), only CD103$^+$ CD11b$^-$ CD8α$^+$ DCs cross-present IEC-derived ovalbumin to CD8$^+$ OT-I T cells. Similarly, in the mesenteric lymph nodes (MLN), cross-presentation of IEC-ovalbumin was limited to the CD11c$^+$ MHCII^{hi} CD8α$^+$ migratory DCs, but absent from all other subsets, including the resident CD8α^{hi} DCs. Crucially, delivery of purified CD8α$^+$ LDCs, but not other LDC subsets, into the MLN subcapsular lymphatic sinus induced proliferation of ovalbumin-specific, gut-tropic CD8$^+$ T cells *in vivo*. Finally, in 232-4 mice treated with R848, CD8α$^+$ LDCs were uniquely able to cross-prime IFN-γ-producing CD8$^+$ T cells and drive their migration to the intestine. These results clearly demonstrate that migrating CD8α$^+$ intestinal DCs are indispensable for cross-presentation of cellular antigen and, in conditions of inflammation, for the initial differentiation of effector CD8$^+$ T cells. They may therefore represent an important target for the development of anti-viral vaccinations.

OR.81. Nasal Tissues Contain Unique Subsets of Dendritic Cells with Distinct Phenotypic Characteristics and Functional Behavior

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Intranasal (i.n.) vaccination generates immunity across local, regional and distant sites. However, nasal dendritic cells (DC), pivotal for the induction of intranasal vaccine- induced immune responses, have not been studied in detail. Here, using a variety of parameters, we define nasal DCs in mice and humans. Distinct subsets of "classical" DCs, dependent on the transcription factor zbtb46 were identified in the murine nose. The murine nasal DCs were FLT3 ligand-responsive and displayed unique phenotypic and functional characteristics including the ability to present antigen, induce an allogeneic T cell response and migrate in response to LPS or live bacterial pathogens. Importantly, in a cohort of human volunteers, BDCA-1$^+$ DCs were observed to be the dominant nasal DC population at steady state. During chronic inflammation, the frequency of both BDCA-1$^+$ and BDCA-3^{hi} DCs was reduced in the nasal tissue, associating the loss of these immune sentinels with chronic nasal inflammation. The present study is the first detailed description of the phenotypic, ontogenetic and functional properties of nasal DCs and will inform the design of preventative immunization strategies as well as therapeutic modalities against chronic rhinosinusitis.

OR.82. Regional Differences in Dendritic Cell Subsets, Phenotype and Function in the Healthy Human Colon

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Information concerning dendritic cell (DC) distribution and function throughout the healthy human gastrointestinal (GI) tract is scarce. Eighty-four paired sets of proximal (right/ascending) and distal (left/descending) human colonic biopsies from healthy subjects were taken; DC subsets, phenotype and function were assessed by flow cytometry and microbiota composition assessed by 16S rRNA gene sequencing. Colonic DC ($CD45^+DR^+lineage^-$) were myeloid ($CD11c^+CD123^-$) and further distinguished according to CD103 and SIRPα expression: $CD103^-SIRP\alpha^+$ DC predominated and together with $CD103^+SIRP\alpha^+$ DC were $CD1c^+ILT3^+$. By contrast, $CD103^+SIRP\alpha^-$ DC constituted a minor subset of $CD141^+ILT3^-$ cells. Proximal colon had higher numbers of DC and fewer $CD103^+SIRP\alpha^+$ cells. Proximal colonic DC were also more mature than distal DC with higher stimulatory capacity for $CD4^+CD45RA^+$ T cells. However, DC and DC-invoked T cell expression of mucosal homing markers (β7, CCR9) was lower for proximal DC paralleled by lower e-Cadherin and CCL25 mRNA expression. Proximal colon produced higher levels of cytokines and carried a lower microbiota load but with no differences in microbiota composition between compartments. DC numbers were also higher in proximal than distal colon of C57BL/6 male mice but differences were abrogated in the absence of the microbiota in germ-free animals. Proximal colonic DC subsets differ from those in the distal colon being more mature. Studies addressing the immune system of the GI-tract should therefore reflect immune compartmentalization throughout the colon.

OR.83. TGFβ Promotes the Development of $CD103^+$ $CD11B^+$ DCs in the Intestine

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Dendritic cells (DC) play a key role in regulating intestinal immune responses. The intestine contains a unique subset of DCs that expresses both CD103 and CD11b, but it is unclear what factors in the local environment might drive the differentiation of such unusual DCs. TGFβ is highly abundant in the intestine and induces the expression of CD103 on T cells. Here we show that deletion of the TGFβRI on $CD11c^+$ cells leads to a selective defect in $CD103^+CD11b^+$ DCs in the small and large intestinal mucosa, with preserved numbers of $CD103^-CD11b^+$ DCs. The defect in $CD103^+CD11b^+$ DCs was cell intrinsic and was not due to altered migration of intestinal DCs, as identical reductions occurred in mucosa and draining lymph nodes. Isolated down regulation of CD103 does not explain the apparent defect, as microarray and phenotypic analyses showed reduced expression of other markers normally specific to this population, including Siglec F and TREM-1. Preliminary studies indicate that $CD11b^+$ DCs from CD11c-cre-TGFβRI mice have a defective ability to induce the generation of $FoxP3^+$ Treg. Together our data indicate that TGFβ plays a crucial role in the terminal differentiation of potentially tolerogenic $CD103^+CD11b^+$ DCs from a $CD103^-CD11b^+$ precursor in the intestine.

OR.84. IRF8-Dependent DCs Play a Key Role in the Regulation of CD8 T Cell Responses to Epithelial-Derived Antigen in the Steady State but not in Inflammation

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The intestinal immune system has the complex task of generating tolerance towards harmless antigens derived from our diet, commensal microflora or tissue, while maintaining the ability to mount protective immune responses to mucosal pathogens. Much of our understanding regarding the regulation of mucosal T cell responses stems from studies on $CD4^+$ T cells. However, the intestinal mucosa is a major entry site for intracellular pathogens, whose control requires cross-presentation of cell-associated antigens for the induction of protective $CD8^+$ T cell responses. To assess the regulation of mucosal $CD8^+$ T cell priming and differentiation in the steady state and inflammatory setting, we utilized IFABP-tOva mice, in which Ovalbumin (Ova) is expressed as an epithelial-derived antigen in the small intestine. In this model Ova-specific $CD8^+$ T cells were found to differentiate into two distinct subsets, $CD107a/b^+$ cytotoxic T cells (CTLs) and $FoxP3^+$ $CD8^+$ T cells with regulatory potential. Interestingly, neither IRF8 nor

IRF4 expression by intestinal dendritic cells (DCs) was crucial for the expansion of CTLs. In contrast, presence of IRF8- but not IRF4-dependent DCs was critical for the development of FoxP3$^+$ CD8$^+$ T cells in the steady state. However in the inflammatory setting, expansion of the FoxP3$^+$ subset was not affected by the absence of IRF8-dependent DCs, suggesting that other subsets of intestinal antigen presenting cells (APCs) can compensate their function in an inflammatory milieu. Collectively these findings further our understanding of the mechanisms regulating CD8$^+$ T cell responses in the intestinal mucosa and have potential implications for mucosal vaccine design.

W7. The Role of Intestinal Dendritic Cells in Inducing Th2 Responses to Injected *Schistosoma mansoni* Eggs
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Schistosoma mansoni eggs (SME), which are the main cause of chronic Schistosomiasis, provoke a strong Th2 immune response that can lead to granulomatous reactions and fibrosis in affected organs. Recently, CD11c depletion has been shown to severely disrupt Th2 immune responses against Schistosoma mansoni, suggesting that antigen presenting cells, which express CD11c, mediate this immune response. Dendritic cells (DCs) are the most likely CD11c-positive candidates, as they continuously migrate from epithelia, carrying antigens, to the draining lymph nodes where they activate naive T cells. However, the precise roles of DCs in inducing immune responses against SME have not been studied. We have developed an *in vivo* mouse model where we inject SME in the subserosal layer of the small intestine of C57BL/6 mice, the anatomical location where SME naturally become lodged. This provokes a Th2 response in the draining mesenteric lymph nodes (MLNs). To test the role of DCs in this response, we will purify migrating DCs from egg challenged mice and control animals and then inject these cells into the subcapsular region of the MLN of naive mice. Detection of an antigen-specific Th2 response in those mice will indicate that migratory DCs are sufficient to carry and present egg antigens and drive Th2 responses in our model. This knowledge will contribute to a better understanding of how the early immune response against SME is mediated. This information can be applied to battle chronic Schistosomiasis and may help understand Th2 driven responses against other parasites or Th2 allergic responses.

W8. Polyphenol Administration Impairs T Cells Proliferation by Imprinting a Distinct Dendritic Cell Maturational Profile
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Intestinal immune cells are exposed to numerous factors able to imprint a specific maturational profile to intestinal resident Dendritic Cells (DCs). Those factors include nutritionally derived compounds whose effects are still to be completely characterized. In the present study, we examined the effects of DCs exposure to Quercetin and Piperin Reconstituted Oil Bodies (ROBs-QP). In particular we studied the LPS induced maturation of DCs exposed or not to ROBs-QP. ROBs-QP administration skews the DCs maturational profile towards an alternative impaired inflammatory response. By microarray analysis we compared LPS response of ROBs-QP Vs vehicle treated DCs. We obtained a decreased ratio between pro- Vs anti-inflammatory cytokines, an impaired chemokine receptor switch, a decreased ability to recruit T cells and present antigens. This profile is unique of the ROBs-QP exposed DCs. Finally, by two-photon intravital microscopy and OT-II adoptive transfer we demonstrate that ROBs-QP administration reduces the number of stable immunological synapse and, consequently, antigen specific T cells divisions. Our data demonstrate that polyphenols exposure can educate DCs towards a new anti-inflammatory molecular profile responsible for dampening the inflammatory response, thus paving the way for complementary nutritional approaches for the treatment of chronic inflammatory syndromes.

W9. The Role of CD103$^+$ Conventional Dendritic Cells in Host Antiviral Responses in the Gut
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Dendritic cells (DCs) are key modulators of the immune response. DCs are heterogeneous cells and can be divided into several subsets with unique functions. In this context, which DC subset(s) has the capacity to elicit adaptive immune responses during viral challenge at the mucosa is not fully understood. Rotavirus (RV) almost exclusively infects and replicates in the small intestinal villi, resulting in both cellular and humoral adaptive immunity. We used a RV infection mouse model to study the DC-elicited antiviral responses in the gut. By using diphtheria toxin (DT) - treated CD11c-DTR and Zbtb46-DTR chimeric mice, we demonstrated that conventional DCs (cDCs) were crucial in RV-specific CD8$^+$ T cell activation and proliferation as well as RV clearance. Deficiency of

$CD103^+CD11b^-$ cDCs ($Batf3^{-/-}$ mice) can partially recapitulate the phenotype found in DT-treated Zbtb46-DTR chimeric mice, suggesting that $CD103^+CD11b^-$ and $CD103^+CD11b^+$ cDC subsets cooperate with each other in defending against this viral pathogen. Ongoing work is examining the RV response in huLangerin-DTA mice, whose $CD103^+CD11b^+$ cDCs are absent while $CD103^+CD11b^-$ cDC subset remains intact. Given the importance of RV infection in children, our future direction will focus on dissecting the role of gut DCs under RV challenge in neonatal mice.

W10. Intestinal Mucin-Dendritic Cell Crosstalk in Gut Homeostasis

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Intestinal dendritic cells (DCs) are essential in sampling luminal antigens and promoting the appropriate immune response to different signals present in the intestinal environment. Intestinal DCs are in close contact with intestinal mucus, a protective barrier mainly composed of the goblet cell-secreted mucin MUC2, which may also play important regulatory functions. Thus, a possibility is that interactions between DCs and mucins modulate DC function, with recent published data suggesting that mucins may trigger anti-inflammatory pathways in DCs. To identify important pathways by which DCs are modulated by intestinal mucins, MUC2 obtained from intestinal cell lines and mouse intestinal mucin was purified and used to treat human monocyte-derived DCs. We now find that expression of pro-inflammatory markers CD86 and CD83 is significantly upregulated on human DCs in the presence of human MUC2 and mouse intestinal mucins, together with the expression of the pro-inflammatory chemokine interleukin 8 (IL-8). Additionally, IL-8 produced by mucin-treated DC is able to enhance neutrophil migration *in vitro*. Thus, in contrast to recent published results, we find that intestinal mucins are capable of inducing important pro-inflammatory functions in DC. Further investigation is therefore required to explore mucin-DC interactions during health and infection.

W11. Characterization of Human Fetal Intestinal Dendritic Cells

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The gastro-intestinal tract harbors 10^{14} commensal bacteria, but also an active immune system to challenge harmful pathogens. Disturbance of this fine balance contributes to the development of inflammatory bowel disease (IBD) via the induction of commensal-specific T cell-mediated immune responses. T cell responses are initiated by antigen-presenting dendritic cells (DCs) that sense and take-up microbes in the tissues that line epithelia. In adults, 3 different intestinal DC subsets have been defined, $CD103^+CD141^+SIRP\alpha^-$ cross-presenting DCs, $CD103^+$ $SIRP\alpha^+$ tolerogenic DCs and $CD103^-SIRP\alpha^+$ DCs of which the function is less clear. However, the development of human intestinal lamina propria DCs remain poorly understood. Here, we analyzed the phenotype and function of the human fetal gut DCs. Our preliminary results show that $CD103^+CD141^+SIRP\alpha^-$ subset is absent in human fetal intestinal lamina propria, while this subset is an important cross-presenting cells in the adult human gut. $CD103^-$ SIRP α^- dendritic cells are abundantly present in fetal gut, which is absent in adult human gut, which may be precursors of various mucosal DC subsets. Meanwhile, fetal intestinal DCs only produce cytokines in response to PGN, but not to LPS and poly(I:C). CD103-SIRP α^+ subset is able to produce more cytokines and more capable to induce proliferation in naïve $CD4^+$ T cells than the other two subsets. The understanding of the role of these subsets and the development of human intestinal DCs will help to define strategies for the treatment of intestinal pathologies and contribute to improved design of mucosal vaccines.

W12. Loss of TGFβ Signaling in Dendritic Cells Leads to Increased Susceptibility to Epithelial Injury and Deficient Production of IL-22 and IL-17a by the Innate Immune Cells

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Selective loss of TGFβ signaling in dendritic cells ($Tgfbr2^{\Delta DC}$, JI 2012,189:3878) leads to increased activation of T cells and autoimmunity. DCs interact with other innate immune cells to provide the first line of defense against infection and translocation of commensals during mucosal injury. The mechanisms that mediate this crosstalk are not clearly understood. Cre^- and Cre^+ $Tgfbr2^{\Delta DC}$ mice on $Rag2^{-/-}$ background (DKO) were evaluated for their susceptibility to 2% dextran sodium sulfate (DSS). Compared to DSS-treated Cre^- mice, DSS-treated Cre^+ mice exhibited significantly more severe phenotype with accelerated and greater body weight loss. Histological analysis revealed severe inflammation and infiltration of the distal colon, extensive mucosal erosions cells and a

complete loss of goblet cells. Despite the similar neutrophilic infiltration based on the H&E analysis, immunohistochemistry, and mucosal MMP8 mRNA expression, contrary to Cre^- DKO, Cre^+ mice failed to upregulate IL-22 and IL-17a, cytokines implicated in mucosal protection during epithelial barrier breach. Although the exact mechanism and the type of innate immune cells affected are still under investigation, loss of TGFβ signaling in DCs as an initial steps in mucosal inflammation may lead to insufficient initial protective innate immune responses and contribute to the establishment of chronic disease.

W13. TLR4/TRIF/IL15-Dependent Activation Leads to Diminished TGFβ Signaling in Dendritic Cells; Contribution to T Cell-Mediated Autoimmune Inflammation in the Gut

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TGFβ signaling in DCs is critical for the maintenance of self-tolerance. Here, we describe that in experimental colitis, DCs migrating to mesenteric lymph nodes show diminished levels of pSmad2. LPS- or poly(I:C)-induced activation of bone marrow-derived DC *in vitro* leads to refractory response to TGFβ stimulation in a TRIF-dependent and MyD88-independent fashion. Maturation of BMDC was associated with increased expression of IL15 and IL15Rα, and antibody blocking IL15/IL15Rα complex restored post-LPS TGFβ signaling. To determine the consequences of reduced TGFβ in DC in T cell mediated colitis, we crossed $Tgfbr2^{\Delta DC}$ mice (JI 2012,189:3878) with $Rag2^{-/-}$ mice and adoptively transferred $CD4^+$, $CD8^+$, and $CD3^+$ T cells from naïve mice. Only $CD4$ (or 1:1 mix $CD4^+$ + $CD8^+$) T cells were required to induce colonic pathology, in addition to pancreatitis and hepatitis. $CD3^+$ T cell transfer induced a significant increase in cDC and pDC expression of MHCII, CD80, CD86, and CD40 in Cre^+ mice. Co-transfer of WT $CD8^+$ with $CD40L^{-/-}CD4^+$ T cells did not induce auto-inflammatory response in recipient mice. In conclusion, DC maturation results in IL15-mediated refractory response to TGFβ. Reduced TGFβ signaling in DC leads to $CD4^+$ and $CD8^+$ T cell-dependent inflammation and requires intact CD40L expression in $CD4^+$ lymphocytes.

W14. Interplay of Plasmacytoid and Conventional cDC1 in the Secretory IgA Response to Rotavirus

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Rotavirus (RV), a double-stranded RNA-virus, primarily infects the small intestinal epithelium. RV specific secretory IgA is sufficient to mediate long-term protection from reinfection and resolution of primary infection in mice and likely in humans. Dendritic cells (DCs) guide the initiation of adaptive immunity. We have previously shown that plasmacytoid dendritic cells (pDCs) are necessary for optimal RV-specific IgA induction following infection, a process depending on the production of type I IFNs by pDCs. We now show that BATF3 deficient mice lacking $CD103^+CD11b^-$ conventional dendritic cells (intestinal cDC1 DCs) also show diminished RV-specific IgA responses, suggesting a role for these accessory cells in viral clearance through humoral immunity induction. Interestingly, full activation of cDC1 conventional dendritic cells in response to RV infection depends on both pDCs and TLR3. The gut is a highly immune challenged organ, requiring careful fine-tuning of immune responses to avoid inflammation in the absence of pathogens. A dual requirement for 1) cDC stimulation by type I IFNs from responding pDCs, and 2) simultaneous direct TLR signaling within cDC for full cDC activation in response to RV may function as a safety net to avoid aberrant inflammation of the gut wall.

W15. A New Antigen-Uptaking Macrophage-Like Cell Population Contacts $CD103^+$ Conventional Dendritic Cells Around Airways

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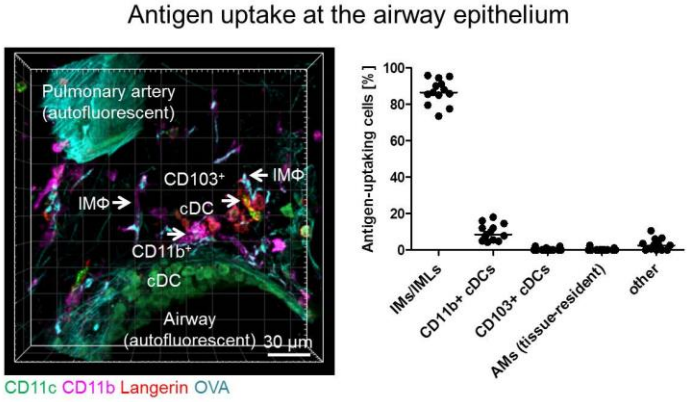

Primary data of DC localization in the lung is patchy. Because cell localization is directly linked to cell function, we aimed to localize DC and macrophage (MΦ) subtypes and determine the antigen-uptaking population by immunohistochemistry. Using an immunohistochemistry-optimized panel of markers we identified $CD11b^+$ and $CD103^+$ conventional (c)DCs, alveolar macrophages

(AMΦs) plasmacytoid (p)DCs and monocytic (mo)DCs. We observed the accumulation of $CD11b^+$ and $CD103^+$ cDCs between the airway and pulmonary artery, but $CD103^+$ cDCs were rarely found in the airway epithelium itself. Interestingly, around blood vessels and airways, $CD103^+$ cDCs were often in close contact with a previously unrecognized $CD11b^+CD11c^-MHC^+$ cell population, which resembles interstitial macrophages. To examine functional differences in pulmonary DC populations, antigen uptake was examined following administration of labeled ovalbumin (OVA) *ex vivo*. After OVA administration AMΦs were the main antigen-uptaking population in the lung. Interestingly, most of the OVA around the airways was taken up by the new interstitial macrophage like (IML) population. As a similar population has been described to induce tolerance in the gut via cross-talk with $CD103^+$ cells, we speculate that our novel antigen-uptaking IML cell population may similarly regulate tolerance in the lung.

W16. Fc Alpha Receptor I Co-Stimulation Promotes Inflammatory Responses by Human $CD103^+$ Mucosal Dendritic Cells

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Maintaining a proper balance between inflammation and tolerance is essential in the gastrointestinal tract due to the high concentrations of residing bacteria. Disturbance of this delicate balance can lead to over-activation of immune responses as observed in inflammatory bowel disease (IBD). Crucial for regulation of intestinal tolerance are $CD103^+$ dendritic cells (DC), which reside in the lamina propria of the gut. However, upon infection of the lamina propria the local tolerogenic response should be converted into a pro-inflammatory response. Yet, whether and how this requires alterations in $CD103^+$ DCs or activation of other cells is still largely unknown. Here we have identified a specific combination of stimuli that promotes inflammatory responses by $CD103^+$ DCs. Bacteria that enter the lamina propria are rapidly opsonized by IgA, which subsequently bind to and activate Fc alpha receptor I (FcαRI) on $CD103^+$ DCs. While stimulation of FcαRI alone did not affect monocyte-derived $CD103^+$ DC responses, FcαRI stimulation synergized with Toll-like receptors for the selective production of pro-inflammatory cytokines TNFα, IL-1β, IL-6 and IL-23. Strikingly, FcαRI-induced cytokine production was completely independent of gene transcription, but instead was mediated by modulation of gene translation. Taken together, we have identified a specific combination of stimuli that promotes pro-inflammatory cytokine production by $CD103^+$ DCs, which involves the antibody IgA and its main receptor FcαRI. Our identification of the molecular mechanism behind this process could be important in the pathogenesis of IBD and may provide potential new targets for treatment of this disease.

W17. Loss of Hypoxia Inducible Factor 1 in Dendritic Cells Leads to Impaired Activation of Protective Regulatory T Cells in Murine Colitis

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Dendritic cells (DCs) play a crucial role in connecting innate and adaptive immunity and in maintaining intestinal homeostasis. DCs are highly involved in the pathogenesis of inflammatory bowel disease (IBD). IBD is associated with hypoxic inflammation where gene expression in DCs is regulated by the transcription factor hypoxia inducible factor (HIF)-1. Recent studies have described a protective role for epithelial HIF-1 in mouse models of IBD. To investigate how HIF-1α, the regulatory subunit of HIF-1, in DCs influences the development of an experimental colitis, control mice ($HIF\text{-}1\alpha^{+f/+f}$) and knock-out mice, which had a deficiency of dendritic HIF-1α ($CD11cCre/HIF\text{-}1\alpha^{+f/+f}$), were treated with 3 % dextran sodium sulfate for 7 days to induce colitis. Knock-out mice showed significantly higher weight loss at day 6 and 7 of the experiment. Colonic expressions of pro-inflammatory cytokines and mucin production were significantly increased in knock-out mice. Induction of regulatory T cells was impaired, and the number of forkhead box P3 regulatory T cells, which have protective effects on colitis, was diminished by dendritic HIF-1α knock-out. Our findings demonstrate that in DCs HIF-1α is necessary for the induction of sufficient numbers of regulatory T cells to control intestinal inflammation.

W18. Intraepithelial Processes of Dendritic Cells in Pigs Exposed to the Mycotoxin Deoxynivalenol

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The mycotoxin deoxynivalenol (DON) is contaminating crops and has effects on the intestinal immune system. DON-exposure inhibits maturation and function of dendritic cells (DC), thus affecting the initiation or suppression of a mucosal immune response (Bimczok et al., Immunobiology, vol. 212 pp 655-666, 2007). There is evidence that DC extend processes into the gut epithelium to sample luminal antigens. We conducted an *in vivo* study with pigs (~40kg BW) fed a DON-containing diet (4 mg DON/kg feed) and infunded with either NaCl or LPS (7.5 µg/kg BW) at the end of the experiments. Paraffin-embedded sections of jejunum were analyzed microscopically with MHC II and laminin antibodies to estimate DON effects on intraepithelial DC-processes. Signals of intraepithelial MHC II immunoreactivity indicating DC-processes per 1000 µm basement membrane were counted in the epithelium. In DON-fed pigs the immunoreactivity was markedly reduced in comparison to control animals. After systemic intravenous LPS challenge the signal frequency was higher in control-fed pigs than in DON-fed. Further work is under progress to characterize the intraepithelial MHC II positive processes with respect to the interaction between DC and epithelial cells.

W19. IRF8-Dependent Migratory CD103$^+$CD11b$^-$ Dendritic Cells are Required for Intestinal Intraepithelial Lymphocyte Homeostasis

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CD103$^+$CD11b$^-$ IRF8 dependent dendritic cells (DCs) represent one of the major migratory DC subsets in the intestinal mucosa however their role in intestinal immune homeostasis remains unknown. To assess this we generated CD11c-Cre.Irf8$^{fl/fl}$ mice which lacked CD103$^+$CD11b$^-$ DCs in the small intestine and draining mesenteric lymph nodes (MLN) and had normal numbers of migratory CD103$^+$CD11b$^+$ intestinal DCs. CD11c-Cre.Irf8$^{fl/fl}$ mice displayed a dramatic reduction in 'conventional' small intestinal CD8αβ$^+$TCRαβ$^+$ intraepithelial lymphocytes (IEL), one of the major pools of conventional tissue resident memory T cells in the body. In contrast numbers of 'natural' CD8αα$^+$TCRαβ$^+$ and CD8αα$^+$TCRγδ$^+$ IEL remained relatively unperturbed. In an adoptive transfer model, CD8αβ$^+$ ovalbumin (OVA) -specific OT-I cells primed in the MLN of CD11c-Cre.Irf8$^{fl/fl}$ mice expressed reduced levels of the gut homing receptors CCR9 and α4β7 and the homing of these cells to the intestinal epithelium was severely compromised. CD11c$^-$Cre.Irf8$^{fl/fl}$ mice also completely lacked CD4$^+$CD8αα$^+$ IEL a population of conventional CD4$^+$ T cells that have acquired 'CD8 T cell like' CTL activity. Using mixed bone marrow chimeras we found that expression of the TGFβ convertase αvβ8 integrin by intestinal CD103$^+$CD11b$^-$ DCs was critical for the generation of CD4$^+$CD8αα$^+$ IEL. Notably the intestine of Irf8$^{-/wt}$ mice, which lack lymph node-resident but not intestinal derived IRF8 dependent DCs, had a normal IEL subset composition. Collectively our results demonstrate a non-redundant role for migratory CD103$^+$CD11b$^-$ DCs in intestinal intraepithelial lymphocyte homeostasis.

W20. Investigating the Role of *Escherichia coli* in Perpetuating Inflammation in Crohn's Disease

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Crohn's disease (CD) is characterized by chronic intestinal inflammation and dysbiosis. Intestinal dendritic cells (DCs) prime and shape mucosal T cell responses, and a defect in the myeloid compartment in CD implicates them in its pathogenesis. Recent work has linked Escherichia coli with perpetuating inflammation in CD. To investigate how interaction between E. coli and DCs may maintain chronic intestinal inflammation, we characterized colonization dynamics of a strain of E. coli isolated from a CD patient, NRG857c, in C57BL/6 mice. Mice were treated with streptomycin 24 h before oral infection with NRG857c. After 7 days, infected mice showed faecal shedding at 10^7 CFU g^{-1}. NRG857c was recovered from all intestinal tissues, but caecum and colon were most heavily colonized (>10^6 CFU g^{-1}). As little is known about DC subsets in the murine caecum, we examined this tissue and show that caecal DC subsets mirror those found in the colon, and that CD103$^+$CD11b$^+$ DCs are absent from the caecal patch. We further show

that caecal DCs migrate to the colon-draining MLN in the steady state. This work provides a basis for characterizing the immunological consequences of NRG857c colonization *in vivo*, and for identifying factors that could contribute to CD pathogenesis.

W21. Urban Particulate Matter (PM10) Induces a Distinct and Complex Programme of Activation in Human Dendritic Cells
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Fossil fuel derived particulate matter (PM) is present within airway myeloid cells of individuals exposed to urban pollution and is linked with inflammatory disease. We hypothesized that exposure of airway dendritic cells (DC) to PM enhances their ability to induce inflammatory responses. We examined the effects of urban ambient PM of different sizes (<10µm, PM10; <2.5µm, PM2.5) on human DC, differentiated from monocytes (MoDC) or FACS-sorted (HLA-DR$^+$Lin$^-$ cells which express ZBTB46) from induced sputum cells (RT-DC). DC phenotype was assessed by flow cytometry and qRT-PCR; stimulatory capacity determined in a mixed leukocyte reaction. PM10 from two UK locations induced 'classical' MoDC maturation indicated by dose-dependent up-regulation of MHC class II, CD40, CD86 and CCR7. Neither PM2.5 nor carbon black particles, representing the particulate core of PM, activated DC. Consistent with their more mature phenotype, PM10 treated MoDC were significantly more stimulatory for naive CD4$^+$ T cells. Like the TLR4 agonist LPS, PM10 induced MoDC production of IL-6, and IL-12 but in contrast to LPS, PM10 also induced the release of cytokines associated with inflammasome activation (IL-1β and IL-18) as well as IL-23. Unlike LPS, PM10 additionally induced aryl hydrocarbon receptor (AhR) signaling in MoDC, as indicated by AhR-dependent induction of the target gene CYP1A1. PM10 also induced CYP1A1 expression by RT-DCs *in vitro*. Thus, components of urban PM10 induce a complex programme of DC activation that includes classical maturation, inflammasome dependent cytokine release and AhR signaling. In the airway, such changes may lead to altered responses to inhaled antigen.

W22. Syk Signaling in Dendritic Cells Instructs Steady-State IL-17 and IL-22 Cytokine Expression in the Intestinal Tract
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The cytokines interleukin 17 (IL-17) and IL-22 play an important role in host protection in barrier tissues against certain pathogens. More recently, they have emerged as key players in steady state to maintain the barrier function of the intestinal epithelium and to prevent systemic dissemination of commensal bacteria. Although IL-17 and IL-22 production is directly controlled by the presence of microbial components, the mechanism of regulation and the involved host response pathways remain unknown. Here we show that dendritic cells (DCs) are critical for coordinating the production of IL-17 and IL-22 via a Syk-coupled pathway that induces a bimodal activation of IL-17 and IL-22 expression in intestinal T cells and innate lymphoid cells, which involves IL-6 and IL-23p19 respectively. Defects in this pathway result in diminished antimicrobial defense and in impaired systemic immune function as a consequence of defective IL-17 and IL-22 induction. Thus, Syk-mediated signaling in DCs orchestrates intestinal and systemic immune homeostasis via the regulation of IL-17 and IL-22.

W23. Repeated Antigen Painting and Sublingual Immunotherapy Convert Sublingual Dendritic Cell Subsets and Induce Antigen-Specific Tolerance
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Purpose: The sublingual mucosa (SLM) is utilized as the site for sublingual immunotherapy (SLIT) to induce tolerance against allergens. The contribution of SLM-dendritic cells (SLM-DCs) has not been clarified. The aim of this study was to examine the dynamics and phenotype of SLM-DCs after topical antigen (Ag) painting and SLIT. SLM-DCs were histologically evaluated after FITC painting. A novel murine Japanese cedar pollinosis (JCP) model was generated and change in SLM-DCs after SLIT was examined. [Results] The density of SLM-DCs was clearly lower compared with the buccal mucosa and dorsal surface of the tongue. Topical FITC painting on the SLM induced maximal recruitment of sub-mucosal DCs (smDCs) at 6 h, but most smDCs had vanished at 24 h. Repeated painting on the SLM induced exhaustion and conversion of the smDC phenotype. CD206^{high}CD11c^{low} round-type

cells with fewer dendrites and less lymph node migration capacity became dominant. In the murine model of JCP, SLIT efficiently inhibited clinical symptoms and allergen-mediated immunological responses. SLIT markedly reduced the number of SLM-DCs, converted to the round-type dominant phenotype and inhibited the activation of regional lymph node DCs. [Discussion] Topical antigen painting on the SLM induced rapid exhaustion and conversion of smDCs and the unique dynamics of SLM-DCs may contribute to tolerance induction in SLIT. We will further report Ag-specific T cell tolerance by repeated antigen painting using a DO11.10 T cell transfer model.

W24. High-Dose Vitamin D_3 Treatment Decreases $CD103^+$ Intestinal Dendritic Cells in Healthy Subjects
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Background: Vitamin D has been shown to modulate intestinal dendritic cells (DCs) and induce regulatory T cells (Treg) *in vitro* and in animals. However, it is still unclear whether vitamin D treatment affects human intestinal DCs *in vivo*.
Methods: 10 healthy subjects consumed a total of 12 mg vitamin D_3 over 15 days and underwent endoscopy with colonic biopsies before and after the intervention. Mononuclear cells were isolated from the biopsies and stained with the DC surface markers HLA-DR, CD11c and CD103 and analyzed with flow cytometry. Snap-frozen biopsies were analyzed with q-PCR for DC and Treg related genes. Results: Vitamin D_3 treatment significantly decreased the total number of $CD103^+$ DCs ($p = 0.01$) as well as the fraction of $CD103^+$ DCs ($p = 0.002$) of all DCs and of all live cells ($p = 0.006$). The transcription of DC and Treg related genes did not change significantly. Conclusion: Oral high-dose vitamin D_3 treatment significantly decreases the number of $CD103^+$ DCs in the colonic mucosa of healthy subjects without changing the transcription of DC-related genes. Thus, further studies are needed to clarify the underlying mechanisms of the observed changes.

W25. Gender Differences in Intestinal Immune Cell Frequencies
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Although gender biases in systemic immune responses are described, it is unknown whether immune differences are also present in the intestine. Therefore we studied immune cell populations in Peyer's patches (PP) in male and female mice in two strains. In the spleen and PP frequencies of myeloid ($CD11c^+/CD11b^+$) and lymphoid ($CD11c^+/CD11b^-$) DCs and their CD80 and CD103 expression were measured using flow cytometry. In T helper (Th) cells, we measured frequencies of Th1 ($Tbet^+$), Th17 ($RoRyT^+$) and Treg ($FoxP3^+/CD25^+$) cells. Moreover, the percentage of $NKp46^+$ natural killer (NK) cells was determined. Results were tested using 2-way-ANOVA, $p<0.05$. Faecal microbiota composition was determined using MITchip and ileum gene expression was measured using gene array. Males showed increased $CD80^+$ DCs in both PP and spleen and increased $CD103^+$ DCs in the spleen. Furthermore, males showed increased Th1 cell frequencies in both PP and spleen and increased Tregs in the spleen. NK cells were decreased in the spleens and increased in the PP of males. Clear gender differences in faecal microbiota composition and small intestinal immunological gene pathways were found. Our results show that gender differences in immune populations, microbiota composition and gene expression can be detected in the intestinal immune system.

W26. Dynamic Surface Expression of CD103 on Human Dendritic Cells Due to Endosomal Recycling
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CD103 (αE integrin) is expressed by the majority of murine intestinal dendritic cells (DCs), whereas CD103 expression by human DCs is more variable. Specifically, CD103 by human gastric DCs was extremely low, as our recent study revealed. Since integrins may undergo recirculation through endosomal pathways, we asked whether human DCs contained intracellular pools of CD103 that recycle through the cell membrane. Indeed, intracellular expression of CD103 was detected in $53^{\pm}12\%$ of gastric and $73^{\pm}9\%$ of MoDCs, whereas surface expression was significantly lower, at $13^{\pm}2\%$ and $8^{\pm}3\%$, respectively. These data were confirmed by imaging flow cytometry and confocal microscopy, which revealed endosomal intracellular staining patterns for CD103 in both gastric DCs and MoDCs. Furthermore, MoDCs incubated with fluorescent labelled anti-CD103 accumulated fluorescence over time, indicating endosomal recycling. Similar results were obtained for β7, which forms the functional αEβ7 integrin heterodimer with

CD103. αEβ7 recycling through the DC membrane may enable dynamic interactions between human mucosal DCs and E-cadherin expressed by mucosal epithelia, allowing efficient DC immunosurveillance of the epithelial barrier.

W27. CD40-Signaling Induces Loss of Lamina Propria CD103$^+$ Dendritic Cells, Abrogation of iTreg Induction and Fatal Colitis
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Self/non-self-discrimination is a hallmark of the immune system, where encounter with self-antigen induces tolerance, while the appropriate recognition of non-self antigen triggers immune reactions. A tight control of induction of immunity versus tolerance is especially important at body surfaces such as the intestinal tract, where foreign, commensal-derived antigens must be tolerated. Dendritic cells (DCs) have key roles in this important equilibrium as they can induce both, immunity and tolerance, depending on their maturation status. To further study signals in DCs that might contribute to tolerance in steady-state we focused on signaling via CD40. Although CD40-signaling in DCs is important for DC-licensing in combination with inflammatory signals, there is evidence that CD40-signaling alone induces incomplete maturation of DCs with regard to cytokine production. To investigate the influence of CD40 signals on DCs without the reported systemic, necro-inflammatory effects induced by injected anti-CD40 mAb on CD40$^+$ non-DCs, we generated transgenic mice, where selectively DCs receive a tonic CD40-stimulus. DCs in spleens of these transgenic LMP/CD40-animals showed no signs of DC-activation with respect of costimulatory molecules and cytokine production. But all animals developed severe colitis, characterized by abundant IFNg$^+$ and IFNg$^+$IL17$^+$ T cells in gut accompanied by highly elevated levels of pro-inflammatory cytokines. Disease development depended on presence of commensal bacteria as well as on B and T cells.

EFFECTOR T CELLS AND CYTOKINES

OR.41.The Role of Ly49E Expression on Intestinal Intraepithelial Lymphocytes in the Development and Progression of Inflammatory Bowel Diseases, Tumor Immune Response and Bacterial Infection
Aline Van Acker. Ghent University, Ghent, Belgium

The Ly49E NK receptor is a unique inhibitory receptor, presenting with a high degree of conservation among mouse strains and expression on both NK and intraepithelial-localized T cells. Amongst intraepithelial-localized T cells, we show that the Ly49E receptor is abundantly expressed on CD8αα-expressing innate-like intestinal intraepithelial lymphocytes (iIELs), with even higher expression in the colon as compared to the small intestine. CD8αα-positive iIELs contribute to front-line defense at the mucosal barrier. Here, we have investigated a potential role for Ly49E expression on iIELs in the context of inflammatory bowel diseases, tumor immune response, and bacterial infection. Making use of Ly49E-deficient mice, we show that Ly49E expression on iIELs does not influence the development or progression of DSS- or TNBS-induced colitis, nor that of TNF$^{\Delta ARE}$ ileitis. Exploring a role for Ly49E in intestinal tumor immune response, we made use of the azoxymethane-induced colorectal cancer model and Apc$^{Min/+}$ transgenic mice. We show that colorectal cancer development is unaltered between Ly49E WT and Ly49E KO mice, and that Apc$^{Min/+}$ Ly49E KO mice display with a similar tumor immune response as compared with Apc$^{Min/+}$ Ly49E WT mice. Finally, investigating a role for Ly49E expression on iIELs in bacterial infection, we show that Ly49E expression does not affect the kinetics of Citrobacter rodentium or Salmonella typhimurium infection. In conclusion, although Ly49E is highly expressed on CD8αα^{pos} iIELs of the colon and small intestine, this NK receptor does not influence the development or progression of inflammatory bowel diseases, intestinal tumor immune response or intestinal bacterial infection.

OR.42. Generation and Characterization of Th22 Cells Identifies a Unique Molecular Profile
Maximilian Plank[1], Gerard Kaiko[2], Steven Maltby[1], Hock Tay[1], Jessica Stewart[1], Scott Durum[3] and Paul Foster[1]. [1]University of Newcastle, Newcastle, Australia; [2]Washington University St. Louis, St. Louis, MO; [3]National Cancer Institute, Frederick, MD

Interleukin-22 (IL-22) belongs to the IL-10 cytokine family and is expressed in many tissues during chronic inflammatory diseases, including asthma and rhinitis. IL-22 can have both pro-inflammatory and tissue protective roles, which depend on the inflammatory context, tissue tropisms, and local cytokine milieu. How IL-22 exerts its varying effects remains poorly understood. Recently, a novel lineage of CD4$^+$ T helper cells (known as Th22 cells) have been identified that predominantly express IL-22, but not IL-17. Th22 cells have subsequently been associated with the pathogenesis of asthma, atopic dermatitis and psoriasis. The functional role

of these Th22 cells in inflammatory responses remains largely unknown, due to an inability to characterize their function directly *in vitro* or *in vivo*. Knowledge of the factors controlling Th22 cell differentiation *in vitro* remains limited, making phenotypic and functional characterization of these cells difficult. We have identified, for the first time, critical differentiation factors that promote the polarization and outgrowth of large numbers of Th22 cells *in vitro*. Through fate mapping studies, we demonstrate that Th22 cells develop independently of the Th17 lineage. Further, using novel T cell transgenic reporter mice, we have specifically isolated purified Th22 cells using FACS. Phenotypic characterization of the Th22 transcriptome identified a unique expression profile, distinct from Th17 cells. Our study provides valuable techniques for the assessment of Th22 function and its role in immunity. An improved understanding of Th22 function in mucosal tissues in asthma and rhinitis will provide insight into disease processes and potential novel targets for therapeutic intervention.

OR.43. Notch-STAT5b Signaling Regulates the TCRαβ$^+$CD8αα$^+$ Intraepithelial Lymphocytes in the Small Intestine.
Chieko Ishifune and Koji Yasutomo. Tokushima University, Tokushima Japan

Intraepithelial lymphocytes (IELs) in the small intestine are divided into several subsets. It has been reported that TCRαβ$^+$CD8αα$^+$IELs and TCRγδ$^+$IELs have suppressive roles in colitis model. However, the molecular mechanisms underlying IELs development and their functions are largely obscure. We found that TCRαβ$^+$CD8αα$^+$IELs and their maturation from Thy1$^+$ to Thy1^{neg}Granzyme B $^+$ cells in the small intestine were markedly decreased in CD4-Cre dependent Notch1, 2-Rbpj signal deficient mice compared with control mice. We did not find any reduction of IELs precursors in the thymus and their ability to differentiate toward CD8αα$^+$ cells in CD4-Cre Rbpj$^{f/f}$ mice *in vitro*. Villin1-Cre dependent Jagged1 and/or Dll1 deficient mice had normal number of IELs. Those data indicate that both Notch1 and Notch2 regulate the differentiation of TCRαβ$^+$CD8αα$^+$Thy1^{neg}IELs but Jagged1 or Dll1 in the intestinal epithelium is not involved in the differentiation. TCRαβ$^+$CD8αα$^+$IELs from CD4-Cre Rbpj$^{f/f}$ mice have reduced expression of STAT5b and phospho-STAT5 but not STAT5a and receptors for IL-2, 7, or 15. CD4-Cre Stat5a/b$^{f/f}$ mice had few numbers of TCRαβ$^+$CD8αα$^+$IELs, suggesting that STAT5a/b is required for the regulation of TCRαβ$^+$CD8αα$^+$IELs. Furthermore, overexpression of constitutive-active STAT5b in CD4-Cre Rbpj$^{f/f}$ mice could partially rescue the reduction of TCRαβ$^+$CD8αα$^+$IELs but not their maturation. These data indicate that Notch1 and Notch2 signaling controls the differentiation of TCRαβ$^+$CD8αα$^+$IELs in the small intestine without affecting the development of its precursor. Furthermore, Notch-mediated differentiation of TCRαβ$^+$CD8αα$^+$IELs would be, at least partly, regulated by the activation of Stat5 signaling.

OR.44. Retinoic Acid Signaling is Required for the Efficient Differentiation of CD4$^+$ T Cells into Pathogenic Effector Cells During the Development of Intestinal Inflammation
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Epidemiological studies of vitamin A-deficient populations have illustrated the importance of the vitamin A metabolite retinoic acid (RA) in mucosal immune responses. However, RA seems to be a double-edge sword in CD4$^+$ T cell biology. While it sustains the development of foxp3$^+$ regulatory T cells, it was also very recently reported to be essential for the stability of the Th1 lineage and to prevent transition to a Th17 program. Here we explored the role of RA signaling in CD4$^+$ T cells during the development of intestinal inflammation in the T cell transfer colitis model. We found that RA signaling-deficient CD4$^+$ T cells are less potent at inducing intestinal inflammation compared to their RA signaling-competent counterparts and exhibit a differentiation skewing towards more IFNγ$^-$ IL-17$^+$, IL-17$^+$IFNγ$^+$ and foxp3$^+$ cells, while their capacity to differentiate into IL-17$^-$IFNγ$^+$ Th1 cells is compromised. *In vitro* studies confirm the inefficacy of RA signaling-deficient T cells to generate bona fide Th1 cells and demonstrate their aberrant increased RORγt expression while their differentiation into Th17 remains unaffected. Surprisingly, RA signaling-deficient CD45RB^{lo} regulatory T cells (Tregs) are however as efficient as their RA signaling-competent counterparts to inhibit colitis development. Together our results indicate that RA, through its receptor RARα, negatively regulates the early expansion of CD4$^+$ T cells during colitis and is necessary for the generation of colitogenic Th1/Th17 cells, while it is dispensable for the protective function of Treg cells. We are currently deciphering the mechanisms of these effects of RA on CD4$^+$ T cells.

W28. IL17B, a Novel Member of the IL17 Cytokine Family, Promotes Type 2 Inflammation

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The IL17 family of cytokines is involved in host defense mechanisms against bacteria, fungi and other pathogens. While the IL17A, IL17F and IL17C family members induce overlapping inflammatory cascades that promote neutrophil mediated immunity, IL17E, or IL25, contributes to Type 2 immune responses and eosinophil activity. Numerous genetic and pharmacological studies have demonstrated the importance of these cytokines in mucosal immune responses. In contrast, little is known about the biology of the related family member, IL17B. We now report that IL17B shares many functional properties with IL25, including induction of Type 2 cytokine responses *in vivo* and receptor usage. We demonstrate that akin to IL25, IL17B activity requires the IL17RA and IL17RB receptor subunits. These data are consistent with other reports describing IL17RA as a shared receptor subunit for this cytokine family, and the *in vitro* data demonstrating physical interaction between IL17RB and IL17B. In cellular assays IL17B induces Type 2 cytokine expression, and can augments IL25 activities. These data suggest that IL17B also participates in Type 2 inflammatory responses, poising this as another IL17 family member contributing to mucosal immune responses. Our data shed light on the biological properties of this novel cytokine member.

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Fig. 1a

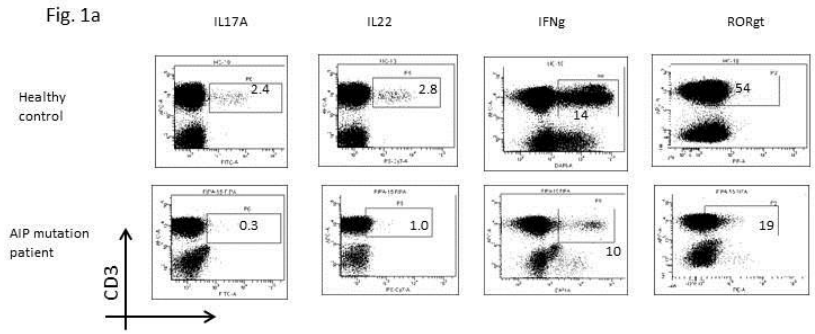

Fig. 1b

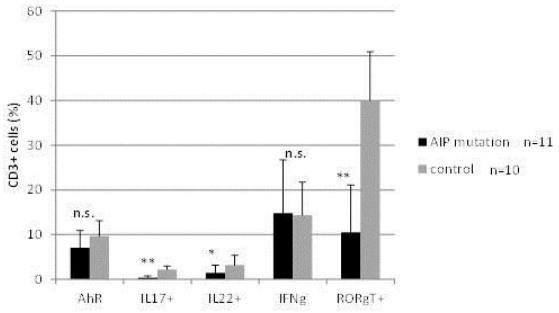

Fig. 1. Peripheral blood T cells from AIP-mutation patients produce significantly less IL17A and IL22 and have lower expression of the transcription factor RORgt.
A. Peripheral blood mononuclear cells (PBMCs) from healthy controls and AIP-mutation patients were treated with PMA/ionomycin/monensin for 6h before surface staining of CD3 and intracellular staining of IL17A, IL22, IFNg, and RORgt. One representative experiment is shown.
B. Expression of the AhR, IL17A, IL22, IFNg and RORgt in CD3+ T cells of AIP-mutation patients and controls as measured by flow cytometry. N=10 (HC) and 11 (AIP mut.). * p<0.05; ** p< 0.01. Student t-test, +SD.

W29. The Role of AIP (Aryl Hydrocarbon Receptor (AhR) Interacting Protein) in the Human T Cell Response

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Heterozygote loss-of-function germline mutations in the aryl hydrocarbon receptor (AhR) interacting protein (AIP) gene can result in familial pituitary adenomas. We have a cohort of 200 subjects with various AIP mutations. AIP's partner, AhR, is known to play an important role in the development of T cells which make IL-17-family cytokines. We hypothesized that our patients might have immune abnormalities due to deficiency of AIP. Our preliminary data using blood samples from AIP-mutation-positive subjects show that PMA-stimulated T lymphocytes display a markedly reduced IL22 and IL17 production and reduced expression of RORγt (the transcription factor which drives IL22/IL17 production) (Fig. 1A,B). IL17 and IL22 concentrations in plasma samples of AIPmut$^+$ patients are also significantly lower compared to controls. Furthermore, our preliminary data demonstrate that the analysis of IL17/IL22 production in T cells of relatives of AIPmut$^+$ patients can accurately predict who is a carrier of the mutation. In sporadic cases of pituitary adenomas with an AIP mutation, the analysis of IL17/IL22 could be crucial in determining whether it is a functional relevant germline mutation or single nucleotide polymorphisms(SNP). IL-17 appears to be important in resistance to fungal infections. We found that AIPmut$^+$ patients have mild but significantly increased levels of C-reactive protein (CRP) and raised Candida albicans antibody levels suggesting impaired immunity and inflammation. In summary, our study on AIPmut$^+$ patients demonstrates a role for this protein in the IL17/IL22 immune response in humans.

W30. A Role for Non-Cognate CD4 T Cell Activation in Bacterial Clearance
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CD4 Th1 cells are required for protective immunity to a variety of microbial pathogens but the relative contribution of cognate and non-cognate CD4 T cell activation is poorly understood. We previously demonstrated that Salmonella-specific CD4 T cells can be activated to produce IFN-g in a Myd88-dependent manner by a variety of innate stimuli. Here, we used mice with Myd88 deficiency in CD4 T cells to examine the contribution of non-cognate stimulation in protection against a variety of different infections. While bacterial clearance was significantly delayed following Salmonella or Chlamydia infection, CD4-myd88-deficient mice displayed normal clearance of Brucella and Plasmodium. Delayed clearance of Salmonella and Chlamydia correlated with a marked reduction in IFN-g production by CD4 Th1 cells in response to innate stimuli. This deficiency was specific in infection models since Th1 cells that were generated by sub-unit vaccination were able to produce IFN-g normally. Together, these data support a model where non-cognate CD4 T cell activation develops in response to bacterial replication and makes a significant contribution to bacterial clearance.

W31. Molecular Regulation of T Helper Cell Self-Control
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Secretion of the immunosuppressive cytokine interleukin(IL)-10 by pro-inflammatory Th1 and Th17 cells is an essential mechanism to self-limit T cell-mediated inflammation. However, the transcriptional regulation of IL-10 expression in T cells is insufficiently understood. By directly comparing IL-10-producers with non-producers we found that Th1 and Th17 cells employ different transcriptional networks to regulate IL-10 expression. In Th1 cells, IL-10 expression specifically depends on the transcriptional regulator Blimp-1 downstream of IL-12/IL-27 signaling. Hence T cell-specific Blimp-1 deficiency resulted in enhanced inflammation and immunopathology during T. gondii infection. In contrast in Th17 cells TGF-b antagonized Blimp-1 expression and instead induced a switch to a Blimp-1-independent and c-Maf-dependent IL-10 expression. Despite this differential transcriptional regulation of IL-10 production in distinct Th cell lineages we found that the Notch signaling pathway acts as a potent universal amplifier of IL-10 expression in both Th1 and Th17 cells. Our data illustrate how T helper cells integrate various signals from the environment into a coherent but highly flexible transcriptional regulation of this critical immunosuppressive cytokine.

W33. Intestinal Side Effects of IFNα2a (Roferon) are Mediated by the Activation of Mucosal T Lymphocytes and the Onset of a Th1 Response
Anne Jarry[1], Florent Malard[2], Virginie Dehame[2], Chantal Bou-Hanna[1], Christian Laboisse[2], Mohamad Mohty[3] and Céline Bossard[4]. [1]EA4273 Biometadys, Nantes, France; [2]INSERM U892 CHU Nantes, Nantes, France; [3]INSERM U938, CHU St Antoine, Paris, France

IFNα2a, a subtype of IFN type I, plays an important role in innate immunity. In the clinics, IFNα2a, called Roferon, part of the treatment of various diseases, has side-effects including intestinal disorders. The aim of this work was to explore the potential deleterious effects of IFNα2a on intestinal mucosa homeostasis, using an *ex vivo* 3-D model system of human normal colonic mucosa explant culture. We showed that treatment of 24h explant cultures with IFNα2a dose-dependently induced a marked alteration of the surface colonic epithelium and crypt base, through an apoptotic process. In addition, IFNα2a i) elicited a dose-dependent IFNY response, associated with an IL-18 response, variable among individuals, and ii) strongly increased the number of Tbet+ lamina propria lymphocytes. Furthermore, a pharmacological approach demonstrated that both the IFNα-induced Th1 response and epithelial damage were subordinated to the inflammasome (caspase-1 / IL18) pathway. Finally, preliminary experiments, both *ex vivo* and *in vitro*, strongly suggested that the epithelial barrier apoptosis resulted from the IFNα-mediated Th1 (IFNY) response. Altogether, these findings provide the first demonstration that the intestinal side-effects of Roferon can be accounted for by epithelial barrier disruption via the activation of mucosal T lymphocytes and the onset of a Th1 response.

W34. Double Positive and Double Negative T Cells in the Intestinal Mucosa: Differences Between Ileum and Colon in Health and Inflammatory Bowel Diseases.

Anna Carrasco, Fernando Fernández-Bañares, Yamile Zabana, Montserrat Aceituno, Mercè Rosinach, Xavier Andújar and Maria Esteve. Mutua de Terrassa, Terrassa, Spain

Introduction: Double Positive (DP, $CD3^+CD4^+CD8^+$) and Double Negative (DN, $CD3^+CD4^-CD8^-$) T cells are two rare T cell subsets with a poorly understood role in intestinal and systemic immunity. Aim: To evaluate DP and DN T cells presence in different bowel locations and in inflammatory bowel disorders. Methods: 20 Crohn's Disease (CD), 7 ulcerative colitis (UC), 5 infectious colitis (IC) and 14 collagenous colitis (CC) patients were included in the study, all of them with active disease and without treatment. Ileum, right and left colon samples from 16 healthy controls (HC) were compared in a paired manner. DP and DN T cells were analyzed by flow cytometry in biopsy specimens and in peripheral blood. Results: A reduction of peripheral DN T cells was found in CD patients compared to HC (p=0.036). DN T cells were reduced in colonic CD (p=0.005) but not in ileal CD compared to HC (p=0.452). In contrast, DN T cells were increased in CC compared to HC (p=0.038), whereas no differences were found in IC or UC. No differences in DP T cell peripheral or mucosal lymphocytes were found between healthy and patients with inflammatory bowel disorders. However, a significant increase of DP T cells was observed in ileal samples compared to colonic samples, both in HC (0.028) and CD (0.016) groups. Conclusions: DN T cells are reduced in colonic CD and increased in colonic CC, suggesting that this subset could have a role in those intestinal disorders. The ileal increase in healthy and inflamed mucosa suggests that DP T cells could have a role in intestinal homeostasis regulation.

W35. Feedback Regulation of IFNα/ß Signaling by Axl Receptor Tyrosine Kinase Modulates HBV Immunity

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Hepatitis B virus (HBV) is known to cause age-dependent infection outcomes wherein most infection during young age results in chronicity. The age-related immune mechanism underlying the differential outcome remains elusive. By using hydrodynamic injection of the replication-competent pAAV-HBV, we established a mouse model in which HBV persistence was generated in 4-5 w/o C57BL/6 young mice, but not in adult mice of over 10 w/o. The HBV-tolerant young mice expressed higher interferon (IFN)-a/b in the hepatocytes and intrahepatic plasmacytoid DCs (pDCs) than the adult after pAAV-HBV injection. Paradoxically, excessive IFN-a/b expression of the young mice was associated with induction of the Axl regulatory pathway and expansion of intrahepatic Treg cells. In line with these findings, augmented IFN-b expression increased HBV persistence of the adult mice and IFN-a/b signaling blockage decreased HBV persistence of the young mice. Accordingly, Axl overexpression decreased HBV clearance of the adult mice whereas Axl silencing enhanced HBV clearance of the young mice. *In vitro*, IFN-b priming of bone marrow-derived pDCs enhanced Treg cell differentiation. These findings suggest that the age-dependent HBV chronicity is attributed to IFN-b-Axl immune regulation, which is selectively induced in the young mice by excessive IFN-a/b production at early stage of HBV infection.

W36. Expansion of Human Regulatory T Cells Protects Humanized Mice from Experimental Colitis

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Many autoimmune and inflammatory disorders result from failure to establish or maintain central or peripheral tolerance and some have been attributed to altered regulatory T cell (Treg) number or function. Accordingly, many studies have focused on technologies to expand or promote Treg development/function. Here, we have developed humanized murine systems to assess directly *in vivo* technologies to expand Tregs. Low-dose IL-2 in humans has been shown to be an effective therapeutic strategy for Treg expansion *in vivo* for the treatment of chronic graft vs. host disease and HCV-induced vasculitis. However, the use of this modality for Treg expansion in the intestinal compartment to suppress colitis has not been evaluated. To test this approach, we generated immunodeficient mice that lack murine major histocompatibility complex II and instead express human HLADR1 and reconstituted these mice with human $CD4^+$ T cells. Mice treated with TNBS developed weight loss, colitis, with the human T cells recovered from the colonic lamina propria expressing increased TNF and IFNg. Administration of low-dose IL-2 prior to TNBS was protective against TNBS-induced weight loss and colitis development. We also showed in this humanized system that activation of the aryl hydrocarbon receptor using the agonist ITE expands human regulatory T cells and protects against TNBS-induced colitis. These data demonstrate that human $CD4^+$ T cells can promote colitis development in a humanized murine system and can serve as

a pre-clinical model in which to evaluate immunomodulators or novel therapeutics aimed at promoting intestinal immune homeostasis.

W37. Intestinal Microbial Signals Sustain Inflammation and Autoimmunity Induced by Hypomorphic Rag Defects
Rosita Rigoni[1], Virginia Maina[2], Veronica Marrella[2], Graziano Pesole[3], Simone Guglielmetti[1], Luigi Poliani[4], Fabio Grassi[5], Anna Villa[2] and Barbara Cassani[2]. [1]Universitá degli Studi di Milano, Milan, Italy; [2]Humanitas Clinical and Research Center, Rozzano, Italy; [3]Universita di Bari, Bari, Italy; [4]University of Brescia School of Medicine, Brescia, Italy; [5]Institute for Research in Biomedicine, Bellinzona, Switzerland

Hypomorphic RAG mutations cause profound immunodeficiency associated with autoimmune-like manifestations, mediated by oligoclonal activated T cells, in humans and mice. The role of microbial signals and gut homeostasis in the disease pathogenesis is debated. The $Rag2^{R229Q/R229Q}$ mice developed an inflammatory bowel disease characterized by marked infiltration of $CD4^+$T cells producing both Th1 and Th17 cytokines. Similar pro-inflammatory profile was also evident in the periphery and a significant proportion of splenic effector T cells expressed the CCR9 and α4ß7 receptors, underlying the abnormal lymphocyte trafficking to this environmental interface in mutant mice. Moreover, deficiency in mucosal B cells and IgA secretion enhanced intestinal permeability and resulted in reduced microbial biodiversity. Fecal transplant into wild-type mice increased host Th1/Th7 mucosal responses, indicating the pathogenic potential of microbiota populating the gut of $Rag2^{R229Q/R229Q}$ mice. Consistently, administration of broad-spectrum antibiotics significantly limited gut lymphocytic infiltration, and ameliorated both the intestinal and systemic inflammation by diminishing the frequency of Th1/Th17 cells. These results suggest that gut microbiota play a key role in the immune dysregulation distinctive of these conditions.

W38. T_H17 Response Is Diminished in Absence of PTAFR Signaling in DSS-Induced Colitis
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Platelet activating factor (PAF) has been shown to be important in various inflammatory conditions. However, its exact role in inflammatory bowel diseases (IBD) is unclear. We examined how the absence of PAF receptor signaling ($PTAFR^{-/-}$) or excessive PAF receptor activation ($PLA2G7^{-/-}$) affects DSS-mediated colitis *in vivo* and T cell activation by a-CD3/a-CD28 *in vitro*. The inflammatory response in the colon and in *in vitro* activated cells was assessed by quantifying cytokine gene expression by real time PCR. In WT mice treatment with DSS leads to an increase in cytokines characteristic for the T_H17-response, while in $PTAFR^{-/-}$ mice we observed a complete absence of IL17 and IL22. The TH17-specific chemokine CCL20 and the TH17-specific chemokine receptor CCR6 were significantly increased in the colons of $PLA2G7^{-/-}$ mice, and they were significantly decreased in colons of $PTAFR^{-/-}$ mice, as compared to wild type (WT) colons. *In vitro* stimulated T cells showed a non-polarized increase in cytokine transcripts in $PLA2G7^{-/-}$ and a non-polarized decrease in $PTAFR^{-/-}$, as compared to WT. Our results suggest that signaling through PAF receptor is required for maximal non-polarized T cell activation *in vitro* and for a maximal T_H17-type polarized response in the colon *in vivo*. This is best explained by a role for PAF in T_H17-specific homing to the intestine and not by selective activation of T_H17 cells. A precise delineation of PAF-specific mechanisms in the polarity of T cell-mediated inflammatory response may lead to novel treatment strategies for IBD.

W39. Ets1 Regulates the Expression of ICOS and Controls the Maturation, Homeostasis, and Function of Invariant NKT Cells
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The transcription factor Ets1 is essential for the development of iNKT cells. However, its detailed role and mechanism of action are unknown. We used two genetic approaches to generate iNKT cells with either impaired Ets1 activity or deficient in Ets1. Here we show that Ets1 is dispensable for the expression of Va14Ja18 TCR and thymic selection of iNKT cells, but is essential for the maturation of post-selected iNKT cells. This function of Ets1 can be partly compensated by a Vα14Jα18 TCR transgene and is independent of its N-terminal Pointed domain. Ets1 also promotes the production of type 1 and type 2 cytokines but suppresses the expression of IL-17A by a Pointed domain-dependent mechanism. Furthermore, Ets1 directly transactivates ICOS and Ets1-deficient peripheral iNKT cells are prone to apoptosis. Taken together, Ets1 has Pointed domain-dependent and independent functions in iNKT cells and regulates their peripheral survival by promoting the expression of ICOS.

W40. Type 1Interferons are Major Regulators of Human Mucosal-Associated Invariant T Cells Functions
Christelle Gross, Gauthier Chazal, Justine Oliva and Emmanuel Treiner. Inserm UMR1043, Toulouse, France

Human Mucosal-Associated Invariant T (MAIT) cells represent a subset of $CD8^+$ innate-like T cells found in blood, mucosae and liver. They display anti-bacterial functions, owing to the recognition of highly conserved microbial metabolites associated to the monomorphic MHC class-I like molecule MR1 (MHC-Related 1). Hence, MAIT cells may represent an important line of defense against various pathogens, mostly intracellular bacteria. MAIT cells activation is also regulated by cytokines and innate microbial signals, in particular by TLR8 ligands and the combination of $IL12^+18$, which activate MAIT cells in the absence of TCR stimulation. We now describe the prominent role of type 1 IFN as major regulators of MAIT cells functions. In the absence of TCR signals, IFNα/ß activate MAIT cells, in synergy with the combination of IL-1ß and IL-12. More strikingly, IFNα very strongly potentiates TCR signaling in MAIT cells, in a manner that is not found in other $CD8^+$ T cell subsets. MAIT cells respond poorly to anti-CD_3 stimulation, but IFNα dramatically increases the number of IFNgamma-producing MAIT cells; a similar effect is observed on degranulation. IFNα acts directly on MAIT cells with STAT phosphorylation. The TLR agonists poly I:C and R848 also potentiate MAIT cells response to anti-CD_3 stimulation in an IFNα-dependent manner. Finally, exogenous IFNalpha potentiates MAIT cells responses to heat-killed bacteria. Altogether, these results demonstrate that MAIT cells display a major and specific sensitivity to type 1 IFN, which is probably highly relevant to infections with intracellular bacteria but also in other chronic inflammatory disorders.

EPITHELIAL CELLS IN INNATE IMMUNITY

PS.5. Role of RIPK2 in Protecting the Intestinal Epithelium from Caspase-8 Mediated Apoptosis
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NOD1 and NOD2 are cytosolic innate immune sensors of bacteria. Downstream of NODs, RIPK2 is an essential adaptor that triggers NF-κB and MAPK signaling. Accumulating evidence emphasizes that dysregulation of NOD-RIPK2 immune axis in the intestine can lead to inflammatory bowel diseases. While NOD receptors were extensively studied for controlling innate immunity and intestinal homeostasis, the role of RIPK2 in preserving homeostasis and protecting from gut inflammation remains elusive. Here, we show that impairment of intestinal epithelial barrier integrity predisposes RIPK2-deficient mice to acute experimental colitis, resulting in increased intestinal epithelial cell (IEC) death, intestinal inflammation and mortality. Blockade of caspase activation rescued $Ripk2^{-/-}$ mice from colitis by promoting IEC proliferation and tissue repair, whereas RIPK3 ablation did not prevent excessive intestinal inflammation and tissue damage caused by RIPK2 deficiency. Colonocytes depleted of RIPK2 were highly sensitive to TNFα-induced apoptosis through engagement of caspase-dependent processes. Interestingly, RIPK2 negatively regulated apoptosis of colonocytes independently of NOD signaling. Moreover, macrophages from wild-type and $Ripk2^{-/-}$ mice were equally resistant to TNFα- or MDP-mediated cell death. Mechanistically, RIPK2 interacted with caspase-8 to activate NF-κB signaling in a kinase-independent manner, thereby contributing to apoptotic resistance and ensuring IEC survival. Thus, our results define a unique scaffolding property of RIPK2 in protecting IECs from caspase-8-mediated apoptosis and reveal the RIPK2-caspase-8 complex as a critical hub at the crossroad of apoptosis and innate immunity.

OR.5. Functional Differences in Type I Versus Type III Interferon Mediated Immunity in Intestinal Epithelial Cells
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Type I interferon (IFN) mediated innate immunity is ubiquitous. On the contrary, type III IFN signaling is confined to epithelial cells due to the restricted expression of its receptor. It is currently believed that both cytokines have fully redundant functions. However, the epithelium specificity of type III IFNs strongly suggests that both IFNs must have functional differences at epithelial surfaces. Here, we use human intestinal epithelial cells (IECs) to study both the antiviral innate immune response and the functional differences of both IFNs at epithelial surfaces. We found that IECs, upon enteric virus infection, selectively secrete only type III IFNs, although both type I and III IFNs are transcriptionally upregulated. Interestingly, IECs can respond to both IFNs and pre-treatment of the cells with either IFN protects against viral infection. Biochemical analysis revealed differences in Jak/STAT signaling between both IFNs. Moreover, while transcript profiling revealed that both IFNs induced the same set of IFN stimulated genes (ISGs), Q-PCR analysis revealed that the kinetics of induction and regulation of a large set of ISGs and transcription factors differs depending on

the IFNs. Type I IFN induces a strong stimulation of ISGs while type III IFNs signaling induces just enough ISGs to confer IECs an antiviral state. In this work we define, for the first time, functional differences between type I and type III IFN signaling in epithelial cells and we propose that type III IFN signaling is specifically tailored to efficiently combat infection without inducing an excessive pro-inflammatory response.

OR.6. Intrinsic Regulation of Ileal Epithelial Cell Proliferation by the Bacterial Peptidoglycan Sensor Nod2

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Polymorphisms in the gene encoding for the cytosolic peptidoglycan receptor NOD2 are known to confer significant risk of developing ileal Crohn's disease. While much of the research into this gene's function has been focused on the hematopoietic compartment, NOD2 is also known to be highly expressed by intestinal epithelial cells (IECs), and recent evidence suggests that it plays a significant role in epithelial proliferation and maintenance of barrier integrity. To examine this further, we used the Cre-loxP system to generate an IEC-specific Nod2 knockout mouse ($Nod2^{\Delta IEC}$), in which we induced small intestinal inflammation by ip. injection with 10mg/kg of doxorubicin hydrochloride. Surprisingly, $Nod2^{\Delta IEC}$ mice were significantly protected from doxorubicin-ileitis as compared to Nod2-sufficient littermate controls at all-time points as assessed by body weight changes and examination of H&E-stained microscopic sections. As doxorubicin is toxic to rapidly dividing cells, we hypothesized that $Nod2^{\Delta IEC}$ small intestinal crypts might have a lower proliferation rate than those from littermate controls, thus resulting in decreased levels of crypt cell death. In support of this, we found that small intestinal crypts in naïve $Nod2^{\Delta IEC}$ mice display significantly lower levels of numbers of proliferating cells than their Nod2-sufficient littermates as measured by immuno-histochemical staining for the presence of the proliferation marker Ki-67 and the incorporation of the exogenously added thymidine analog BrdU. Taken together, these results suggest that the proliferation rate of small intestinal epithelial cells can be modulated by NOD2 in a yet to be determined mechanism.

OR.7. Properdin Deficiency Protects from 5-Fluorouracil-Induced Small Intestinal Mucositis in a Complement Activation Independent but IL-10 Dependent Mechanism

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We used properdin deficiency to interrogate the impact of complement in mucositis, a dose-limiting side effect of anti-cancer drugs that in particular damages the epithelium. C57BL/6 (WT), IL-$10^{-/-}$, properdin knockout (P^{KO}) or IL-10/properdin double knockout (DKO) mice were injected daily with 5-fluorouracil (5-FU). The animals' weight and presence of rectal blood was recorded. Twenty four hours after the last injection serum and small intestines were collected. During 5 days of 5-FU injections both WT and P^{KO} mice lost weight; however, at the end of the regimen P^{KO} mice had less rectal bleeding. C3a and C5a were significantly increased in intestines of both strains indicating complement was activated, yet the histology revealed that P^{KO} mice were less inflamed. Additionally, P^{KO} mice had less intestinal TNF and higher IL-10 levels (IL-1β was unchanged and IL-6 and interferon-γ undetectable). We reasoned that if increased IL-10 is necessary for protection in P^{KO} mice then DKO mice ought to be as susceptible as IL-$10^{-/-}$ mice to mucositis, and this proved to be true. Thus while complement is activated during mucositis, properdin deficiency protects from mucositis in a complement activation-independent, IL-10-dependent mechanism. Further investigation is required to determine if blocking complement can de-couple mucositis from the cancer cell target of chemotherapeutic drugs.

OR.8. Alveolar Overexpression of the Polymeric Immunoglobulin Receptor in the Inflamed Lung as a Novel Mechanism for Antibody-Mediated Immune Exclusion of Bacterial Pathogens

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Community-acquired pneumonia caused by bacterial pathogens represents a major comorbidity in individuals with chronic respiratory diseases. In order to improve our understanding regarding potential alterations in the immune response to bacterial pathogens in hosts with preexisting conditions in the lung, we performed Streptococcus pneumonia infections in mice with established $CD4^+$ T cell-mediated lung inflammation. Despite increased pulmonary leak and in direct contrast to our expectation, mice with preexisting lung inflammation showed improved anti-pneumococcal resistance. This became evident by a better control

of bacterial growth in the airways and prolonged survival of infected mice. Enhanced phagocytic activity of alveolar macrophages as an underlying mechanism for anti-pneumococcal resistance could be excluded. Whole genome transcriptional profiling of lung tissue and comprehensive analysis of the bronchioalveolar lavage (BAL) protein composition from diseased mice and healthy controls revealed a striking overrepresentation of the polymeric immunoglobulin receptor (pIgR) in the inflamed lung. Elevated pIgR expression was localized to alveolar epithelial cells and resulted in increased mucosal transport of secretory IgM and IgA into the bronchioalveolar space of diseased mice. sIgM and sIgA binding assays revealed increased pneumococcal binding by secretory antibodies present in the BAL fluid of mice with preexisting lung inflammation. In conclusion, we propose inflammation-induced overexpression of pIgR in alveolar epithelial cells as a novel mechanism for antibody-mediated immune exclusion of bacterial pathogens that efficiently prevents bacterial adhesion to and penetration of the pre-injured airway epithelium.

OR.20. Studying Communication Circuits of Intestinal Macrophages and their Environment in the Healthy and Inflamed Gut
Biana Bernshtein, Mor Gross, Caterina Curato, Eyal David and Steffen Jung. Weizmann Institute of Science, Rehovot, Israel

Intestinal macrophages reside in the connective tissue underlying the gut epithelium, which separates them from the diverse microbiota populating the gut lumen. This complex and dynamic environment necessitates intimate and precise inter-cellular communication. Unlike other tissue macrophages, gut macrophages derive from monocytes, most likely recruited by tonic low-grade inflammatory stimuli. Upon arrival in healthy gut tissue, monocytes adopt a non-inflammatory fate that is critical for maintenance of gut homeostasis[1]. We recently established a murine colitis model by taking advantage of CX3CR1-Cre animals[2] to generate mice that harbor macrophage-restricted Interleukin 10 receptor (IL10R) deficiency. IL10R-deficient gut macrophages are pro-inflammatory, causing severe, early onset colitis that resembles the pathology of children carrying IL10R mutations[3]. To investigate how macrophage dysregulation affects the epithelium, we performed RNAseq of small and large intestinal epithelial cells of mice harboring IL10R-deficient macrophages. Epithelial cells readily respond to pro-inflammatory macrophages by up-regulation of anti-microbial peptide secretion, and alterations in their differentiation program. To further dissect the specific communication modules between macrophages and epithelial cells, we take advantage of cell ablation and reconstitution models developed in our lab[4]. By transferring IL10R-deficient and sufficient monocytes to mice ablated of mononuclear phagocytes, we are able to follow the differentiation steps of these cells while interacting with their close environment. Bibliography: (1) Zigmond E. et al. Immunity 37, 2012; (2) Yona S. et al. Immunity 38, 2013; (3) Zigmond E, Bernshtein B et al. Immunity 40, 2014; (4) Varol C. et al. Immunity 31, 2009

OR.77. MUC2 Mucin Regulates Cathelicidin in the Colon Under Normal and Disease Conditions
Eduardo Cobo, Kris Chadee, France Moreau and Vanessa Kissoon-Singh. University of Calgary, Calgary, AB, Canada

Embedded in the colonic mucus layers are cathelicidins, small cationic peptides secreted by neutrophils and colonic epithelial cells. Humans and mice have only one cathelin-related antimicrobial peptide (CRAMP), LL-37/hCAP-18 and Cramp respectively, with related structure and antimicrobial and chemotactic functions. Altered production of MUC2 mucin and antimicrobial peptides is characteristic of colonic inflammation and disease. However, the interactions between MUC2 mucin and cathelicidins in conferring innate immunity are not well characterized. In this study, we quantified whether MUC-2 expression and release can regulate the expression and secretion of cathelicidin LL-37 in human colonic goblet cells. Even though LL-37 was released at basal levels it was enhanced when goblet cells were stimulated with sodium butyrate (a normal product of colonic bacterial fermentation) or IL-1β (hallmark pro-inflammatory cytokine in colitis). Activation of cyclic adenylyl cyclase (AMP) and mitogen-activated protein-kinase (MAPK) signaling pathways, but not phosphatidylinositol 3-kinase, was necessary for the simultaneous expression of MUC2 and cathelicidins. In animals, Muc2 production regulated cathelicidin evidence by markedly reduced Cramp in $Muc2^{-/-}$ as compared to Wt $Muc2^{+/+}$ littermates. A similar response was noted in DSS-induced colitis with a reduced mucus barrier and in closed colonic loops inoculated with the colonic parasite Entamoeba histolytica. These studies show a regulatory mechanism between MUC-2 and cathelicidins in the colonic mucosa where an intact mucus barrier is essential for expression and secretion of cathelicidins. Defects in MUC2 mucin, hallmarks in IBDs, may impair cathelicidin response and influence the outcome of abiotic and infectious colitis.

OR.78. Cell Shedding Arrested in the Absence of GGTase-Prenylation Within IECs

Rocio Lopez Posadas[1], Christoph Becker[1], Alastair JM Watson[2], Martin O Bergo[3], Markus F. Neurath[1] and Imke Atreya[1]. [1]Friedrich-Alexander University, Erlangen, Germany; [2]Norwich Medical School University of East Anglia, Norwich, United Kingdom; [3]University of Gothenburg, Gothenburg, Sweden

Introduction: Epithelial integrity is essential for barrier function in the gut, contributing to the maintenance of tissue homeostasis. Such integrity needs continuous self-renewal of the epithelial layer, guaranteed by equilibrium of cell proliferation, migration, death and shedding. These processes require adjusted signaling pathways and cytoskeleton function in intestinal epithelial cells (IECs). In this context, Rho GTPases and their regulation by prenylation represent attractive candidates to be analyzed *in vivo*.
Methods: Conditional GGTase-I KO mice were generated by crossbreeding Pggt-Iβ$^{flx/flx}$ and VillinCre-ERT2 mice. Pggt-Iβ^{TiΔIEC} mice were extensively analyzed *in vivo and* ex *vivo*.
Results: Deletion of the prenylation-enzyme GGTase-Iβ in IECs led to increased intestinal permeability and lethal enteric disease in mice. Our data support alteration of cell shedding and cytoskeleton remodeling as key mediator underlying this phenotype. Live imaging of the gut demonstrated that breakdown of barrier function (passage of topically applied rhodamine-dextran) went along with an accumulation of leakage points (epithelial gaps) and appearance of dextran-permeable IECs. The accumulation of arrested (early) versus completed (late) shedding events in Pggt-Iβ^{TiΔIEC} mice suggested an impaired completion of cell extrusion or arresting of cell shedding. Preliminary data suggested that LPS-induced pathological cell shedding does not contribute to the phenotype in Pggt-Iβ^{TiΔIEC} mice, and let us to speculate about the involvement of physiological cell shedding in GGTase-Iβ deficient epithelium.
Conclusions: Epithelial integrity depends on a sufficient level of GGTase-Iβ-mediated-prenylation within IECs. Lethal intestinal pathology in GGTase-Iβ deficient epithelium in mice is driven by arresting of cell shedding due to cytoskeleton remodeling.

OR.79. Critical Role of Commensal Flora-Dependent Type 3 Innate Lymphoid Cells (ILC3) for the Induction and Regulation of Paneth Cells

Mariko Kamioka[1], Yoshiyuki Goto[1], Jun Kunisawa[1,2], Aayam Lamichhane[1], Shintaro Sato[1] and Hiroshi Kiyono[1]. [1]University of Tokyo, Tokyo, Japan; [2]National Institute of Biomedical Innovation, Osaka, Japan

Intestinal epithelial cells (ECs) have fucosylation which is one of glycosylation pattern for the creation of cohabitation and protective niches. Our previous research showed that fucosylation of Peyer's patch M cells and columnar ECs was distinctly regulated by two forms of α(1,2) fucosyltransferase: Fut1 and Fut2, respectively. However, further analysis using Fut1- and Fut2-deficient mice revealed that fucosylation of Paneth cells is regulated by both Fut1 and Fut2, and Paneth cells can be at least divided into two subsets, Fut1 only, and Fut2 expressed double positive cells. We also revealed that Fut2 expressing Paneth cells is induced and regulated by ILC3 in commensal bacteria-dependent manner. Moreover, the expression of Reg-III family, which is a pivotal player of the immunosurveillance in the intestine, is associated with Fut2-expressing Paneth cells by the commensal flora-ILC3 axis dependent mannar. Taken together, our findings suggest that the commensal flora-ILC3 axis plays critical roles for induction and regulation of Fut2- and Reg-III-positive Paneth cells. Our current study is aiming at the molecular and cellular understanding of the commensal flora-ILC3 axis dependent Paneth cells for their contributions in the creation of healthy intestinal environments.

OR.80. Intestinal Epithelial Cell Tyrosine Kinase 2 Transduces Interleukin-22 Signals to Protect from Acute Colitis

Eva Hainzl[1], Isabella Rauch[2], Susanne Heider[3], Caroline Lassnig[1], Silvia Stockinger[1], Lukas Kenner[3], Thomas Decker[2], Birgit Strobl[1] and Mathias Müller[1]. [1]University of Veterinary Medicine Vienna, Vienna, Austria; [2]University of Vienna, Vienna, Austria; [3]Ludwig Boltzmann Institute for Cancer Research, Vienna, Austria

In the intestinal tract, IL-22 activates signal transducer and activator of transcription 3 (Stat3) to promote intestinal epithelial cell (IEC) homeostasis and tissue healing. The mechanism has remained obscure but we demonstrate that IL-22 acts via tyrosine kinase 2 (Tyk2), a member of the Janus kinase (Jak) family. Using a mouse model for colitis, we show that Tyk2 deficiency exacerbates inflammatory bowel disease (IBD). Colitic Tyk2$^{-/-}$ mice have less phosphorylated Stat3 (pY-Stat3) in colon tissue and their IECs proliferate less efficiently. Tyk2-deficient primary IECs show reduced pY-Stat3 in response to IL-22 stimulation and expression of IL-22-Stat3 target genes is reduced in IECs from healthy and diseased Tyk2$^{-/-}$ mice. Experiments with conditional Tyk2$^{-/-}$ mice reveal that IEC-specific depletion of Tyk2 aggravates colitis. Disease symptoms can be alleviated by administering high doses of recombinant IL-22-Fc, indicating that Tyk2 deficiency can be rescued via the IL-22 receptor complex. The pivotal function of Tyk2 in

acute colitis was confirmed in Citrobacter rodentium-induced disease. Thus, Tyk2 protects against acute colitis by amplifying inflammation-induced epithelial IL-22 signaling to Stat3.

W41. Cell Shedding Arrested in the Absence of GGTase-Prenylation Within IECs

Rocio Lopez Posadas[1], Christoph Becker[1], Alastair J.M. Watson[2], Martin O. Bergo[3], Markus F. Neurath[1] and Imke Atreya[1]. [1]Friedrich-Alexander-University, Erlangen, Germany; [2]Norwich Medical School University of East Anglia, Norwich, United Kingdom; [3]University of Gothenburg, Gothenburg, Sweden

Introduction: Epithelial integrity is essential for barrier function in the gut, contributing to the maintenance of tissue homeostasis. Such integrity needs continuous self-renewal of the epithelial layer, guaranteed by equilibrium of cell proliferation, migration, death and shedding. These processes require adjusted signaling pathways and cytoskeleton function in intestinal epithelial cells (IECs). In this context, Rho GTPases and their regulation by prenylation represent attractive candidates to be analyzed *in vivo*. Methods: Conditional GGTase-I KO mice were generated by crossbreeding Pggt-Iβ$^{flx/flx}$ and VillinCre-ERT2 mice. Pggt-Iβ^{TiΔIEC} mice were extensively analyzed *in vivo* and *ex vivo*. Results: Deletion of the prenylation-enzyme GGTase-Iβ in IECs led to increased intestinal permeability and lethal enteric disease in mice. Our data support alteration of cell shedding and cytoskeleton remodeling as key mediator underlying this phenotype. Live imaging of the gut demonstrated that breakdown of barrier function (passage of topically applied rhodamine-dextran) went along with an accumulation of leakage points (epithelial gaps) and appearance of dextran-permeable IECs. The accumulation of arrested (early) versus completed (late) shedding events in Pggt-Iβ^{TiΔIEC} mice suggested an impaired completion of cell extrusion or arresting of cell shedding. Preliminary data suggested that LPS-induced pathological cell shedding does not contribute to the phenotype in Pggt-Iβ^{TiΔIEC} mice, and let us to speculate about the involvement of physiological cell shedding in GGTase-Iβ deficient epithelium. Conclusions: Epithelial integrity depends on a sufficient level of GGTase-Iβ-mediated-prenylation within IECs. Lethal intestinal pathology in GGTase-Iβ deficient epithelium in mice is driven by arresting of cell shedding due to cytoskeleton remodeling.

W42. The Short-Term Effect of Peptidoglycan Injected into the Intestinal Lumen Against Host Defense Responses

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Numerous indigenous bacteria in the animal intestine are regulated by host defense responses. In our previous studies, some host defense responses, such as the acceleration of epithelial cell migration and the transient secretion of bactericidal peptides from Paneth cells, were induced in the ileal intestinal villi with hyper-proliferation of indigenous bacteria. In the present study, 1 mg/ml peptidoglycan (PGN), the dominant constituent of the bacterial cell wall, especially in Gram-positive bacteria, was administered into the lumen of intestinal loop made in a fasted rat jejunum and reacted for 30 min. Then, the effect of PGN against host defense responses was investigated. The results showed that Toll-like receptor (TLR)-2, a receptor for PGN, was detected in the striated borders of the villous columnar epithelial cells in the rat jejunum. The number of proliferating epithelial cells in the intestinal crypt was greater in the PGN-injected group than the negative control group. On the other hand, the disappearance of lysozyme-immunopositivity and the formation of vacuoles in Paneth cells were not increased by PGN injection. From these findings, PGN might induce acceleration of the epithelial cell cycle-mediated host defense response in a short period of time, probably via TLR-2 expressed in the intestinal villi.

W43. Endoplasmic Reticulum Stress Increases Inflammatory Responses by Bronchial Epithelial Cells

Michael Weitnauer, Vedrana Mijosek, Konrad Bode and Alexander Dalpke. University Hospital Heidelberg, Heidelberg, Germany

Airway epithelial cells (AEC) provide a first line of defense against respiratory pathogens. Their main function is to provide a physical barrier. In addition these cells display and mediate a tolerant phenotype towards microbial products, e.g. LPS, in the pulmonary system. Contradictory, an important pro-inflammatory function of AECs during pulmonary inflammatory processes has been demonstrated in the literature. Recently, several pulmonary diseases have been correlated with the unfolded protein response (UPR). UPR is an essential adaptive intracellular signaling pathway activated in response to accumulation of un- or misfolded proteins (ER-Stress). UPRhas been shown to have pro-inflammatory potential. Therefore we wondered whether the tolerant phenotype of AECs could be reverted during conditions of ER stress. Induction of UPR by Thapsigargin resulted in an increased response of Beas2B cells to LPS with respect to IL-6 and IL-8 induction, indicating a hyperactive phenotype. These

observations could be confirmed using differentiated murine tracheal epithelial cells cultivated in air-liquid interface. UPR induction resulted in an increase of p38 and ERK phosphorylation by LPS, however NFκB activation was not significantly affected. The importance of p38 and ERK activation were further verified using pharmacological inhibitors. Investigating which ER-stress sensor mediates this process we could demonstrate that PERK is critically important for the LPS and UPR mediated increase in IL6 and IL8 expression. Taken together our results indicate that ER-stress is able to reverse the tolerant phenotype of AECs and this process might be of crucial importance in several pulmonary diseases.

W44. Dysregulated Gastrointestinal Innate Immune Function in Human Type 1 Diabetes
Christina Graves, Melissa LaPato, Jian Li, Andrea Knowlton, Mark Wallet and Shannon Wallet. University of Florida, Gainesville, FL

The host-environment dialogue is thought to be a critical mediator in the initiation and progression of auto-inflammatory and autoimmune disease, including Type 1 Diabetes (T1D), though the exact mechanisms of (mis)communications taking place largely remain elusive. Therefore, we aim to decipher the global intestinal immune environment in T1D, focusing on the innate immune function of intestinal epithelial cells (IEC) and local immune cell milieu. Using fresh human duodenal tissue, we evaluated the soluble mediator profiles of and immune cell populations within the gastrointestinal environment. Our data indicate a whole organ T1D-associated reduction in tolerogenic mediators concomitant with elevated IEC-induced pro-inflammatory mediators. Significant alterations in intestinal leukocyte populations were observed, whereby increased frequencies of pathogenic T cells and alterations in tolerogenic DCs were noted. We also evaluated IEC-specific innate immune responsiveness using primary IEC culture, where T1D-derived cultures demonstrated a lack of tolerogenic responses concomitant with induction of inflammatory responses to microbial ligand stimulation as compared to non-T1D derived cultures. Together, these data suggest a loss of gastrointestinal homeostasis in T1D potentially as a result of a dysfunctional IEC-mediated host-environment dialogue, though whether this is a cause or consequence of disease remains to be defined.

W45. The Role of IL-37 on Cytokine Responses Downstream of TLR4 and TLR5 Signaling in the Colon Epithelial Cells
Sezin Gunaltay[1], Mohammed Ghiboub[2], Olof Hultgren[1] and Elisabeth Hultgren Hornquist[1]. [1]Orebro University, Orebro, Sweden; [2]Frenche Comte University, Besancon, France

Microscopic colitis (MC), comprising collagenous colitis (CC) and lymphocytic colitis (LC), is characterized clinically by chronic watery diarrhea, abdominal pain, and weight loss. IL-37 (IL-1F7) is a newly described anti-inflammatory cytokine of the IL-1 family. Previously, reduced gene expression of IL-37 has been observed by our group in the colonic mucosa of MC patients, which was suggested as one of the reasons for the chronicity of the colonic inflammation. We here investigated the role of IL-37 on pro-inflammatory cytokine responses mediated by TLR2, TLR4, or TLR5 signaling pathways in the Caco-2 colon epithelial cell line upon stimulation with peptidoglycan, LPS or Flagellin, which was followed by silencing of IL-37 with siRNA. Gene and protein expressions of IL-37, CXCL8, IL-1β, TNF, CXCL11, and CCL20 were detected using qRT-PCR and ELISA. IL-37 gene and protein levels were significantly increased upon LPS and Flagellin stimulations in Caco-2 cells. We didn't observe any changes upon peptidoglycan stimulation. IL-37 silenced Caco-2 cells had significantly increased CXCL8, TNF and CCL20 gene expressions, but not IL-1β upon 100 ng/ml Flagellin stimulation, whereas 500 ng/ml LPS stimulation led to up regulation of CXCL8, TNF and IL-1β but not CXCL11 gene expressions compared to cells without siRNA transfections. Flagellin and LPS stimulations of IL-37 silenced Caco-2 cells resulted in significantly increased CXCL8 protein levels. Altogether, this study show the novel regulatory role of IL-37 on pro-inflammatory cytokine production following TLR4 or TLR5 stimulation, corroborating the importance of reduced IL-37 in MC patients as one of the important immunopathological factors.

W46. The Role of Stromal Cells in the Immune Response to Respiratory Syncytial Virus (RSV) Infection *in vivo*
Fahima Kausar and Cecilia Johansson. Imperial College London, London, United Kingdom

The characteristics of RSV infection and spread in the lung are not well defined. *In vitro* studies have shown stromal cells, especially epithelial cells, to express inflammatory mediators, however their importance to the cytokine and chemokine production *in vivo* during RSV infection has not been established. We have been able to identify several important chemokines, cytokines and growth factors that are expressed by stromal cells in response to RSV *in vivo* using fluorescent activated cell sorting and gene expression analysis, as well as via intracellular cytokine staining. Interestingly, our data show that alveolar type II epithelial cells are the main producers of growth factors while the non-epithelial, non-endothelial stromal cells are the main source of chemokines. In addition,

neutralization experiments of one of these growth factors, granulocyte macrophage colony-stimulating factor (GM-CSF) *in vivo*, influence lung inflammation, suggesting this it is important for regulating cellular responses and viral clearance. Data from these experiments will highlight a novel and previously unknown role of stromal cells in the immune response to RSV infection *in vivo* and go towards future development of therapeutics and vaccines.

W47. Epithelial Interleukin-25 Contributes to the Nasal Polypogenesis in Chronic Rhinosinusitis

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Chronic rhinosinusitis (CRS) with nasal polyps (NP) is associated with Th2 cytokine polarization. Interleukin (IL)-25 was recently reported to induce Th2-type immune responses and to contribute to several allergic diseases including atopic dermatitis and asthma. However, the role of IL-25 and its mechanism in nasal polyposis remain unclear. We investigated IL-25 expression and its cellular origins in NPs of human subjects by immunohistochemistry, qRT-PCR, and ELISA of NP tissues. Correlations between IL-25 and other inflammatory markers in NP tissues were also explored. To confirm the function of IL-25, anti-IL-25 neutralizing antibody was administered in the ovalbumin- and staphylococcal enterotoxin B (SEB)-induced murine NP model. IL-25 expression was upregulated in NP mucosa from patients with CRSwNP compared with the uncinate process tissue from control patients and those with CRSsNP. Overexpression of epithelial IL-25 was confirmed by immunohistochemistry, and double immunohistochemistry staining showed that tryptase positive cells were one of the main source of IL-25 among immune cells. Furthermore, IL-17RB was also increased in immune cells of nasal polyps, compared with control. In NPs, IL-25 mRNA expression positively correlated with the expression of several inflammatory markers, including T-bet, RORC, GATA3, ECP, TGF-b1, and TGF-b2. IL-25 was more abundant in the murine NP tissues than controls. Anti-IL-25 treatment reduced polyp number, mucosal edema, collagen deposition, and inflammatory cell infiltrations. This treatment also inhibited the expression of local inflammatory cytokines, such as IL-4 and IFN-g. Our findings suggest IL-25 in sino-nasal mucosa play a crucial role in the pathogenesis of CRSwNP and could be a novel therapeutic target.

W48. *In vivo* Study of Optineurin in Immunity and Inflammation

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Optineurin is a multifunctional protein with sequence similarity with NEMO, the regulatory subunit of IκB kinase complex. NEMO preferentially binds K63-linked ubiquitin chains, and OPTN was shown to compete with NEMO for poly-ubiquitinated RIP1 dampening TNF-induced NF-κB signaling. Since OPTN is also an NF-κB target gene, it could act as a negative feedback regulator preventing uncontrolled inflammation. OPTN was also shown to be crucial for the phosphorylation of IRF3 and IFN type I production after LPS, and was shown to function as an autophagy receptor needed for the autophagic clearance of cytosolic Salmonella enterica. However, the vast majority of these data comes from *in vitro* studies and no full knockout mouse has been described. Therefore, we generated full OPTN-knockout mice to study the role of OPTN *in vivo*. OPTN-KO mice are indistinguishable from their wild-type littermates, develop normally and do not show any signs of spontaneous organ abnormality. Also upon DSS challenge OPTN-deficient mice do not show higher susceptibility to colitis. Unexpectedly, TNF-treated embryonic fibroblasts show normal NF-κB responses and also *in vivo* no differences could be observed upon TNF injection. However, in primary bone marrow derived macrophages, OPTN-deficiency leads to a reduced TBK1 and IRF3 phosphorylation and IFN type I production after LPS or poly(I;C), suggesting that OPTN is involved in innate immune responses upon pathogen infection. Finally, we inoculated mice with Salmonella enterica sv. Typhimurium and observed that in agreement with a role for OPTN in autophagy and bacterial clearance, OPTN deficiency sensitizes mice to Salmonella infection.

W49. Defining the Intestinal Epithelial Defects in Crohn's Disease

Kelli VanDussen, Richard Head, Ta-Chiang Liu and Thaddeus Stappenbeck. Washington University, St. Louis, MO

Intestinal epithelial defects are likely critical to Crohn's disease (CD) pathogenesis; however, molecular targets have been elusive due to varying degrees of inflammation in the tissue specimens typically used for expression screens. To circumvent this problem, we generated transcriptional profiles of CD patients (n=38) and non-IBD controls (n=33) by both RNA-seq and microarray using unstained, formalin-fixed, paraffin-embedded tissue sections of surgical resection margins. Histological scoring of serial

tissue sections was used to subdivide the CD patients into two additional groups: no inflammation and mild chronic inflammation (but no active disease). We identified three principal gene networks with significant, differential expression between the CD and non-IBD groups. The most prominent was an epithelial cell-associated network present in ~75% of the CD patients that was independent of mild inflammation and contained 418 down-regulated transcripts (compared to controls) with both known and novel disease-relevant functions. We validated this network in biopsy tissue from CD patients who had not undergone resection. Two additional networks, the inflammatory and Paneth cell networks, exhibited a coordinated increase in transcript abundance in CD patients who had mild chronic inflammation and elevated Paneth cell numbers detected histologically. This study uncovered an epithelial signature that represents a partial inhibition of differentiation in most adult CD patients. This signature will be a critical tool to uncover novel aspects of CD pathogenesis and is a potential therapeutic target.

W50. Dual Oxidase 2 (DUOX 2), a Novel Player in IBD: Expression and Functional Implications

Keren Rabinoiwtz, Leah Reshef, Uri Gophna and Iris Dotan. Tel Aviv University, Tel Aviv, Israel

Dual Oxidase 2 (DUOX2) mediates anti-pathogen responses in the intestine by secreting reactive oxygen species (ROS) and may be induced by microbiota. We recently reported an increase in DUOX2 expression in inflammatory bowel disease (IBD) patients. Aim: To decipher DUOX2 expression and function in intestinal inflammation. DUOX2 mRNA expression in the non-inflamed small and large intestine of IBD patients was increased (30 fold, $p<0.05$). This was further augmented in inflamed IBD tissues (≥ 150 fold, $p<0.001$). Decrease in microbial taxa diversity was observed in fecal samples from IBD patients with inactive disease compared to normal controls ($p<0.004$). Further decrease was noticed in fecal samples from IBD patients with active disease ($p<0.0001$). DUOX2 expression by epithelial cell lines significantly increased in response to inflammatory cytokines (IL-1b, TNF-a, IFN-g, IFN-b) (≥300 fold increase, $p<0.001$) and to fecal extracts derived from patients with active IBD (≥10 fold increase, $p<0.001$). ROS production increased in response to fecal extracts derived from patients with active IBD (3 fold, $p<0.001$) but not in response to inflammatory cytokines alone. Increase in DUOX2 mRNA expression and decrease in microbial taxa diversity in fecal samples correlated with IBD activity. DUOX2 expression in epithelial cells is increased in response to inflammatory stimuli and to fecal extracts derived from patients with active IBD. In contrast, ROS production increased only in response to fecal extracts. Thus DUOX2 increased expression and aberrant function are affected by inflammatory stimuli and intestinal microorganisms, and may have a role in further augmenting intestinal inflammation in IBD.

W51. The Role of Epithelial Cells in Mucosal Inflammation in the Zebrafish Gut and Gills

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Mucosal barriers of the intestine and the lung offer the first line of protection in the host defense mechanisms against ingested and inhaled antigens. While long considered innocent bystanders simply forming a physical barrier, it is becoming increasingly evident that mucosal epithelial cells play a crucial role in maintaining immune homeostasis and mediating inflammation. Initially we have found that a single exposure to a diet rich in cholesterol results in the accumulation of myeloid cells in the intestine in both zebrafish and mice. Pharmacological and genetic interventions demonstrated that cholesterol induces inflammasome activation in intestinal epithelial cells which in the longer-term leads to impaired intestinal motility. This model reveals a novel route by which dietary cholesterol can initiate intestinal inflammation. To test whether similar processes could take place in the epithelium of the airways, we performed similar studies in the zebrafish gills, which is a non-ciliated but otherwise equivalent epithelium to that of mammals. Adult zebrafish were exposed to cigarette smoke and we found that inflammatory cytokine production and myeloid cells were affected. We are currently studying the involvement of epithelium in the activation of pathways leading to this observation. Together, our models of mucosal inflammation illustrate the power of the zebrafish system to study pathophysiological responses induced within epithelial cells in a whole organism.

W52. Defect in TLR5 Expression Enhances Spontaneous Visceral Hypersensitivity and Decreases Anxiety Behavior
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Irritable Bowel Syndrome (IBS) and Inflammatory Bowel Diseases (IBD), intestinal disorders associated with colonic hypersensitivity (CHS) and abdominal pain, are believed to result in part from breakdown of homeostasis between intestinal microbiota and the mucosal immune system. In addition, such patients also suffered from several associated disorders, such as anxiety and depression. Among alterations in immune innate function, some are related to the Toll-Like Receptor 5 (TLR5) responsible for the primary defense of the organism against microbial intruders especially potentially virulent invasive bacteria expressing flagellin. Mice deficient for TLR5 (TLR5KO) have been described to uniformly exhibit altered microbiota and increased levels of inflammatory markers. The aim of this work was to assess visceral sensitivity of TLR5KO mice and to determine if visceral pain was associated with anxiety and depressive disorders. TLR5KO mice uniformly exhibited greater CHS in comparison to their WT littermates, revealed by an increase of the visceromotor activity of the abdominal muscle in response to a colorectal distention. Surprisingly, TLR5KO mice showed decreased anxiety while depressive phenotype and cognitive disorders were not observed. This study demonstrated an involvement of TLR5 and innate immunity in CHS and anxiety behavior. Such impact could be direct, depending on its expression at the nervous level, or indirect and related to its role in the modulation of the intestinal microbiota.

W53. Epithelial Autophagy Dampens Chronic Intestinal Inflammation
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Inflammatory bowel disease (IBD) is a chronic disorder of the gastrointestinal tract, thought to arise from an imbalanced immune response to the microflora. Intestinal epithelial cells (IECs) form the physical and chemical barrier separating the microflora from the mucosal immune cells and are a key player in maintaining homeostasis as well as modulating the immune response. Recent genome-wide association studies (GWAS) linked polymorphisms in the autophagy genes ATG16L1 and IRGM with susceptibility to inflammatory bowel disease (IBD). We sought to investigate the role of Atg16l1/ autophagy within the intestinal epithelium during chronic colitis. We employed a tissue-specific Atg16l1-deficient transgenic mouse strain in a Helicobacter hepaticus driven model of chronic colitis. In parallel, we studied the response of the IECs towards inflammatory mediators in an *ex vivo* organoid system. Mice lacking Atg16l1 in IECs ($Atg16l1^{\Delta villin}$) were more susceptible to development of chronic colitis. However, despite severely aggravated pathology in $Atg16l1^{\Delta villin}$ mice, we did not observe elevated adaptive immune responses in the lamina propria, but found elevated levels of epithelial derived chemokines. In accordance, genome-wide RNA sequencing of organoids stimulated with inflammatory cytokines revealed increased activation of Atg16l1-deficient IECs compared to wt IECs. These findings suggest that the IBD susceptibility gene ATG16L1 actively regulates the pro-inflammatory response in the epithelium and thereby dampens chronic intestinal inflammation.

W54. Assessing DNA Methylation in the Developing Human Intestinal Epithelium: Potential Link to Inflammatory Bowel Disease
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DNA methylation is one of the major epigenetic mechanisms implicated in regulating cellular development and cell type- specific gene expression. We investigated the role of DNA methylation in regulating human intestinal epithelial cell function and its potential impact on the development of inflammatory bowel diseases (IBD). Simultaneous genome-wide DNA methylation and gene expression analysis was performed on purified intestinal epithelium from human fetal and healthy pediatric gut, as well as from children newly diagnosed with IBD. Functional impact of DNA methylation on gene expression was assessed using *in-vitro* assays. We observed distinct differences in DNA methylation profiles between fetal and pediatric epithelium. Comparative analysis identified 214 genes for which expression is regulated via DNA methylation, i.e. regulatory differentially methylated regions (rDMRs). Pathway and functional analysis of rDMR- associated genes suggested a critical role for DNA methylation in regulating gene expression and functional development of the human intestinal epithelium. Moreover, analysis on epithelium of pediatric IBD patients revealed altered DNA methylation in genomic loci which overlap significantly with those undergoing methylation changes

during intestinal development. Our study provides novel insights into the role of DNA methylation in regulating functional maturation of the human intestinal epithelium and suggest a possible link to IBD.

W55. The Involvement of Macrophages in the Function and Maintenance of the Intestinal Epithelium
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Our aim is to investigate the involvement of macrophages in the development, function and maintenance of the intestinal epithelium. Our data suggests that macrophages have an influence in the composition of the intestinal epithelium. We treated mice with an anti-CSF1R monoclonal antibody (M279) for 6 weeks which resulted in a complete ablation of macrophages in the gut (Sauter et al. 2013). Subsequently, we observed depletion of Paneth cells in intestinal crypts and reduction in M cell density in the follicular associated epithelium (FAE). These data suggest that macrophages may have a more intimate relationship with Paneth cells than previously understood and influence the intestinal epithelium by providing a scaffold for intestinal crypt proliferation function. In a sister study we treated mice with anti-CCL6 and anti-CCL9 antibodies which were previously described to be expressed exclusively by M cells in the FAE (Kobayashi et al. 2012). These chemokines were suspected to be involved in the chemoattraction of antigen presenting cells to the pockets of M cells to initiate antigen sampling. We found depletion in M cell post anti-CCL9 antibody treatment; however no difference was found in M cell densities in mice treated with anti-CCL6.

W56. Immunosuppressive Agents Induce MicroRNAs Controlling Innate Immunity During Renal Bacterial Infections
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Urinary tract infections (UTI) and pyelonephritis, mainly due to uropathogenic Escherichia coli(UPEC), is the most common bacterial infection observed in renal transplanted patients that may compromise renal graft function. Cyclosporine A (CsA) is an immunosuppressant drug widely used in prevention after organ transplantation, which inhibits T cell proliferation and activation. We already showed that renal collecting duct (RCD) cells are a preferential site of UPECs adhesion and initiation of Toll-like receptor 4 (TLR4)-mediated inflammatory response for host defense. Immunosuppressive therapy post-transplant increases the risk of bacterial infection and eventually subsequent graft failure, but the cellular mechanism by which they favor UTI remains elusive. The aim of the study was to determine the effects of CsA on cellular mechanisms involved in innate host defense against UPEC in RCD cells. The results show that TLR4 expression is down regulated by CsA on RCD, leading to a decrease of UPEC-induced cytokine production and an increase of bacterial proliferation in the kidney. Results were confirmed in a mouse model of urinary tract infection. We also showed that the down-regulation of TLR4 expression by CsA is dependent of the induction of a microRNA specifically targeting TLR4. The injection of an antagomiR to UPEC-infected mice treated with CsA reestablishes the production of cytokines by RCD and favors bacterial clearance. These findings could explain the greater sensitivity of patients to UTI after transplantation and highlight the possible use of microRNAs as therapeutic tool to prevent progressive alteration of the graft.

W57. C-Jun N-Terminal Kinase 2 Mediates Cytoprotective Functions in Intestinal Epithelial Cells and Ameliorates Experimental Colitis
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Objective: C-Jun N-terminal kinases (JNKs) regulate several cellular functions including apoptosis, proliferation, differentiation and cytokine production. JNKs are encoded by three genes, but Jnk3 is not transcribed in the gastrointestinal tract. In this study we analyzed the role of JNKs in the intestine using knockout mice. Design: Complete deletion of JNKs in intestinal epithelial cells (IECs) was achieved by crossing mice with a conditional IEC-specific Jnk1 and a complete Jnk2 deletion ($Jnk1^{DIEC}$ /$Jnk2^{-/-}$). We analyzed these mice during homeostasis and dextran sodium sulfate (DSS)-induced colitis and compared their phenotype with wild-type (Wt) and single knockout animals ($Jnk1^{-/-}$, $Jnk2^{-/-}$ and $Jnk1^{DIEC}$). Furthermore, we performed bone-marrow transfers to create chimeric $Jnk2^{-/-}$/Wt animals. Results: In the colon JNK2 was more strongly expressed then JNK1 and highly induced upon inflammation. All untreated mice were healthy, developed normally and did not show any signs of intestinal inflammation or barrier dysfunctions. However, $Jnk1^{DIEC}$ /$Jnk2^{-/-}$ and to a lesser extent $Jnk2^{-/-}$ mice showed enhanced IEC cell turnover with increased rates of apoptosis and proliferation. Jnk1 deficiency alone had only a minor effect on IEC cell turnover. After DSS challenge $Jnk2^{-/-}$ mice

exhibited increased pathology, IEC apoptosis, goblet cells loss, inflammation, ER stress and barrier dysfunction. Investigation of chimeric Jnk2$^{-/-}$/Wt mice showed that JNK2 expression in non-hematopoietic cells mediated the protective effects. Jnk1 deficiency alone or in combination with Jnk2deletion showed only a slightly aggravated colitis. Conclusion: JNKs, and in particular JNK2, have cytoprotective functions in IECs and ameliorate chemical induced acute colitis.

W58. SIRT1 Attenuates Nasal Polypogenesis by Suppressing HIF-1-Mediated Epithelial-to-Mesenchymal Transition
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Background: Nasal polyps imply a refractory clinical course in chronic rhinosinusitis (CRS). Previously, we showed that hypoxia-inducible factor (HIF)-1 could mediate nasal polypogenesis via epithelial-to-mesenchymal transition (EMT). SIRT1, a histone deacetylase, reportedly suppresses the transcriptional activity of HIF-1. Thus, the authors hypothesized that SIRT1 attenuates nasal polyposis by inhibiting HIF-1-induced EMT. Methods: The effects of SIRT1 on nasal polypogenesis were investigated in previously developed murine models. Immunohistochemistry, immunoblotting and immunoprecipitation were done to evaluate SIRT1 level, EMT and hypoxic markers in human nasal epithelial cells or sino-nasal tissues from the mice and the CRS patients with or without nasal polyp. Results: SIRT1 transgenic (TG) mice had significantly fewer mucosal lesions with epithelial disruption and fewer nasal polyps than wild-type (WT) mice. In addition, resveratrol (a SIRT1 activator) treatment suppressed nasal polypogenesis in WT mice; however, sirtinol (a SIRT1 inhibitor) administration increased the polyp burden in SIRT1 TG mice. In CRS sinonasal specimens, SIRT1 was down regulated in the mucosa from patients with polyps as compared with patients without polyps. SIRT1 overexpression or activation reversed hypoxia-induced EMT in human nasal epithelial cells (hNECs). The intranasal transfection of a sh-SIRT1 lentiviral vector induced more nasal polypoid lesions in SIRT1 TG mice. Finally, mucosal extracts from CRS without nasal polyps increased SIRT1 expression in nasal epithelial cells, and those from CRS with nasal polyps did not. Conclusion: SIRT1 suppressed nasal polyp formation, possibly due to inhibition of HIF-1-induced EMT. Thus, nasal epithelium SIRT1 may be a therapeutic target for nasal polyps.

W59. Intestinal Organoids as a Model for Bacterial Infection and Innate Immune Responses
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Recently, intestinal organoid cultures have been employed as a new experimental model of the intestinal epithelium. By isolating and cultivating intestinal stem cells from the intestinal crypts of conventional or transgenic mice, or from human patient biopsies; primary epithelial cell cultures can be established to model the epithelium from either the small intestine or colon. These resultant organoid cultures include many of the cell types normally found in the intestinal epithelium, including enterocytes, goblet cells, Paneth cells and enteroendocrine cells. Furthermore, the resultant epithelium polarizes and forms a crypt structure similar to the *in vivo* intestinal epithelium. In our research, we have evaluated these organoid cultures as a model for infection and innate immunity. We have employed them as a model of infection and cell invasion for the common enteric pathogen Campylobacter jejuni. Additionally, we have tested the response of these cells to pro-inflammatory stimuli ranging from innate receptor ligands and cytokines, to bacterial factors to test their responses to stimuli relative to their *in vivo* counterparts. In conclusion, this novel technique of primary epithelial cell culture, promises new methods for the study of infection and innate immunity.

W60. Exocytosis of MUC2 is Regulated by VAMP8 and Plays a Critical Role in DSS-Induced Colitis
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The gastrointestinal mucus layers provide the first line of innate host defense by spatially separating potential threats and pathogens away from the surface epithelium. Goblet cells produce, store and secrete MUC2 mucin constitutively and in response to noxious substances and pathogens by an unknown mechanism. In this study, we characterized the exocytosis machinery that is responsible for the release of MUC2 as SNAP23, Syntaxin 3, VAMP8 and Munc18b by pull-down and confocal microscopy. Mucin exocytosis was driven by both calcium and PKCδ through independent mechanisms. Knock down of SNAP23 and VAMP8 by shRNA in colonic goblet cells significantly decreased both constitutive and induced MUC2 exocytosis. In Vamp8$^{-/-}$ mice the colonic epithelium showed gross structural deformities in crypt architecture, goblet cell hypertrophy, enlarged granules and surface epithelium erosion as compared to Vamp8$^{+/-}$ and Vamp$^{+/+}$ littermates. Vamp8$^{-/-}$ mice were highly susceptible to DSS-induced colitis and all animals succumb to disease by day 6. This coincided with higher disease activity index scores with increased neutrophil

infiltrate and MPO levels. These results demonstrate that the MUC2 exocytosis machinery is highly regulated and highlight the importance of the mucus barrier in normal and disease states.

W61. TLR-Independent Intestinal Epithelial TRAF6 Signaling Protects Mice from DSS-Induced Colitis
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Multiple studies in mice demonstrated that the gut microbiota modulates host susceptibility to intestinal inflammation. However, the cell types and the signaling pathways orchestrating this bacterial regulation of intestinal homeostasis remain poorly understood. Here we investigated the function of epithelial TLR responses in the Dextran Sodium Sulfate (DSS)-induced mouse model of colitis. Blocking epithelial TLR signaling by simultaneous deletion of MyD88 and TRIF specifically in intestinal epithelial cells (IECs) did not alter DSS-induced colitis severity, unequivocally showing that microbiota-driven epithelial TLR signaling does not influence inflammation in this model. In contrast, mice lacking the downstream ubiquitin ligase TRAF6 in IECs showed exacerbated DSS-induced inflammatory responses that were driven by the gut microbiota and that resulted in the development of chronic colitis. Together, these results reveal that epithelial TRAF6-dependent but MyD88/TRIF- and thus TLR-independent signaling pathways are critical for preventing propagation of DSS-induced colon inflammation by the gut microbiota.

W62. Role of Estradiol on Intestinal Permeability and Bacterial Translocation During Systemic Inflammation in Female Rats
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The intestinal epithelium exerts a critical role as a barrier to prevent the dissemination of microorganisms from the gut. The integrity of this epithelium may be compromised during systemic inflammation, increasing intestinal permeability. The inflammatory mediators produced during systemic inflammation mediates the intestinal barrier disruption. Estradiol has been demonstrated as an anti-inflammatory hormone and responsible for maintaining the architecture of intestinal epithelium. In present study, we evaluated the role of estradiol on the intestinal permeability and bacterial translocation during systemic inflammation induced by lipopolysaccharide administration (LPS; 1.5 mg/kg, intravenous) in female rats. The female rats were ovariectomized and allowed to recover for 10-12 days before the experiment. For three consecutive days, rats were pre-treated with estradiol cypionate (50 or 100 µg/kg, subcutaneous) or corn oil (vehicle). The intestinal permeability was determined at 6h after LPS or saline administration by injecting FITC-dextran 4 kDa in the colon or ileum. Furthermore, the bacterial translocation was evaluated by the number of colony forming units in the mesenteric lymph nodes also at 6h after LPS administered. Our results demonstrate that estradiol reduces intestinal permeability in both gut segments, colon and ileum. Moreover, estradiol also prevents the bacterial translocation induced by LPS. This study suggests that estradiol participates in the gut protection against the deleterious effects of systemic inflammation on the intestinal epithelium.

W63. Cell Wall from CRL431 Induce Both Intestinal Epithelial and Macrophage Cells Activation
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Cell wall (CW) molecules from probiotic are crucial to interact through host-receptors and induce signaling that result in the immune system modulation. The knowledge of bacterial components as immunomodulators offers interesting opportunities to elucidate a novel structure as oral adjuvant. In the current study we aimed to study the impact of CW from Lactobacillus casei CRL431 (Lc431) in the activation of intestinal epithelial cell (IEC) and peritoneal macrophages (MQP) to be the sentinels of innate immune cells. CW fraction was obtained from Lc431 (CW431). *Ex vivo*, phagocytic and antimicrobial activities of MQP were determined. *In vitro*, IL-6 levels of supernatant of MQP were determined. IEC activation was observed trough transmission-electron-microscopy (TEM). Lc431 and CW431 groups showed phagocytic and microbicidal activity significantly higher in those groups when compared with the control mice, while values for IL-6 do not improved respect to control group. However, Lc431 and CW431 showed for TEM a higher activation of IEC after 30 min of stimulation when compared with IEC control. Probiotic are able to activate and improve the activity of IEC that is a key cell in mucosal immune system activation. This work demonstrated that cell wall fraction, could be interesting alternative as oral mucosal immunomodulators.

W64. Cholecystokinin Prevents Intestinal Barrier Dysfunction Induced by Systemic Inflammation in Rats
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Systemic inflammation consists in a primary injury to the intestinal epithelium structure. The inflammatory mediators synthesized in the acute phase contribute to the increase in intestinal permeability and bacterial translocation. Cholecystokinin (CCK) was firstly described as a gastrointestinal hormone, but immune cells express their receptors, suggesting a possible involvement of this peptide in pathophysiological processes. Our aims were to evaluate the role of CCK on intestinal permeability, bacterial translocation and production of cytokines during systemic inflammation induced by lipopolysaccharide administration (LPS). Male Wistar rats were pre-treated with proglumide (P) (non-selective CCK receptor antagonist; 30 or 50 mg/kg) or CCK (0.4 or 40 µg/kg) before LPS administration (1.5 mg/kg, i.v.). At 4 and 24h after endotoxemia induction, the intestinal permeability was evaluated by injecting FITC-dextran 4 kDa in the colon or ileum, mesenteric lymph nodes were collected for microbiological analysis and also cytokines were quantified in the plasma. Our results demonstrated that CCK administration reduces the permeability only in the colon, while P showed the opposite effect. The plasma concentrations of TNF-α, IL-1β, IL-6 and IFN-γ were significantly reduced in CCK-treated rats, while the P group showed increased synthesis of these cytokines. Furthermore, CCK prevented the bacterial translocation to the mesenteric lymph nodes at the both time-point investigated in comparison to LPS group. This data suggests a protective role for CCK preventing the intestinal barrier dysfunction induced by systemic inflammation, possibly modulating the inflammatory response.

W65. Hydrolysates from Different Food Sources Show Protective Effects on the Intestinal Epithelial Barrier
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Recently, dietary molecules are more and more recognized to, besides having a nutritional function, act directly on functionalities of the intestine. For example, several hydrolyzed dietary proteins (hydrolysates) were found to show immunomodulating effects. Increased permeability of the epithelial barrier, an important aspect of the intestinal immune system, is associated with various intestinal and systemic diseases. We investigated the effect of hydrolysates on epithelial permeability in the T84 intestinal epithelial cell monolayer model. Polarized T84 cells were pre-incubated with hydrolysates and stimulated with the pro-inflammatory phorbol-12-myristate-13-acetate (PMA). Trans-epithelial electrical resistance (TEER) was analyzed as a measure for tight junction–mediated barrier function. Hydrolysates from different sources (soy, wheat and cow's milk) attenuated the PMA induced permeability increase. For cow's milk hydrolysates, the observed effect seems to depend on the cow's milk fraction (whey or casein) and hydrolysis. Overall, specific hydrolysates show protective effects on the epithelial barrier. However, the underlying mechanisms are unknown. Since Toll-like receptor 2 and 9 activation is associated with increased epithelial integrity, and the protective hydrolysates stimulate these receptors, we hypothesize that protective hydrolysates confer their effect via these TLRs. More research is needed to confirm this. Ultimately, this knowledge will lead to a better understanding of how nutrition can contribute to improving health via direct effects on the intestines.

W66. Lactate and Short Chain Fatty Acids Modulate TLR-Mediated Activation of Epithelial and Myeloid Cells *in vitro*
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Short chain fatty acids (SCFA) are produced by intestinal microbiota in the large intestine where they can interact with players of innate immune response. In order to determine if SCFA (butyrate, propionate, acetate and lactate) can modulate the activation of myeloid or epithelial cells, bone marrow derived macrophages (BMMs) or bone marrow derived dendritic cells (BMDCs) were treated with 100 ng/mL of E.coli LPS and different concentrations of each SCFA. In all cases SCFA modulated surface expression of CD40 in BMDCs ($p<0.05$). Effects of butyrate and propionate were evident at lower concentrations than lactate and acetate. Butyrate and propionate modulated IL6 generation at concentrations higher than 10 mM ($p<0.05$), without affecting IL12 production. Lactate modulated IL6, IL1b and IL12 production in BMM, at concentrations higher than 20 mM ($p<0.05$) which correlated with modulation of the increase in glycolytic rate observed upon TLR4-dependent activation. Flagellin NFkB-mediated activation of intestinal epithelial cells was modulated by all SCFA treatments. Butyrate and propionate showed these effects at concentrations higher than 1 mM whereas lactate and acetate modulated CCL20, CXCL2 and CXCL10 expression at concentrations

higher than 10 mM. SCFA can modulate TLR-activated epithelial and myeloid cells at concentrations usually found in large bowel lumen.

W67. Anti-Inflammatory Effect of Dietary Fibers is Toll-Like Receptor Dependent

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A range of dietary carbohydrate fibers are present in fruits and grains and thus constitute an important part of our diet. As such these fibers are responsible for many health beneficial effects and are attributed to have anti-inflammatory effects in the gut. However, the molecular mechanism of interaction between dietary fibers and the immune system is not known yet. Recently, we have shown that β2-1 fructans protect the barrier function of epithelial cells through activation of Toll-Like receptors (TLR). Therefore, we hypothesized that the carbohydrate fibers interact with innate immune receptors like TLR in the intestine to impart their health beneficial effects. TLRs play an important role in maintaining the tight junctions in intestine and also immune homeostasis of the gut. To analyze the interaction of dietary fibers with TLRs we used TLR reporter cell lines. Only particular dietary fibers were found to inhibit TLR activation, leading to reduced NF-κB activation. To confirm the anti-inflammatory effects, we studied the effect of the identified dietary fibers administration in a Doxorubicin induced mucositis model. Doxorubicin is a chemotherapeutic agent which in addition to its anti-cancer effect, causes intense inflammation in the intestine leading to mucositis. Administration of the selected dietary fibers in mice was found to be beneficial to reduce inflammation symptoms of mucositis. Thus, we propose a novel anti-inflammatory food intervention for doxorubicin induced mucositis.

F128. The Intestinal Epithelial Cells and Toll-Like Receptors TLR-2 and TLR-4 Involvement in Immune Modulatory Effect of AHCC

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Active Hexose Correlated Compound (AHCC®) is a cultured mushroom extract that is commercially available and promoted for immune support. AHCC supplementation is shown to affect immune cell populations and immune outcomes, including NK cell response to infection. The mechanism of the immunomodulatory effect is not well understood. The present work aimed to characterize the activity of AHCC in the gut and to study the effects of AHCC on Toll-like receptor (TLR) signaling in intestinal epithelial cells (IECs). BALB/c mice were fed AHCC by gavage. *In vivo* activities were assessed by immunohistochemistry and cytokine production. The effects of AHCC on *ex vivo* primary cell culture from IECs were examined after challenge with LPS or E. coli alone or in the presence of anti-TLR-2 and TLR-4 blocking antibodies. Feeding AHCC resulted in increased IgA+ cells in the intestine and increased sIgA, IL-10, and IFN-γ in the intestinal fluid. In IECs, contact with AHCC increased IL-6 production but not to the pro-inflammatory level of positive controls, LPS and E. coli. Blocking TLR-2 and TLR-4 reduced the induction of IL-6 by AHCC, suggesting that these innate receptors are involved in generating the immune response of IECs to AHCC. It was suggested that AHCC plays a role in the immune response and the maintenance of immune homeostasis in part by priming the TLR-2 and TLR-4 gate at the intestinal epithelium. The response is likely due to the recognition of non-pathogenic food-associated molecular patterns (FAMPs) such as those found associated with other mushroom or yeast-derived compounds.

FOOD ALLERGY

OR.53. Deletion of Wiskott-Aldrich Syndrome Protein in Regulatory T Cells Results in Spontaneous Allergic Sensitization to Food Antigens.

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Patients and mice genetically deficient in Wiskott-Aldrich syndrome protein (WASp) have significantly elevated levels of serum IgE of poorly defined antigenic specificity. Here we show that WAS patients and $WASp^{-/-}$ mice mount IgE and IgG responses against common human food allergens such as wheat and soy. Spontaneous food allergy in $WASp^{-/-}$ animals developed independently of genetic background or colitis, and phenocopies human food allergy with small intestinal mast cell accumulation and improvement of allergic symptoms upon elimination diet. Sensitization was preserved in germfree $WASp^{-/-}$ mice, was not transferrable to co-housed wild-type mice and could not be prevented by fostering of $WASp^{-/-}$ pups by wild-type mothers. The antigen-specific IgE produced following oral ovalbumin administration in $WASp^{-/-}$ mice mediated equally effective type I hypersensitivity reactions *in vitro* and *in vivo* when compared to an adjuvant-based model of food allergy in wild-type mice. $Foxp3^{+}$ Tregs are critical to disease pathogenesis, as conditional deletion of WASp in Tregs resulted in exacerbated disease. We demonstrate that IgE/FcεRI-mediated signaling is impaired in WASp-deficient mast cells, accounting for the disparity in severity of intestinal Th2-type inflammation between complete and Treg-conditional $WASp^{-/-}$ mice. Classification of food allergies in a cohort of 25 WAS patients revealed a prevalence of clinical food allergy of 20%, indicating that relative dampening of allergic responses by $WASp^{-/-}$ mast cells is likely to be operative in human patients. In conclusion, our study demonstrates allergic sensitization to food as a feature of WASp-deficiency and establishes Treg-specific $WASp^{-/-}$ mice as a spontaneous model of food allergy.

OR.54. Lack of Thymic Stromal Lymphopoietin (TSLP) Down-Regulation of Mucosal Pro-Inflammatory Cytokines in Refractory Coeliac Disease (CD)

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Background & Aims. TSLP is down-regulated in untreated CD mucosa, whereas its levels are unknown in refractory CD (RCD). We evaluated duodenal TSLP and TSLP receptor (TSLP-R) expression in RCD, and we studied TSLP effects on pro-inflammatory cytokine production by RCD biopsies. Methods. TSLP and TSLP-R expression was studied on duodenal biopsies from 12 RCD, 14 uncomplicated CD patients and 14 control subjects by qRT-PCR, confocal microscopy and immunoblotting. IL-17A, IL-6 and TNF-α were measured by ELISA in the supernatants of duodenal biopsies from 6 RCD patients cultured *ex vivo* with or without rhTSLP. Results. TSLP gene and protein expression was significantly reduced in the duodenum of RCD and untreated CD patients compared to treated CD patients and control subjects, without differences between RCD and untreated CD patients. TSLP-R was expressed in the duodenal mucosa, without significant differences between RCD, untreated and treated CD patients and control subjects. rhTSLP significantly inhibited IL-17A, IL-6 and TNF-α release by RCD biopsies (from 62±6 to 20±3 pg/ml; from 1892±341 to 632±172 pg/ml; from 181±42 to 67±15 pg/ml, respectively). Conclusions. Reduced TSLP expression may sustain the pro-inflammatory cytokine increase observed in RCD. Further studies are needed to clarify the influence of TSLP reduction on mucosal immunosurveillance in RCD.

W68. The C5a/C5aR1 Axis Drives Experimental Food-Induced Anaphylaxis

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Food-induced anaphylaxis is a life-threatening allergic reaction mainly driven by antigen cross-linking of IgE-loaded Fce-receptors on mast cells (MCs). Such MC activation leads to the release of pro-inflammatory mediators eventually resulting in anaphylaxis. The complement fragment C5a can activate MCs through its cognate C5a receptor 1 (C5aR1). Here, we subjected $C5aR1^{-/-}$ and wild-type

(wt) Balb/c mice to oral ovalbumin (OVA)-induced anaphylaxis. In wt mice but not in C5aR1$^{-/-}$ male mice, we observed a severe clinical phenotype. More specifically, 78% of wt but only 9% of C5aR1$^{-/-}$ mice suffered from diarrhea and hypothermia. The lower incidence of evidence of anaphylaxis in C5aR1$^{-/-}$ mice was associated with decreased OVA-specific serum IgE and MC activation (MCPT-1 levels). Further, histamine treatment resulted in a milder temperature drop in C5aR1$^{-/-}$ than in wt mice. To link the observed phenotype with MC function, we examined activation and degranulation of bone marrow-derived MCs (BMMCs) in response to FceR cross-linking. Degranulation of C5aR1$^{-/-}$ BMMCs was reduced by 50% and IL-6 production was reduced by 35% as compared with wt cells. Our findings identify a critical role of the C5a/C5aR1 axis in IgE-mediated oral antigen-induced anaphylaxis. C5aR1 targeting may prove useful to suppress the inflammatory response in food-induced anaphylaxis.

W69. Preventive and Therapeutic Effects of Dietary Omega3 Fatty Acid-Originated 17,18-Epoxyeicostetraenoic Acid on the Control of Intestinal Allergy

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Omega-3 polyunsaturated fatty acids (PUFAs) have anti-allergic properties, but the metabolic progression from dietary oil to host immune system remains to be elucidated. Here we show that 17, 18-epoxyeicosatetraenoic acid (17,18-EpETE) as an anti-allergy metabolite generated in the colon preferentially from dietary omega-3 alpha-linolenic acid (ALA). Incidence of allergic diarrhea was decreased in mice fed with ALA-rich linseed oil (Lin-mice) when compared with mice fed with conventional soybean oil. MALDI-IMS-based imaging analyses revealed increase of ALA and its metabolites, especially eicosapentaenoic acid (EPA), in the colon of Lin-mice. Further, LC-MS/MS-based mediator lipidomics identified the increase of EPA-derived metabolites including 17,18-EpETE in the colon of Lin-mice. Administration of synthetic 17,18-EpETE showed preventive and therapeutic effects on the development of allergic diarrhea. These data suggest that metabolizing dietary w3 PUFAs generates 17, 18-EpETE, which is an endogenous anti-allergic metabolite and potentially is a therapeutic agent to control intestinal allergic diseases.

W70. Suppression of the Allergic Reaction in a Food Allergy Mouse Model Through the Oral Administration of a Heat-Killed *Tsukamurella inchonensis*

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Food allergy, a worldwide immune adverse reaction to certain foods, is a growing clinical concern with no approved standardized immunotherapy. In this work we aimed to modulate milk allergy in a mouse model through the oral administration of a heat-killed Actinomyces bacteria (Tsukamurella inchonensis-Ti). Balb/c mice were sensitized with cow´s milk proteins (CMP) plus cholera toxin by gavage, and orally challenged with CMP to evidence hypersensitivity. Thereafter, Ti was orally administered during two months for immunomodulation. Mice were challenged and treatment efficacy was *in vivo* (clinical score and cutaneous test) and *in vitro* (serum specific antibodies and cytokines by ELISA, and cell analysis by flow cytometry) evaluated. Clinical signs and serum specific IgE levels were lower in Ti-treated mice compared with sensitized mice ($p<0.05$), with a concomitant reduction of IL-4 and IL-5. Ti-treated mice showed a reduction of intestinal $CD4^+$ $CD25^+$ $CD69^+$ Teff cells with an increased frequency of lamina propria $CD4^+$ $CD25^+$ $FoxP3^+$ T cells (9.61±2.15% vs 6.15±0.25% Ti-treated and Sensitized, respectively). Intestinal IL-10 and IL-10^+ $FoxP3^+$ T cells were up-regulated. In conclusion, Ti induced Treg that controlled the Th2-medited allergic responses, with suppression of IgE. These findings may constitute the basis for a potential immunotherapy for food allergies.

W71. Consequences of the Introduction of a New Protein During the Recovery Period of a Chronic Intestinal Inflammation

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Introduction New foods introduced to an inflamed gut may result in sensitization to this food. AIM Evaluate the time required for recovery to develop tolerance to new proteins after a chronic gut inflammation. Methods: Male C57BL/6 mice(n=30) were immunized with 100µg of peanut protein. Half received a 30-day raw-peanut-challenge-diet (CD) (inflamed-I) while the other received mouse chow (controls-C) (Teixeira 2008). They were further divided and received sweetened OVA (new protein) (20% egg white, 5% sucralose, H_2O, v/v/v) orally for 7 days, on day 0 (I1/C1), 10 (I2/C2) or 20 (I3/C3) post CD. Body weight, food intake,

antibodies and T cell phenotype of mesenteric lymph nodes (MLN) and spleen (SPN) was assessed. ANOVA with Tukey post-test was performed ($p<0.05$). Approval of local Ethical Committee #00147-09. Results: OVA consumption was significantly lower in I1 ($3.21^{\pm}1.0$) compared to I2 ($6.53^{\pm}1.19$), I3 ($6.82^{\pm}2.3$) and C ($7.5^{\pm}1.7$). The MLN showed a significant increase of $CD8^{+}$T cells of I1 ($29.49^{\pm}4.1$) and I2 ($31.72^{\pm}4.0$) compared to I3 ($21.53^{\pm}3.6$) and C ($25.65^{\pm}5.4$) with no significant difference in $CD4^{+}$T cells (I $37.34^{\pm}5.7$ and C $38.35^{\pm}5.1$) and $CD4^{+}CD25^{+}$ T cells (I $6.86^{\pm}1.5$ and C $7.00^{\pm}1.9$). Increase of SPN $CD4^{+}$T cells in I1 ($38.67^{\pm}2.5$) compared to I2 ($25.93^{\pm}3.48$), I3 ($24.48^{\pm}5.9$) and C ($26.25^{\pm}12.2$) was observed. The I-1 showed gut inflammation, increased intraepithelial leukocytes, destruction/flattening of the villi and decrease of goblet cells. I-2 and I-3 were similar to C. Conclusions: Aversion to novel proteins in the context of gut inflammation may be a protective mechanism to multiple allergies while the recovery period was of at least 10 days.

W72. Inflammation or Dysbiosis: What Comes First?

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The complex ecosystem formed by the interaction between resident microbiota and the mucosal epithelium is essential for the maintenance of the intestinal homeostasis. Aim: investigate if Dysbiosis occurs in the gut of mice submitted to a gut, antigen specific chronic inflammation. Methods: C57Bl/6 mice received 100µg Peanut Protein Extract (PPE) or saline twice subcutaneously. 21 days after the booster inoculation half of the animals of each group received a raw-peanut, 30 day, challenge diet, while the other half continued eating mouse chow. After a macroscopic inspection of the peritoneal cavity, gut samples were retrieved for histological and microbiological analysis (DcodeTM Universal Mutation Detection System - BioRad). Results: PPE inoculated groups presented significantly higher anti-peanut antibodies compared to saline groups, irrespective of their diet. PPE inoculated animals that ate peanuts showed significant reduction of the villi number, shortening of the villi and increase in the mononuclear cell infiltrate in the epithelial layer and lamina propria altering the mucosal architecture. A significant qualitative shift of the bacterial profile was only observed in PPE inoculated animals challenged with the peanut diet. We are currently undertaking the quantitative analysis to determine which components of the microbiota altered most. Conclusion: the antigen specific chronic inflammatory bowel disease, developed by our group, is a good model to study the microbial shift of the gut microbiota during food allergies.

W73. *In vitro* and *in vivo* Screening of Candidate Probiotic Strains for their Anti-Allergic Properties

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There is considerable interest in generating efficient approaches that may reduce the risk of developing food allergy which is responsible for significant effects on morbidity and quality of life. Alterations in the sequential bacterial colonization of the gut could be responsible for a T-helper balance deviation, a major factor in the rise of allergic diseases. Therefore, a modulation of the gut microbiota is appealing for preventing and managing allergic diseases and this notion supports the use of probiotics. Our aim is to select a probiotic strain with preventive properties in allergy using a combination of *in vitro* and *in vivo* approaches. A panel of 33 strains was screened for their immunomodulatory properties on hPBMC and on splenocytes from ovalbumin-sensitized mice. Three *Lactobacillus* and three *Bifidobacterium* inducing a low IL12/IL10 ratio on both models were tested in a murine model of food allergy to beta-lactoglobulin (BLG). Three strains showed a protective impact on sensitization with a decrease in allergen specific IgE and on allergy with a decrease in mast cell degranulation. Among them, L. rhamnosus LA305 decreased the secretion of total and allergen specific IgE as well as blood mouse mast cell protease-1. In sensitized mice, the activation of Th17 pathway in ileum, the suppression of Th2 response in mesenteric lymph nodes, and the production of IFN-γ by BLG-stimulated splenocytes suggest a restoration of the T-helper balance that may, in part, explain the protective effect of LA305 on allergy beneficial. This project is co-funded by the ANRT and PiLeJe Group.

W74. Augmentation of Retinoic Acid Production in the Colonic Epithelial Cells Ameliorates Food Allergy via the Induction of Foxp3⁺ Regulatory T Cells in a Murine Model

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Background & Aim: Regulatory T cells (Tregs) in the lamina propria play a critical role in maintaining peripheral tolerance in the gut, and retinoic acid (RA) is required for induction of Tregs. Although colonic epithelial cells (CECs) produce RA, it remains unclear whether the RA production in the CECs leads to the induction of Tregs. It is reported that puerarin, isoflavone derivatives, exerts anti-inflammatory effects in several epithelial cells. Thus, we investigated the effect of puerarin in a food allergy (FA) mouse model. Methods: BALB/c mice were systemically sensitized and then orally challenged with ovalbumin in the FA mouse model. Results: Puerarin treatment ameliorated FA. The level of ALDH1A1 mRNA, RA generating enzyme, in the CECs and the proportion of $Foxp3^+CD4^+$ cells were significantly higher in the puerarin-treated FA mouse colon. The induction of $Foxp3^+CD4^+$cells by puerarin was reduced by pretreatment of LE540, retinoic acid receptor antagonist. In addition, the therapeutic effect of puerarin was suppressed by pretreatment of LE540 in the FA model. Conclusion: The present findings suggest that the augmentation of RA production in the CECs enhances the induction of Tregs and ameliorates FA in the mouse model.

W75. Interference of the Continuous Administration of Active Vitamin D in Model of Antigen-Specific Intestinal Inflammation

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Although the immune response to food proteins, usually results in tolerance, which is characterized by local hyperactivity with the production of IgA and non-inflammatory cytokines, concomitant with a low systemic production of specific IgG and IgE, proteins routinely tolerated can also induce local inflammatory responses. Aim: evaluate the immunomodulatory role of vitamin D in an antigen specific inflammatory gut model. Methods: Male C57BL/6 mice were subcutaneously administered with vitamin D while being submitted to allergy induction protocol that consisted in the immunized with 100µg of peanut protein. The sensitized groups received a 30-day raw-peanut-challenge-diet (inflamed - I) while the negative control group received mouse chow (controls - C). During the experimental protocol both groups received continuous administration of 75ng, 150ng or 300ng of vitamin D. Results: vitamin D decreased the expected body weight loss in sensitized/inflamed groups, which remained similar to the negative control group C(-). During the same period, there were no significant differences between control and experimental groups in food consumption. With regard to serology, the sensitized groups presented higher IgG titers when compared to negative control group ($p<0,01$) Furthermore, the solution with vitamin D did not affect the TGO/AST and TGP/ALT values. The results for flow cytometry showed that Vitamin D apparently has a modulatory effect on subpopulation of regulatory T cells when the treated groups were compared to the positive control group. Conclusions: the continuous administration of vitamin D during the experimental period can modify the pattern of total IgG titers.

W76. Chronic Ingestion of Low Doses of Cadmium Alters the Gut Microbiome and Immune Homeostasis for Enhanced Allergic Sensitization

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Low doses of cadmium can be ingested due to its presence in contaminated water and its accumulation in leafy vegetables, fish and grains. Environment factors, including pollutants are believe to contribute to the increased incidence of allergy diseases. We addressed whether chronic ingestion of low doses of cadmium could impact allergic sensitization and thus, favor the development of allergic diseases. Conventional C57BL/6 mice given low doses of cadmium in the drinking water for 28 days exhibited a significant reduction of bacterial diversity, and an alteration of the Firmicutes to Bacteriodetes ratio. This treatment also activated both the canonical and the non-canonical NF-κB pathway and promoted pro-inflammatory cytokine and antimicrobial responses in the gut. The effects of cadmium were at least partially independent of the gut microbiome since germ-free C57BL/6 mice subjected to the same treatment developed the same profile of responses although at a lower degree. Finally, conventional mice chronically treated with low doses of cadmium developed higher antigen-specific IgE responses upon oral sensitization with OVA and cholera toxin as adjuvant. Furthermore, upon nasal antigen, cadmium-treated mice developed higher airway allergic response, which were characterized by the increased levels of IL-17 and Th1 responses control mice. In summary, ingestion of low doses of the

environmental pollutant cadmium can be a major regulator of gut immune homeostasis and a cause for increased allergic responses at distant mucosal sites.

HIV

OR.13. HSV-2 Rectal Infection Increases Susceptibility to Rectal SIV Infection Even in the Context of SIVΔnef Infection
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$CD4^+$ T cells that express high levels of $\alpha_4\beta_7$ are particularly susceptible to HIV/SIV infection and their frequency at the rectal site of SIV exposure correlates with SIV acquisition. Blocking $\alpha_4\beta_7$ reduces susceptibility to vaginal SIV infection. We have shown that vaginal and rectal HSV-2 infection in macaques increases the frequency of $\alpha_4\beta_7^{high}$ $CD4^+$ T cells and that, in vaginal tissue, this correlates with increased susceptibility to $SHIV_{SF162P3}$. Using our unique model of rectal HSV-2 infection in macaques in combination with the SIVΔnef model of attenuated SIV infection, we found that HSV-2 rectal infection increases the susceptibility of macaques to rectal SIV wild-type (SIVwt) even in SIVΔnef-infected animals. SIVwt/HSV-2 co-infected animals had increased concentrations of IL-17, EGF, and CXCL8 in their rectal fluids, although, intriguingly, they had lower SIV viral loads in mesenteric lymph nodes than animals only infected with SIVwt. Thus, HSV-2 increases susceptibility to rectal SIV infection undermining the protective effects of SIVΔnef. Analysis of additional data will reveal if HSV-2-driven increase in $\alpha_4\beta_7$ and/or other HSV-2 induced changes in the rectal mucosa correlate with the HSV-2-associated increase in SIV infection. This work will help to identify key determinants of HIV susceptibility suggesting novel paths to HIV prevention.

OR.14. Broadly Neutralizing Antibodies Can Prevent HIV Infection in Human Colonic and Foreskin *ex vivo* Explant Infection Models
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In non-human primate models, passive transfer of selected neutralizing antibodies against SIV have shown protection from repeated low dose rectal challenges. To estimate the functional concentrations required to prevent infection in humans, we have used the human colonic and foreskin explant models to test multiple antibodies and assess their ability to impair HIV infection in an ex-vivo system. We compared VRC01 (CD4 binding site), PG9 (V1V2 loop), PGT121 (V3 loop), 10e8 (MPER), PGT151 (trimer) that neutralize the tier 2 JRCSF virus at IC50 of 0.02-0.14 ug/ml in the TZM-bl assay. We show that antibody concentrations 10-50 times above the IC50 can prevent infection on colonic explants from 9 healthy South African donors. Antibody concentrations 5-10 times above the IC50 can prevent infection of foreskin explants from 14 healthy South African donors. The required antibody concentrations are dependent on mucosal activation levels, as addition of PHA significantly increases the antibody concentrations required for protection. Together, these results provide functional estimates of the concentrations of neutralizing antibodies required at site of viral entry in men, and provide a goal for both passive protection and HIV vaccine efforts in mucosal surfaces.

OR.15. Urethral Macrophages Constitute an Unrecognized Anatomical Reservoir for HIV-1 in the Human Male Genital Tract
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HIV-1 eradication, or at least functional cure, requires the elimination/reduction of the HIV-1 reservoir pool. Residual viremia in HIV-1-infected-HAART-treated patients originates not only from memory $CD4^+$ T cells, the best-studied cellular HIV-1-reservoirs, but also from other cell types, especially tissues-resident macrophages. We recently identified male urethral macrophages as a novel

and highly efficient entry site for HIV-1. We now evaluated whether the urethra serves as a yet unrecognized anatomical reservoir for HIV-1 in direct link with the initial HIV-1 infection of urethral macrophages. Accordingly, HIV-1 was detected by ISH in $CD68^+$macrophages but not in T cells in urethral tissues from HIV-1^+ patients under effective anti-viral therapy. In these tissues, macrophages contained the HIV-1 capsid protein p24 (by IF); formed conjugates with $CD3^+$T cells (by IHC); and harbored virions in virus-containing-compartment (VCC)-like structures (ultrastructurally by EM). Hence, despite HARRT, HIV-1 persists in urethral macrophages. Importantly, HIV-1 could be amplified from single-cell suspensions prepared from urethral tissues, following LPS treatment and subsequent contact with activated $CD4^+$T cells. Macrophages polarization in tissues plays a crucial role in orientating the local immune response towards pro- or anti-inflammatory activities. Cell surface characterization of macrophage subsets in normal urethra by flow cytometry showed that M1 outnumber M2-macrophages, and revealed the presence of an new M1/M2 'intermediate' subset. In infected urethral tissues from HAART treated patients, the $CD163^+$M2 population disappeared whereas the proportion of the mixed M1/M2 intermediate subset increased. Altogether, these results suggest an active role for resident urethral macrophages in HIV-1 infection, which also serve as novel HIV-1 reservoirs.

OR.16. The Intestinal Microenvironment Restrict HIV-1 Replication in Human Dendritic Cells.

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We recently showed that human colonic lamina propria (LP) $CD11c^+$ DC actively shuttle R5 HIV-1 across an intact epithelial barrier and transfer infection to $CD4^+$ T cells (Cavarelli, EMBO MolMed 2013). However the susceptibility of intestinal DC to HIV infection has been poorly investigated, due to difficulties in isolating mucosal DC. Supernatant obtained from an *ex vivo* culture of healthy human colonic mucosa was used to condition monocyte-derived DC in an *in vitro* model as to mimic the exposure of DC to intestinal microenvironment. Conditioned–DC (C-DC) were analyzed by flow cytometry for the expression of HIV-1 receptors and activation markers, and incubated *in vitro* with either R5 or X4 HIV-1 to study their susceptibility to infection. C-DC displayed an activated phenotype, a significant down-regulation of CCR5 and CD4 an up-regulation of CXCR4 and a moderate modulation of DC-SIGN expression compared to unconditioned DC. Interestingly, both R5 and X4 HIV-1 integrated their genome and replicated less efficiently in C-DC compared to unconditioned DC. Colonic supernatants contained the CCR5-binding chemokines Mip1b and MCP-1 whereas the CXCR4 ligand SDF-1a was absent. IL-10 and IL-2, described to induce CXCR4 up-regulation on DC, were also detected. This specific intestinal milieu may partially explain the observed phenotype. Interestingly, the CDK inhibitor p21Cip1/WAF1, described to restrict HIV replication in human macrophages, is induced upon conditioning at both transcriptional and protein level. Our results point to a role of p21Cip1/WAF1 as an inhibitory factor of HIV-1 infection in intestinal DCs.

W77. Examining the Impact of HPV Infection and Clearance on HIV Susceptibility in the Female Genital Tract

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Vaginal human papillomavirus (HPV) infection is associated with a 2-fold increased risk of HIV acquisition. We hypothesized that this might relate to HPV-associated genital immune alterations, either during chronic infection or immune clearance. 59 HIV-uninfected women were recruited from the African/Caribbean community in Toronto; 36 were HPV uninfected, and 23 were HPV infected. Cervical mononuclear cells collected by cytobrush were stained with T cell markers (CD3/ CD4/ CCR5/ CD69/ CD38/ HLA-DR/ CD25/ CD39), and dendritic cell markers (CD1a/ CD14/ CD11c/ DC-SIGN/ langerin /mannose receptor). HPV-positive participants returned after 6 months; 11 had cleared HPV, 8 were persistently infected with the same HPV type, 2 were lost to follow-up, and 2 partially cleared infection. There was no difference in cervical CD4 T cell subsets between HPV uninfected and infected women, or between women with persistent vs. cleared HPV infection, although there was an increase in Langerhans cells among women who cleared HPV compared to HPV uninfected women (P=0.015) and those with persistent HPV infection (P=0.023). Neither HPV infection nor clearance were associated with increased endocervical $CD4^+$ HIV target cells, although Langerhans cell numbers were increased. Enhanced HIV susceptibility in HPV-infected women may be due to factors other than target cell recruitment.

W78. NOD2 Ligand Induces Long Lasting Immunity and Amplifies Mucosal and Systemic Immune Responses After Sub Cutaneous Immunization in Mice

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Most successful vaccines are able to induce persistent antibody responses that can last lifetime. Emerging evidences indicate that activation of immune cells through pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) or NOD-like receptors (NLRs) may be critical mechanisms. Among PRRs, the use of TLR ligands as adjuvants is already largely described whereas the use of NLRs ligands remains largely unexplored regarding the induction of long lasting immunity. The added value of encapsulation of NOD1 and NOD2 receptor ligands into Poly(Lactic Acid) (PLA) biodegradable nanocarriers to induce persistent antibody responses was explored. *In vitro*, NOD ligands encapsulated into PLA nanoparticles induced strong up-regulation of maturation markers and enhancement of pro-inflammatory cytokine secretion by DCs, with a better effect with NOD2 receptor ligands. *In vivo*, co-injection of encapsulated NOD2 ligands, in mice, with PLA particles carrying Gag p24 HIV-1 antigen allow a 100 fold increase in antibody responses in comparison to Alum and induction of mucosal immune responses. The same study with gp140 HIV-1 glycoprotein show enhancement of antibody responses and induction of long-lasting immunity. Our results provide a rational approach for broader application of particulate vaccines using encapsulated NODs receptor ligands as potent tools to induce long lasting immunity.

W79. Common Features of Mucosal and Peripheral Antibody Responses Elicited by Candidate HIV-1 Vaccines in Rhesus Monkeys

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Human mucosal surfaces represent the major portal of entry for human immunodeficiency virus type 1 (HIV-1) and a prophylactic vaccine will likely need to elicit protective antibody responses at mucosal sites. However, mucosal humoral immune responses following vaccination are poorly characterized. In this study we collected sera and colorectal mucosal secretions from adult rhesus monkeys immunized with intramuscular injection of either 2×10^{10} vial particles of adenovirus serotype 35 (Ad35) at week 0 and Ad26 at week 24 encoding SIV Env/Gag/Pol (Ad/Ad) or 0.25 mg recombinant HIV-1 Env protein with adjuvant at weeks 0, 4, 8, 12, 16 and 20. ELISA assays were performed to assess of the magnitude, durability and isotype of vaccine-elicited antibody responses. Neutralizing assay was performed to determine the functionality of antibody responses and peptide microarrays were performed to determine the epitope specificities of vaccine-elicited antibodies. We found that both Ad/Ad and protein immunization elicited potent and durable Env-specific IgG and IgA responses in sera and mucosal secretions. Vaccine-elicited mucosal IgG and IgA responses correlated with their counterparts in sera. Both Ad/Ad and protein immunization elicited IgG1 and IgG3 responses in serum and mucosal secretions. Lastly, mucosal secretions and sera from vaccinated monkeys exhibited neutralizing activity and vaccine-elicited Env-specific mucosal and serum IgG shared similar epitope specificities. These data suggest that intramuscular immunization of both Ad-vectored and protein-based candidate HIV-1 vaccines elicit potent and durable antibody responses at colorectal mucosa mirroring those in serum and vaccine-elicited peripheral and mucosal humoral immune responses are likely immunologically coordinated.

W80. The Effect of Timing of Antiretroviral Therapy on $CD4^+$ T Cell Reconstitution in the Intestine of HIV-Infected Patients

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Whether and to what extent gut mucosal $CD4^+$ T cells of human immunodeficiency virus (HIV)-infected patients can be restored by combination antiretroviral therapy (cART) is not well understood. We studied absolute numbers, differentiation, and activation of mucosal $CD4^+$ T cells at different stages of infection and assessed the effect of timing of cART initiation on their reconstitution. Mucosal $CD4^+$ T cell numbers were severely reduced at all stages of chronic infection, but normal in patients with acute infection. In patients with initiation of cART during chronic infection, mucosal $CD4^+$ T cells restored to less than half of the numbers in controls. However, in patients who initiated cART during acute infection, mucosal $CD4^+$ T cell numbers were fully preserved. Mucosal effector memory $CD4^+$ T cell proportion normalized only if cART was initiated at >350 $CD4^+$ T cells/µl but not with delayed

treatment. In all treated patient groups, the activation pattern of mucosal $CD4^+$ T cells did not return to normal. In conclusion, mucosal $CD4^+$ T cell numbers can be preserved if cART is initiated in acute HIV infection. In chronically HIV-infected patients, early cART improves mucosal $CD4^+$ T cell differentiation but cannot prevent the persistent lack of total $CD4^+$ T cells.

W81. Impact of Short-Term Antiretroviral Therapy During Early HIV Infection on Microbial Translocation, Gut Immunology and Biomarkers of Serious Non-AIDS Illnesses

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HIV infection is characterized by the simultaneous reduction of mucosal Th22 cell numbers and Th17 cell function, and impaired epithelial integrity leading to inflammation and serious non-AIDS illnesses (SNAs). We examined the impact of antiretroviral therapy (ART) initiated during early HIV infection (N=22) on mucosal immune function (Th17 and Th22 cells), gut IL-22 signaling, and blood biomarkers of epithelial integrity (I-FABP), microbial translocation (LPS) and SNAs (IL-6 and D-dimer). Prior to ART, gut Th22 cell number and Th17 functionality (ability to co-produce IL-22, IFNg and TNFa) were reduced compared to HIV-uninfected controls, and plasma levels of LPS, I-FABP, IL-6 and D-dimer were elevated. After ART initiation, gut Th22 cell numbers were restored, but there was no change in gut Th17 function or plasma LPS and IL-6 levels, and blood D-dimer and I-FABP levels actually increased. Immunofluorescence microscopy demonstrated decreased expression of IL-22 receptor on gut epithelial cells after ART, which we hypothesize may impair IL-22 signal transduction and epithelial repair. Therefore, early HIV infection was associated with substantial gut mucosal immune dysfunction, microbial translocation and systemic inflammation, and short-term ART had a limited impact on these parameters.

W82. T Follicular Helper Cells and IgA^+ B cells in Gut-Associated Lymphoid Tissue are Within Normal Ranges in HIV^+ Subjects on Suppressive Antiretroviral Therapy

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$CD4^+$ T cells in gut-associated lymphoid tissue (GALT) are depleted early in HIV-1 infection, and possibly not fully reconstituted on antiretroviral therapy (ART). Whether this includes the T follicular helper (Tfh) subset, which helps B cell responses in germinal centers, is not known. Fifteen healthy controls (HC) and thirteen $HIV\text{-}1^+$ subjects on ART provided ten pinch biopsies each from 3 sites: left colon (LC), right colon (RC) and terminal ileum (TI), via endoscopy and colonoscopy. Single cell suspensions were prepared by collagenase digestion, and $CD45^+$ve/EpCam-ve lymphocytes analyzed by flow cytometry, including cell counts of $IgA^+CD19^+CD27^+$ B cells and Tfh ($CD3^+CD4^+CD45RO^+CXCR5^+PD^-1$high). Median Tfh cell counts from HC subjects were 2743, 4526 and 9714 from LC, RC and TI biopsies respectively, which were not significantly different to corresponding median Tfh cell counts from $HIV\text{-}1^+$ subjects (6840, 5236, and 3703, respectively). Median IgA^+ B cell counts from HC subjects were 29704, 67780 and 30367 from LC, RC and Ti, respectively, and not significantly different to $HIV\text{-}1^+$ subject cell counts (45333, 43641 and 35266, respectively). Cell counts for CD38high IgA^+ plasmablasts were also not significantly different between the two subject groups. In conclusion, HIV^+ subjects on ART appeared to have intact GALT B cell responses *in vivo*, including Tfh and IgA^+ B cell levels, comparable to healthy adult controls.

W83. Cytotoxic Molecule Expression and *ex vivo* Cytotoxicity of Rectal Mucosal $CD8^+$ T Cells in HIV-1 Infection

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We previously demonstrated that HIV-specific $CD8^+$ T cell responses are detected in gastrointestinal mucosa, and reflect clinical status. Intriguingly, however, the majority of rectal $CD8^+$ T cells do not express perforin, an effector molecule essential for killing infected cells. To elucidate effector functions of gastrointestinal $CD8^+$ T cells, we examined expression of perforin; granzymes (Grz) A, B, K; CD107a; and T-bet in rectal tissue and blood from HIV-infected and healthy adults. HIV-specific T cells were stimulated with peptide pools or control stimuli; a redirected lysis assay was used to test cytotoxicity. Perforin production in response to TCR stimulation was reduced in mucosa compared to blood, as was expression of T-bet. *Ex vivo* killing was significantly reduced in rectal

CD8⁺ cells compared to blood, regardless of HIV status or disease progression category. Although rectal CD8⁺ T cells had predominantly 'effector'; 'effector memory'; or 'transitional memory' phenotypes, perforin expression within all subsets was reduced compared to blood. However, expression of cytotoxic effector proteins was elevated in HIV-infected subjects relative to controls. In summary, CD8⁺ T cells in rectal mucosa expressed lower levels of perforin and GrzB and had reduced cytotoxic capacity relative to their counterparts in blood. These differences were not due to reduced frequencies of effector T cells in mucosa, but may be related to lower expression of T-bet in rectal CD8⁺ T cells. These data suggest that non-cytolytic effector functions rather than direct cytotoxicity may be the major role of CD8⁺ T cells in rectal mucosa, and that antigen load plays a role in driving cytotoxic molecule expression.

W84. Targeting α4β7 Integrin Reduces Mucosal Transmission and Dissemination of SIV

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Activated CD4⁺ T cells in the GALT are depleted during acute HIV and SIV infection, and fail to reconstitute even with optimal antiviral therapy. Along with the CD4 receptor and CCR5 co-receptor, these cells express integrin α4β7, the gut homing receptor. gp120 binds to α4β7 on T cells and α4β7 defines a T cell subset that is highly susceptible to HIV-1 and SIV infection. These findings suggest that α4β7 could be effective both as an antiviral target and in minimizing trafficking of infected CD4⁺ T cells into the GALT. To explore that possibility, we developed a primatized rhesus mAb directed against α4β7 that blocks binding to MAdCAM-1. We evaluated the efficacy of α4β7-mAb therapy in a well characterized NHP model based on repeated, low dose intravaginal challenges with SIVmac251. We report that i.v. administration of α4β7-mAb markedly reduces surface exposure of α4β7 on CD4⁺ T cells in the cervico-vaginal canal, significantly delays or prevents trans-vaginal infection, and, when prevention fails, markedly reduces viral DNA loads in the GALT as well as the rate of peripheral CD4⁺ T cell depletion in SIVmac251-infected macaques. These results suggest that agents directed against α4β7 might be useful for prophylaxis or treatment of HIV infection.

W85. Signaling Through Integrin α4β7 Promotes Replication of HIV in Sub-Optimally Activated CD4⁺ T Cells

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Mucosal transmission of HIV is inefficient. Viral infection of activated CD4⁺ T cells represents a key step in infection. α4β7/CD4⁺ T cells are targeted at an early stage of transmission. A recent study in rhesus macaques demonstrated that a primatized monoclonal anti- α4β7 antibody reduces the efficiency of mucosal transmission of SIV in a low-dose vaginal challenge model. α4β7 mediates homing to gut-associated lymphoid tissue through an interaction with MAdCAM-1, expressed on gut endothelial venules. MAdCAM binding to α4β7 delivers a potent costimulatory signal to CD4⁺ T cells. We hypothesized that the costimulatory activity of α4β7 could explain the enhanced susceptibility of α4β7/CD4⁺ T cells to HIV infection. We evaluated the capacity of α4β7-mediated costimulation to facilitate HIV replication, and the ability of the primatized anti- α4β7 mAb to inhibit viral replication. Costimulation through α4β7 promotes viral replication in a way that is inhibitable by the primatized α4β7 mAb. CFSE dye analysis, upregulation of CD69, and shedding of CD62-L confirmed that α4β7 promotes activation. These results provide a novel mechanism by which α4β7 can facilitate mucosal transmission that may aid in our understanding of the mechanism(s) by which a primatized anti-α4β7 mAb reduced the efficiency of mucosal transmission.

HOST-MICROBIOTA INTERACTIONS

PS.3. Regulation of T Follicular Helper Cell Response in Peyer's Patches by Microbiota Derived ATP via Purinergic P2X7 Receptor

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Follicular B helper T (Tfh) cells in Peyer's patches (PPs) promote B cells expansion, class switch recombination (CSR) and affinity IgA maturation. The anatomical location of PPs allows rapid mucosal amplification of B and T cell responses to small intestine endoluminal content. Adenosine triphosphate (ATP) is a ubiquitous extracellular messenger, which activates purinergic receptors in the plasma membrane termed P2 receptors. The P2X7 receptor subtype is an ATP-gated nonselective cationic channel expressed in

a variety of cell types. In T cells protracted receptor stimulation leads to opening of a pore permeable to molecules up to 900 Da and cell death. We showed that P2X7 regulates Tfh cells abundance in PPs. Lack of P2X7 in Tfh cells enhanced the germinal centre reaction, high affinity IgA secretion and binding to commensals. The ensuing depletion of mucosal bacteria resulted in reduced systemic translocation of microbial components, which affected B1 cells stimulation and serum IgM levels. In the small intestine we detected ATP in the micromolar range; here we show that small intestine commensals release ATP that promotes PPs Tfh cell death via P2X7. Administration of bactericidal antibiotics per os resulted in ATP release by bacterial cell death that significantly impacted on Tfh cells number and endoluminal IgA concentration. Finally, we demonstrate that bacterially derived endoluminal ATP regulates T cell dependent IgA response and affect commensals abundance and composition.

OR.29. Epithelial Cell-Specific Induction of Activating Transcription Factor 6 Signaling Promotes Intestinal Dysbiosis and Colonic Tumorigenesis

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Activation of the endoplasmic reticulum unfolded protein response (erUPR) might contribute to the oncogenic transformation of tissues. However, a causative tumor-promoting role has not been demonstrated. To address the role of activating transcription factor (ATF)6-mediated erUPR signaling in intestinal epithelial cells (IEC), we generated Villin-Cre-driven IEC-specific transgenic mice overexpressing the activated form of ATF6 (nATF6^{IEC}). Homozygous nATF6$^{IEC\text{-}tg/tg}$ mice spontaneously developed colonic adenomas independent of major inflammatory processes, with an incidence of 100% at 12 weeks of age. In contrast, heterozygous nATF6$^{IEC\text{-}wt/tg}$ mice showed no tumor formation but a tumorigenic response to both IL-10 deficiency and chemical wounding using dextran sodium-sulfate. In nATF6$^{IEC\text{-}tg/tg}$ mice, erUPR-related gene expression and increased proliferation of IEC preceded tumor formation. Further, loss of mucin-filled goblet cells was associated with increased microbial penetration of the mucus barrier. High-throughput 16S-rRNA gene sequencing of cecal microbiota revealed a clear separation of bacterial communities according to genotype with reduced bacterial diversity. Finally, antibiotic treatment and germfree housing was shown to alleviate or even prevent tumor formation. In conclusion, the novel nATF6^{IEC} mouse model demonstrates for the first time a causal contribution of altered erUPR signaling to colonic tumorigenesis linked to changes in the gut microbiota.

OR.30. Commensal Bacteria and their Metabolites Reduce Intestinal Permeability to Protect Against Food Allergen Sensitization

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Food allergies are a growing public health concern. We have identified a consortium of spore-forming Firmicutes from the Clostridia class that prevents food allergen sensitization when administered to neonatal antibiotic-treated or germ free (GF) mice. Protection was characterized by reduced serum concentrations of the peanut allergens Ara h 2 and 6 in orally challenged Clostridia-colonized mice and required the induction of IL-22 in the intestinal lamina propria (LP). We examined whether Clostridia mediate their epithelial barrier protective effects through direct interaction with the host or through the secretion of metabolites. Heat-inactivation abrogated Clostridia-induced expression of IL-22, but not IL-12, in the intestinal LP. Heat-inactivated Clostridia also failed to reduce serum concentrations of either FITC-labeled dextran or Ara h 2/6, suggesting a possible protective role for bacterial metabolites. Short chain fatty acids (SCFA) are important immunoregulatory metabolites of this bacterial class. Metabolomic analysis revealed that our Clostridia consortium produces both butyrate and acetate. We found that administration of butyrate, but not acetate, to GF mice induces IL-22 expression and reduces serum Ara h 2/6 concentrations. Our data suggest that the production of the SCFA butyrate is one mechanism by which Clostridia induce a barrier protective response that prevents sensitization to food.

OR.31. Absence of Antigen-Sampling M Cells Compromises Both the Antigen-Specific and Nonspecific Components of the Secretory IgA Response to Monocolonization of Mice with SFB

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The gut mucosal immune system produces abundant secretory IgA that assists in establishing homeostasis with the commensal microbiota. Much of this IgA response is triggered by the microbiota, although the precise cellular pathways associated with

responses to individual bacterial species remain to be characterized. Using a conditional RANK knockout mouse lacking intestinal M cells in Peyer's patches (RANKΔIEC strain), we previously demonstrated that M cells play a key role in efficient initiation of the overall IgA response to commensal microbiota. To further our understanding of the role that M cells play in generation of IgA responses to specific bacterial species, we studied the kinetics of fecal IgA responses in RANKΔIEC mice that were germ-free or monocolonized with segmented filamentous bacteria (SFB). Under germ-free conditions wild-type and RANKΔIEC mice produced equivalent low amounts of secretory IgA, indicating that the low level of production of natural IgA and IgA directed against non-microbiota environmental antigens does not depend on M cells. Since SFB are known to be robust inducers of both secretory IgA and Th17 responses, we also assessed the contribution of M cells to fecal IgA responses occurring in mice monocolonized with SFB. Both the rapid nonspecific total IgA response detected by ELISA and the more gradual specific anti-SFB IgA response assessed by bacterial flow cytometry were depressed, but not ablated, in M-cell deficient mice. Our results indicate that M cells are critical, non-redundant mediators of intestinal antigen sampling required for efficient development of mucosal IgA to antigens associated with the commensal microbiota.

OR.32. Nitric Oxide Regulates Metaplasia in Response to Helicobacter pylori in Mice and Humans

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Chronic infection with the bacterium Helicobacter pylori (HP) in humans or mice can cause atrophy of acid-secreting parietal cells (PCs) in the gastric epithelium, which leads to a series of downstream events that increase risk for development of gastric cancer. We have shown that the epithelium responds to PC atrophy by two distinct mechanisms: 1) the normal, constitutively active stem cell in the isthmus region (near the surface of the stomach) increases proliferation and 2) the post-mitotic, mature digestive-enzyme secreting chief cells in the base first dedifferentiate to a progenitor state and then also begin to proliferate in a process known as pseudopyloric metaplasia. How PC atrophy induces those responses in other cells has not been elucidated. Here we show that, both in mouse models of PC atrophy and in HP-infected human tissue, injured (but not yet apoptotic) PCs rapidly increase expression of the inducible nitric oxide synthase (iNOS) to generate nitric oxide (NO). Inhibition of iNOS in mice with an inhibitor Aminoguanylate or by using $iNOS^{-/-}$ mice results in less proliferation and metaplasia following PC atrophy induction. Conversely, exogenous NO from a donor such as S-Nitroso-N-acetyl-DL-penicillamine is sufficient to increase gastric unit proliferation even in uninjured mice. Once PC atrophy is extensive in mice and in humans, iNOS-expressing macrophages infiltrate the tissue. Accordingly, depletion of macrophages with clodronate liposomes also blocks the epithelial injury response. Our results implicate NO, first from PCs, then from macrophages, as a critical mediator of the pro-proliferative response of the epithelium to HP infection.

OR.45. Dysbiotic Gut Microbiota Causes Transmissive Crohn´s Disease-Like Ileitis Associated With Paneth Cell Failure

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Background: Dysbiosis of the intestinal microbiota is associated with Crohn´s disease (CD). Functional evidence for a causal role of bacteria in chronic ileal inflammation development is lacking. Similar to human pathology, $TNF^{deltaARE}$ mice develop a TNF-driven, CD-like transmural inflammation with predominant ileal involvement. Methods and Results: Heterozygous $TNF^{deltaARE}$ mice and WT littermates were housed under conventional (CONV), specific-pathogen-free (SPF) and germfree (GF) conditions. GF-$TNF^{deltaARE}$ mice were free of inflammation in the gut and antibiotic treatment attenuated ileitis but not colitis, demonstrating that disease-severity and location is microbiota-dependent. SPF-$TNF^{deltaARE}$ mice were free of colitis and developed distinct ileitis-phenotypes associated with gradual loss of Paneth cell function, as assessed by immunofluorescence analysis. Analysis of microbial communities by 16S gene sequencing and metaproteomes revealed specific compositional and functional alterations of bacterial communities in inflamed mice. Monoassociation of GF-$TNF^{deltaARE}$ mice with the human CD-related *Escherichia coli* LF82 did not induce ileitis. Transplantation of disease-associated but not healthy microbiota transmitted CD-like ileitis to GF-$TNF^{deltaARE}$ recipients and triggered Paneth cell failure. Conclusion: We provide clear experimental evidence for the causal role of gut bacterial dysbiosis in the development of chronic ileal inflammation with subsequent failure of Paneth cell function.

OR.46. Colonic Microbiota Modulate Epithelial Barrier Function Through Intraepithelial Lymphocyte Produced IL-6
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Microbial colonization is critical for the development of both the epithelial barrier and mucosal immune function. Antibiotic use is known to influence epithelial homeostasis and increase risk of infection by pathogens through unknown mechanisms. We evaluated the impact of broad-spectrum antibiotics on mucosal immune components and epithelial function in C57Bl/6 mice. Antibiotic administration was associated with profound decreases in intraepithelial lymphocytes (IELs) and increased colonic permeability. Therefore, we hypothesized that colonic bacteria modulate epithelial barrier function through IELs. Our data demonstrate the major subpopulation of IELs in the colon are $CD3^+$, $CD4^-CD8^-$ ($52 \pm 3.5\%$ of total IELs), and $TCR\beta^+$ ($71 \pm 4.5\%$ of total IELs), express cell surface markers consistent with activated lymphocytes ($CD44^+ CD69^+ CD62L^-$), and produce large amounts of IL-6. Administration of antibiotics to mice significantly decreased the number of activated, IL-6-secreting IELs ($p<0.0001$). The influence of antibiotics was reversible, as recolonization resulted in normalization of the IEL numbers, IL-6 secretion, and epithelial barrier. IL-6 was found to signal in epithelial cells, and resulted in increased epithelial barrier integrity in model epithelia. Interestingly, IL-$6^{-/-}$ mice were found to have a profound barrier defect, similar to antibiotic treated mice. We conclude that modulation of host microbiota by antibiotics impairs epithelial barrier function through effects of IEL function.

OR.47. Microbial Metabolites Regulate Intestinal Stem Cells During Tissue Repair
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Studies in germ-free and antibiotic-treated mice reveal important cross-talk between the microbiota and intestinal epithelium but the mechanisms in many cases remain unknown. Intestinal stem cells (ISCs) are the master control unit of the epithelial barrier, however to date there is no evidence for a direct effect of specific microbes or microbial components on ISCs. As the mucus layer for the most part keeps bacteria separated from the epithelium, we investigated whether microbial-derived metabolites may act as soluble effectors and influence the function of ISCs. Using primary mouse cultures of ISCs, we conducted a large scale screen of the effect of all known mouse intestinal microbial-derived metabolites and microbial-associated molecular patterns. We discovered 8 microbial metabolites that significantly inhibited ISC proliferation *in vitro*, the most potent of which was the short chain fatty acid butyrate. Similarly, *in vivo* we demonstrated that elevated luminal butyrate suppressed both ISC proliferation and healing in crypts adjacent to areas of ulceration. In contrast, elevated luminal butyrate did not affect ISC proliferation in areas where the epithelial barrier was intact (non-ulcerated regions). In uninjured tissue terminally differentiated colonocytes actively metabolized butyrate, thus preventing it from reaching the stem cell compartment and exerting its inhibitory effect on proliferation. The mechanism underlying the direct impact of butyrate on ISCs was dependent on histone acetylation and independent of GPCR signaling. Importantly, differentiated colonocytes were resistant to this butyrate-induced histone remodeling. This study suggests that microbial-derived metabolites directly regulate the repair of the intestinal epithelial barrier during tissue damage.

OR.48. Differences in Gut Mucus Microbiota Precede Dysbiosis in Inflammatory Bowel Disease
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Impaired tolerance to the gut microbiota is asociated with inflammatory bowel diseases (IBD), such as Crohn's disease and Ulcerative colitis. The microbiota is complex and in the colon resides in the gut lumen and the thick outer mucus layer covering the epithelium, leaving the inner mucus sterile. The mucus bacteria are likely to have most impact on the epithelium and underlying lamina propria. Studies investigating microbiota in IBD pathogenesis have mainly focused on stool microbial analysis leaving mucus bacteria understudied. To determine the importance of mucus bacteria in IBD development, we investigated colitis progression in the mdr1a$^{-/-}$ spontaneous model of colitis. Microbial differences between mdr1a$^{-/-}$ and wild-type littermate controls were evident before colitis onset but restricted to the mucus. Despite mucus microbial composition differing between controls and mdr1a$^{-/-}$mice, bacterial location and mucus thickness were similar. Upon emergence of early inflammation in mdr1a$^{-/-}$ mice, differences in bacterial composition extended from the mucus to the luminal compartment. Furthermore, the mucus layer became thinner. Although bacterial load was not increased overall during colitis onset, bacterial localization with respect to host tissue was altered with bacteria penetrating the inner mucus. Our results show that microbial dysbiosis starts in specific niches before IBD onset.

OR.92. Gut Peri-Cryptal Fibroblasts Re-Program Intestinal Stem Cells via Interleukin-33

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The small intestine comprises an array of epithelial cells that serves distinct functions during its time course. Many pathways are known to regulate this programming of intestinal stem cell. The balance between epithelium and type 2 cytokines like IL-33 is known to play major role in intestinal homeostasis. However, under pathological conditions the intestinal epithelium is forced to undergo many changes eventually disrupting harmony. We generated an inducible transgenic mouse expressing IL-33 specifically in gut (IL-33Vcre). To analyze the role of IL-33 on epithelium directly we used the in-vitro cultivation of organoids from C5BL/6 mice. Localization of IL-33 was studied in IL-33LacZ/LacZ reporter mice. We observed high expression of IL-33 in the gut of TLR-ligand challenged mice and specific expression of IL-33 by sub-epithelial myofibroblasts in the vicinity of stem cells. IL-33Vcre mice led to an increased expression of anti-microbial peptides and goblet cell markers. Signaling of IL-33 into intestinal epithelial cells (IEC) in- vivo and in organoid cultures in-vitro was associated with altered Notch signaling governing proliferation and differentiation of IECs. Our data demonstrate a novel mechanism of IL-33 signaling and its significant consequences on IEC function. Therefore it plays a role in protecting barrier and tackling foreign challenges.

W86. Mapping the Interplay Between Bacterial and Fungal Microbiota During Health and Intestinal Disease

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The mammalian gastrointestinal (GI) tract is compartmentalized and each compartment provides unique environment where nutrients, metabolites and other factors differ depending on the site. Bacteria, viruses and fungi cohabitate in this environment. It is currently unknown whether bacterial and fungal communities are interdependent, and whether some fungi would prefer certain GI sites over the other. Using deep sequencing technology we surveyed the mycobiomes and the respective bacterial communities in the entire murine GI tract. We found that few abundant fungal genera are ubiquitously present at the intestinal mucosa. Notably, site specificity was also observed and fungal genera such as Fusarium and Candida were always found at specific location. The murine mycobiome structure and diversity were significantly affected by intestinal inflammation and by bacterial dysbiosis. To access whether intestinal pathology affects the mycobiome structure in humans, we analyzed fungal and bacterial communities in a cohort of Ulcerative colitis patients and healthy controls. We conclude that fungal and bacterial communities are interdependent and are both affected by the physiological status of the host. Altogether our results demonstrate specific distribution of fungal genera throughout the GI tract which might be associated with site specific immune responses to fungi.

W87. Plasmacytoid Dendritic Cell-Derived IFNα Modulates Th17 Differentiation During Bordetella pertussis Infection in Mice

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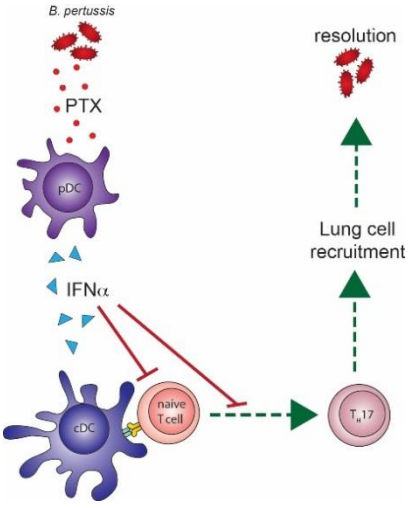

Whooping cough is a highly contagious respiratory disease caused by Bordetella pertussis (B. pertussis). Recent studies have revealed a central role for Th17 cells in the resolution of the infection. Emerging studies document that type I interferons (IFN) suppress Th17 differentiation and IL-17 responses in models of infection and chronic inflammation. Plasmacytoid dendritic cells (pDCs) are a major source of type I IFNs. Therefore, we hypothesize that during B. pertussis infection in mice, pDC derived IFNa inhibits a rapid increase in Th17 cells. We found that IFNa-secreting pDCs emerge in the lungs in the early stages of infection, while a robust rise of Th17 cells in the lungs is detected at 15 days post-infection (dpi) or later. The presence of IFNa led to ablation of Th17 differentiation and proliferation *in vitro*. Furthermore, blocking IFNa produced by pDCs, *in vivo* prior to B. pertussis infection resulted in increase of Th17 frequency, inflammation and reduced bacterial loads in the airways of infected mice. Altogether, this work provides evidence of an inhibitory role for pDCs and pDC-derived IFNa in modulating Th17 responses during the early stages of B. pertussis infection, which may explain the prolonged nature of whooping cough.

W88. Differential Effects of *Escherichia coli* Nissle and *Lactobacillus rhamnosus* Strain GG on Human Rotavirus Infection and B Cell Responses

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Microbiota play a significant role in modulating host-pathogen interactions. We studied the role of gram-positive [Lactobacillus rhamnosus strain GG (LGG)] and gram-negative [Escherichia coli Nissle (EcN)] commensal bacteria on virulent human rotavirus (HRV) infection and immunity using neonatal gnotobiotic (Gn) piglets. Gn piglets were colonized with EcN, LGG or EcN+LGG and challenged with virulent HRV. Mean peak virus shedding titers were significantly lower in EcN-colonized compared to LGG-colonized or uncolonized piglets. Coinciding with lower virus shedding, serum IFNα levels were significantly lower in EcN-colonized piglets compared to LGG-colonized or uncolonized piglets post-challenge. Reduced viral shedding titers were correlated with significantly reduced small intestinal and serum anti-HRV IgA responses in EcN-colonized compared to uncolonized piglets post-challenge. However the total IgA levels post-challenge in intestine and pre-challenge in serum were significantly higher in EcN-colonized than in LGG-colonized piglets. *In vitro* treatment of mononuclear cells (MNCs) with these probiotics demonstrated that EcN, but not LGG, induced IL6, IL10 and IgA, with the latter partially dependent on IL10. Exogenous addition of recombinant porcine IL10 and IL6 to MNCs co-cultured with LGG significantly enhanced IgA responses. Our results suggest that EcN and LGG differentially modulate rotavirus infection and B cell responses.

W89. Microbiota Mediated Protection from Entamoeba histolytica Infection

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Previously our laboratory has demonstrated that colonization of the intestine of mice with a commensal Clostridia-related bacterium, segmented filamentous bacteria (SFB), is protective during E. histolytica infection. SFB colonization was associated with increased cecal levels of interleukin 17A (IL-17A), dendritic cells, and neutrophils. Bone marrow-derived dendritic cells (BMDCs) from SFB-colonized mice exhibited higher levels of IL-23 production in response to stimulation with trophozoites and adoptive transfer of those BMDCs provided protection against E. histolytica infection. IL-17A induction during BMDC transfer was necessary for this protection. This work suggested that alteration of the microbiome may mediate protection against an amoeba infection via extra intestinal effects on bone marrow. In exploring what changes have occurred in the bone marrow of SFB colonized mice we have found increased expression of the GM-CSFR genes CSF2RA and increased expression of the H3K27 demethylase JMJD3 in both whole bone marrow and differentiated BMDCs. Additionally, blockade of GM-CSF during SFB/E.histolytica co-infection abrogated SFB mediated protection. This work suggests that intestinal colonization with SFB may alter bone marrow cells via GM-CSF dependent mechanisms to provide protection from Entamoeba histolytica infection and that these changes might result from long term epigenetic changes to the bone marrow.

W90. Interferon-β Induced by Double-Stranded RNA of Lactic Acid Bacteria Promotes Differentiation of IFN-γ-Producing Th1 Cells

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Double-stranded RNA of lactic acid bacteria (LAB) is recognized by dendritic cells (DCs) via endosomal TLR3 and benefits the anti-inflammatory response through induction of interferon-β (IFN-β). However, how such IFN-β impacts T cell immune responses, and how immune homeostasis is better maintained in the presence of commensal or food-derived LAB is unknown. Here we show that LAB enhances interleukin-12 (IL-12) secretion by DCs and differentiation of IFN-γ-producing T cells in an IFN-β-dependent manner. We demonstrated that IFN-β secreted in response to LAB increased IFN regulatory factor 1 (IRF1) and IRF7 mRNA, which contribute to Il12p35 expression. The resultant induction of Tbet and IFN-β in $CD4^+$ T cells also occurs *in vivo*, where oral administration of LAB augments Th1 immune responses via TLR3 signaling pathway. Th1 induction due to TLR3-mediated IFN-β production may thus confer anti-allergic or anti-inflammatory activity by commensal or probiotic LAB.

W91. Chronic *Trichuris muris* Infection Decreases Diversity of the Intestinal Microbiota and Concomitantly Increases the Abundance of Lactobacilli
Daniel Sorobetea. Lund University, Lund, Sweden

The intestinal microbiota plays critical roles in both health and disease. It has become increasingly clear that pathogen exposure can affect the composition of microbial communities in ways that are poorly understood. We set out to investigate the effect of parasite infection on the murine intestinal microbiota, utilizing the large-intestinal dwelling whipworm Trichuris muris. Our data demonstrate that chronic infection with T. muris results in a profound shift in bacterial communities after 3-4 weeks of infection, with a drop in overall bacterial diversity, and increase in the relative abundance of the bacterial family Lactobacillaceae. In parallel, the immune system was highly affected by the infection, with a marked reduction in the ratio of regulatory to inflammatory immune cells. These changes however appeared independent of the change in the microbiota. These findings shed light on the important role of worm infections on the gut microenvironment, especially with regard to ongoing trials of worm treatment against immune-associated diseases.

W92. Human Cytomegalovirus Potentiates Inflammation in the Gastrointestinal Mucosa
Evida Dennis, Lesley Smythies, Masako Shimamura, William Britt and Phillip Smith. University of Alabama at Birmingham, Birmingham, AL

Background: In immunocompromised conditions such as inflammatory bowel disease and HIV-1 infection, human cytomegalovirus (HCMV) often causes severe mucosal inflammation associated with HCMV-infected lamina propria macrophages (LPMs) and macrophage-derived cytokines. In normal intestinal mucosa, however, LPMs, which are derived from pro-inflammatory monocytes, are profoundly down-regulated for cytokine production (inflammation anergy) due to stromal TGF-β-induced NF-κB inactivation. Here, we investigated the paradox in which LPMs are non-inflammatory in normal mucosa but pro-inflammatory in HCMV mucosal disease. Methods: LPMs were isolated from normal human jejunum, infected with HCMV, stimulated with TLR4/5 agonists, and assayed for cytokine production. Blood monocytes also were infected with HCMV, but then treated with stroma-conditioned media (S-CM, generated from normal intestinal stroma using our established protocol) to differentiate the monocytes into non-inflammatory macrophages. The S-CM-differentiated macrophages were stimulated with TLR agonists and assayed for cytokines. HCMV infection and replication were determined by confocal and electron microscopy, and RT-PCR. Results: LPMs infected with HCMV did not support viral replication or produce cytokines, regardless of TLR stimulation. In contrast, blood monocytes, first infected with HCMV and then S-CM differentiated and TLR stimulated, supported replication and produced significantly higher levels of cytokines compared with mock-infected, S-CM-differentiated, TLR-stimulated macrophages. Conclusions: HCMV non-productively infects LPMs without breaking inflammation anergy. However, when monocytes are infected with HCMV prior to differentiation into intestinal macrophages, inflammation anergy is inhibited. Systemic infection of monocytes by HCMV before recruitment to the mucosa strategically positions pro-inflammatory macrophages to interact with bacteria via TLR4/5, leading to inducible macrophage cytokine responses.

W93. Gut Microbiota is a Modulator of Intraepithelial Lymphocyte Numbers and Phenotype in the Small Intestine
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Intraepithelial lymphocytes (IELs), αβTCR and γδTCR, play a critical role in mucosal barrier maintenance and as revealed by some gnotobiotic studies, their populations are modulated by the presence of the microbiota. Previous research has demonstrated that αβTCR IELs are responsible for infection-associated damage in the gut and are hypothesized to contribute to villous atrophy in celiac disease (CD). Our aim was to determine whether microbiota from CD patients, characterized by dysbiosis, influenced IEL populations in the small intestine (SI). We thus transferred human stools from CD or healthy individuals into germ-free (GF) C57BL/6 mice. Stool from one out of three CD donors (CD_3) increased IELs within the SI villi tips, and increased proportions of αβTCR IELs. 16S rDNA sequencing of SI contents revealed a higher composition of Proteobacteria, specifically Parasutterella, in mice colonized with CD_3 microbiota. Further investigation demonstrated that IEL responses to colonization were dependent on MyD88 and TICAM1 signaling, suggesting that the composition of gut microbiota can alter IEL numbers and phenotype in the SI through a MyD88/TICAM1 pathway. This mechanism may play a pathogenic role in diseases where IEL proliferation and activation is central to disease development such as celiac disease, food allergy or IBD.

W94. Identification of Autoantibodies to Aquaporin-5 in Sera from Patients with Sjögren's Syndrome
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Sjögren's syndrome (SS) is an autoimmune disorder that primarily targets the salivary and lacrimal glands, leading to dryness of the mouth and eye. Although anti-muscarinic receptor 3 autoantibodies have been shown to inhibit the function of acinar cells, the pathophysiology of exocrine dysfunction in SS is not fully understood. Aquaporin-5 (AQP5), a water-channel protein expressed at the acinar cells of the lacrimal and salivary glands, plays a critical role in tear and saliva secretion. Many oral bacterial species express AQPs that have high levels of homology with human AQP5. Therefore, we hypothesized that SS patients may have autoantibodies to AQP5 in sera. To test our hypothesis, the sections of mouse submandibular salivary gland were dual stained with anti-AQP5 antibody and either control or SS patient sera. The signals of AQP5 expressed in the mucous acini, serous acini, and ductal areas showed strong co-localization with the signals stained with SS patient IgG but control sera did not stain the sections. Sera from SS patients, but not from control subjects, also selectively stained the AQP5-EGFP overexpressed in CHO cells. Although both the SS and control sera immuneprecipitated AQP5-EGFP, blind screening of MDCK cells overexpressing AQP5 by immunofluorescence assay revealed that 3 out of 10 SS patients contained higher levels of anti-AQP5 IgG than control subjects. In conclusion, the anti-AQP5 autoantibodies detected in SS sera may be a useful disease biomarker.

W95. NOD2 Does not Influence Bacterial Community Structure in the Salmonella ΔaroA Chronic Inflammation Model
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Mutations in the gene encoding the intracellular bacterial sensor, NOD2, are linked to risk of Crohn's disease. We explored the role of NOD2 in shaping the intestinal microbiota during chronic inflammation induced through infection with Salmonella Typhimurium ΔaroA. We applied a robust experimental design to control for microbial variation by using littermates and a separately housed NOD1 strain for comparison. We found that NOD2 contributes to reducing the pro-inflammatory profile of the cecal environment, but plays no role in decreasing the severity of chronic inflammatory pathology 7 weeks p.i. Shifts in bacterial abundance throughout the acute response to infection, and during chronic inflammation, reflect inflammatory conditions shaped by many aspects of the immune response, independent of NOD2 functions. Curiously, NOD2-strain littermates (all genotypes) consistently showed more intense host and microbial responses to infection compared to the NOD1 strain, including secondary outgrowth of Salmonella that was still evident after 7 weeks. This observation suggests that differences in the microbiota prior to infection may contribute to determining the severity of the inflammatory response or the ability of the pathogen to persist in the host. Thus, when microbial variation is strictly controlled, NOD2 has negligible impact on chronic pathology or microbial abundance in this model.

W96. Bile Acid Imbalance in Morphine Induced Gut Barrier Compromise and Systemic Inflammation: Role of CYP7A1 and F-x-R
Santanu Banerjee, Gregory Sindberg, Fuyuan Wang, Jingjing Meng, Umakant Sharma and Sabita Roy. University of Minnesota, Minneapolis, MN

Despite being predominant drugs of choice for anti-nociception, morphine and its pharmacological derivatives result in severe co-morbidities studied in numerous disease models in mice and humans due to peripheral side effects. Opioids have been shown to promote gram-positive bacterial translocation across the gut mucosa, leading to systemic inflammation and sepsis in a TLR2 dependent manner. We have also shown that bacterial translocation due to the gut mucosal barrier compromise are a part of the commensal flora. In this study, we show for the first time that morphine fosters significant gut microbial dysbiosis and altered cholesterol/bile acid metabolism in WT mice. Recent studies have strongly correlated microbial/bile-acid dysbiosis to gut barrier disruption and host inflammation. In this context, role of hepatic cholesterol-7α-hydroxylase (CYP7A1) and Farnesoid-x-receptor (FxR; hepatic and intestinal) have been strongly implicated in complications arising due to bile acid imbalance. Here, we show the role of bile acid changes due to chronic morphine in gut barrier dysfunction in the context of TLR2/cholesterol hydroxylase/farnesoid-x-receptor modulation.

W97. Short-Term Administration of Probiotics Promote Protective Immunity Against Enteric Bacteria Infection Through Lgr5⁺ Stem Cell Differentiation

Yeji Kim, Yong-Soo Lee, Jin-Young Yang, Su-Hyun Lee and Mi-Na Kweon. University of Ulsan College of Medicine, Seoul, South Korea

The probiotics play an indispensable role in gut homeostasis but underlying mechanisms are still unveiled. In order to clarify an exact role of probiotics for protective immunity in the gut, we fed C57BL/6 mice for five days with human-use probiotic Lacidofil® including Lactobacillus (L.) rhamnosus and L. acidophilus and then orally infected with human-specific pathogen such as enterohemorrhagic Escherichia coli O157:H7 (EHEC) strain. For the positive control, mice were treated orally with antibiotics including ampicillin, vancomycin, neomycin, and metronidazole. While oral O157 challenge resulted in severe pathology in the gut such as epithelium shedding and paneth cells distortion in the nil and antibiotic-fed mice group, pre-feeding with Lactobacillus maintained gut normally. We found that significantly lower numbers of bacteria in the gut-associated lymphoid tissues were detected in Lactobacillus-fed mice than those of nil and antibiotics-fed mice at early time post infection. In addition, predominant numbers of activated paneth cells and mucin-secreting goblet cells were found in the probiotics-fed mice when compared with nil and antibiotic-fed mice. Most interestingly, numbers of Lgr5⁺ cells gut stem cells were significantly increase in the crypt regions of Lactobacillus-fed mice than in those of nil and antibiotic-fed mice. Taken together, pre-feeding with probiotic Lactobacillus play a pivotal role to promote gut stem cell differentiation and subsequently to protect host against enteric bacteria infection.

W98. The Impact of Bacterial Effector Interactions with ELMO1 on Host Mucosal Immune Responses

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The intestinal mucosa maintains immune homeostasis amongst common flora and enteric pathogens. Phagocytes utilize an endocytic process to engulf microbes and generate inflammation but nothing is known in the context of differential immune responses from pathogenic vs non-pathogenic microbes. We found ELMO1 (Engulfment and cell motility protein-1) engulfs enteric bacteria and produces pro-inflammatory responses only following pathogenic bacterial infection. We hypothesize that the interaction of ELMO1 with microbe regulates the innate responses. Myeloid cell specific ELMO1 KO mice produced less pro-inflammatory cytokines (TNF-a, MCP-1) in ileum and spleen after Salmonella infection. Interestingly following infection with commensal flora, the inflammation is significantly reduced and is ELMO1 independent. To understand the mechanism of differential responses, we detected the ELMO1 interacting bacterial effectors as well as host proteins in Salmonella infected murine macrophages. Our in silico analysis, followed by pull down experiment and MALDI-TOF showed that Salmonella effectors SifA and SifB interact with ELMO1. ELMO1 is associated with other host proteins involved in phagocytosis and endocytic pathway during infection. The ELMO1-SifA effector interaction regulates inflammatory responses and bacterial survival inside phagocytes. Our result indicates that the presence of ELMO1 in phagocyte is crucial to maintain the host-microbiota interface and mucosal immune responses.

W99. Effect of Microbiota on Intestinal Immune Cell Phenotype and Intestinal Integrity

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To understand the consequence of intestinal microbiota for epithelial integrity and immune cell homeostasis we assessed the immune cell phenotype in the gut of germfree (GF), specific pathogen free (SPF) mice and GF-mice colonized at age of 5 weeks with SPF-microbiota (COL) in health and intestinal inflammation. The phenotype of lamina propria mononuclear cells was characterized by flow cytometry and immunohistology. Colitis was induced by dextran sodium sulfate (DSS). Barrier function of the colon was analyzed by electrophysiology. In the ileum of GF-mice macrophages were increased with the pro-inflammatory CD11c⁺ and MHCII⁺ subset dominating, whereas, the number of T cells was profoundly decreased. In parallel, in GF-mice the total colonic epithelial resistance was decreased accompanied by increased ^{3}H-mannitol- and decreased HRP-flux suggesting a barrier dysfunction. By exposing these mice to DSS at week 9 of age, an additional barrier-disturbing factor was introduced. GF-mice were significantly more susceptible to DSS than SPF- or COL-mice as indicated by higher mortality, hence indicating that the exposure to

the microbiota forms a prerequisite to develop epithelial integrity. However, the inflammation score was higher in SPF- than in COL-mice. In conclusion, the microbiota is essential for the development of the colonic integrity and local immune cell composition. Future studies will serve to determine i) the window of opportunity for the microbiota to exert these beneficial effects, and ii) the optimal microbiota composition.

W100. Intestinal Goblet Cell and Mucus Alterations in Obesity

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Obesity is associated with changes of the intestinal microbiota, but factors that govern this are largely undefined. We hypothesized that altered intestinal goblet cell function contribute to microbiota changes in obesity. Goblet cell numbers and mucus thickness were quantified in jejunal biopsies of eleven obese and five normal weight human subjects (BMI 45.6±1.9 vs. 25.0±1.7kg/m^2) as well as obese and lean ZDF rats (weight: 350±7 vs. 305±5g, n=6 in both groups) using histological and immunohistochemical techniques. Expression of mucus components and differentiation factors such as FCGBP, REG3-gamma and Klf4 were analyzed by qPCR. The total number of fecal bacteria and translocation as well as the relative abundance of phyla and mucus-associated bacteria were determined by qPCR. Bacteria were localized by FISH targeting 16S rDNA and muc2. Obese subjects and rats displayed reduced jejunal goblet cell numbers compared with lean controls (6.4±0.4 vs. 8.1±0.2 cells/100µm villus, $p<0.05$ and 8.4±0.6 vs. 10.6±0.4 cells/100µm villus, $p<0.01$, respectively). Klf4 expression was lower in the jejunum and colon (0.22±0.05 vs. 0.61±0.07, $p<0.01$ and 0.40±0.05 vs. 0.60±0.05, $p<0.05$, respectively). Muc2 staining intensity and gene expression were reduced in the colon of obese rats (0.97±0.17 vs. 1.50±0.19, $p<0.05$). Bacteria appeared to be dispersed throughout the compromised mucus layer of obese rats, and were found in close proximity to epithelial cells. Interestingly, colonic Muc2 gene expression correlated with the abundance of both Firmicutes (r_s=-0.70, $p<0.05$) and A.muciniphila (r_s=0.78, $p<0.05$). Altogether, the mucus barrier is compromised in obesity, which could contribute to the observed microbiota composition changes and promote bacterial translocation.

W101. Early Life Stressful Events Impaired Enteric Antimicrobial Activity and Triggered Commensal E. coli Overgrowth Responsible for Visceral Hypersensitivity in Adult Mice

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Early life stressful events induce long lasting alterations of intestinal homeostasis associated with susceptibility to develop gastrointestinal disorders at adulthood. Neonatal period is characterized by immature intestinal mucosa. Among others, Paneth cells appear only 2 weeks after birth. Our aim was to analyze the consequences of maternal separation (MS) in mice on enteric antimicrobial activity and its consequences on intestinal microbiota, systemic immune response toward microbiota and visceral sensitivity. In 50-days old mice, MS induced a decrease of enteric antimicrobial activity associated with intestinal E. coli overgrowth and an increase of anti-E. coli IgG and IgA in plasma. Furthermore, MS increased IFNγ and TNFα in ileum and induced visceral hypersensitivity in response to colorectal distension. In order to decipher whether or not those alterations were a consequence of E. coli overgrowth, adult mice were force fed daily with 10^9 commensal E. coli for 15 days. E. coli gavage reproduced intestinal E. coli overgrowth as well as anti-E. coli IgG and IgA increase and visceral hypersensitivity without modification of enteric antimicrobial defense. Altogether our results highlighted that early life stressful events impair the development of antimicrobial defenses and promote commensal bacterial overgrowth leading to abnormal response toward microbiota and visceral hypersensitivity.

W102. Neonatal Colonization with Probiotic Lactic Acid Bacteria Expressing Allergic-Chimers for Prevention of Allergic Poly-Sensitization

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It is well recognized that allergic individuals are at risk to develop multiple allergies and such poly-sensitized individuals are difficult to treat by conventional therapeutic measures. We have recently established mouse models of poly-sensitization and demonstrated that allergic poly-sensitization can be suppressed by mucosal treatment with novel allergen chimers in adult mice.

With respect to neonatal interventions, we previously showed that colonization with recombinant probiotic strain expressing the allergen Bet v 1 successfully prevents allergic responses. In order to investigate whether the concept of neonatal colonization with recombinant probiotic bacteria could be used for prevention of allergic multi-sensitivities, our first aim is to construct a recombinant Lactobacillus plantarum, constitutively expressing a birch (Bet v 1) and grass pollen (Phl p 1 and Phl p 5) chimer. With this respect, we have successfully cloned Lactobacillus plantarum with birch and grass pollen chimer. To test if this recombinant LABs can be used to prevent poly-sensitization the strain will be used for the neonatal colonization in (a) conventional mice and in (b) germ-free mice prior to sensitization with allergens. Apart from testing the effects of these treatments, interaction of recombinant LABs with the host immune system will be studied.

W103. Stratification of Intestinal Microbes with Unique Transcriptional Pattern and Metabolism in the Outer Layer of Intestinal Mucus

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The mammalian lower intestinal tract harbors great density and diversity of commensal microbes, and a physical gel-like barrier named mucus is adopted in terms of limiting direct contact of commensal bacteria to host during host-microbes coevolution. The inner mucus layer with its tight compiled structure is impenetrable to bacteria, whereas a comparable dose of bacteria to luminal contents are living in the colonic outer mucus layer. We asked whether the outer mucus layer was a separate microbial niche regarding bacterial kinetics and behaviors. Our data show that the outer mucus layer had different representations of microbes compare to adjacent luminal contents, and the exchange between the two compartments was very limited due to immobility of bacteria in mucus layer. Furthermore, by comparing the transcriptional and metabolic patterns of two model bacterial species, Escherichia coli and Bacteroides thetaiotaomicron, with exceedingly different mucolytic capability, we showed mucus and contents associated bacteria differentially shaped their metabolic pattern to adapt to the nutrients accessible in the two compartments. This was not limited to the carbon source, including host-derived phospholipids for E. coli and host or dietary glycans for B. theta, but also included essential minerals. Intensive study of host-microbial mutualism in outer mucus layer is important to uncover the mechanism in clinic diseases as the mucus associated bacteria have a physical nearby impacts to the host.

W104. Genome-Wide Association Studies in Healthy First Degree Relatives (FDR) of Crohn's Disease (CD) Subjects Reveal that Host Genetics Polymorphisms are Associated with the Gut Microbiota

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It has been suggested that inflammatory bowel disease (IBD) is due to a genetically determined abnormal interaction between gut immune responses and gut bacteria. In order to determine if the composition of gut microbiota is associated with host genetic makeup we assessed the stool microbiome in a cohort of 1098 FDRs of CD patients. The V4 region of 16S rRNA gene was sequenced from bacterial DNA extracted from the stool of 1098 healthy Caucasian FDRs. MiSeq sequences were processed using PANDAseq and the QIIME pipeline. Polygenic heritability of the microbiota (H2R) was calculated from 271 related individuals using SOLAR software. Single nucleotide polymorphisms (SNPs) were determined with the HumanCoreExome BeadChip (Illumina). Associations between SNPs and microbiota were estimated using two-part log normal model fitted using generalized estimating equations and adjusting for age, sex, family structure and the first three genetic principal components. A total of 91 out of 253 taxa show significant heritability (25%<H2R<67%, 0.05<H2R, p-value<3.2×10^{-6}). The taxa with highest heritability and/or the most abundant taxa were then assessed for association by a genome-wide scan containing 258,510 genetic markers. Several SNPs were significantly associated with microbial taxa (p<10-6). Fine mapping of regions showing significant associations will be required to identify the causal variant for these associations. These results indicate that host genetics contribute to differences in intestinal microbiota composition in healthy subjects. It remains to be shown if any of these genetic/microbiome associations are related to the risk of developing Crohn's disease.

W105. iNKT Cells, the Microbiota & Intestinal Inflammation
Thierry Mallevaey. University of Toronto, Toronto, ON, Canada

Inflammatory bowel disease (IBD) is a spectrum of chronic inflammatory disorders of the gastrointestinal tract that has genetic and environmental components. IBD is typically described as deregulated immune responses towards the intestinal microbiota, whereby T cells play a prominent role. Invariant Natural Killer T (iNKT) cells are a subset of innate lipid-reactive T cells that are very conserved across mammals. iNKT cells are functionally versatile, and can influence immune outcomes in a wide range of diseases, including IBD. These cells appear to be protective and deleterious in T_H1 and T_H2 models of colitis, respectively. Comparison of iNKT cell-deficient and sufficient mice during DSS colitis has yielded inconsistent results. Here we show that CD1d KO mice that lack iNKT cells have profound intestinal microbiota alterations compared to their wild-type counterpart, which is associated with increased sensitivity to DSS. However, microbiota differences and DSS sensitivity are abrogated in CD1d-deficient and sufficient littermate mice. Fecal transplant of CD1d KO microbiota into wild-type recipient mice induces basal intestinal inflammation, which is exacerbated upon DSS treatment. This shows that CD1d KO mice harbor a pro-inflammatory microbiota. Finally, treatment with the prototypical iNKT cell ligand a-galactosylceramide suggests that the microbiota influences iNKT cell response and function during intestinal inflammation. We currently investigate how iNKT cells and the microbiota influence each other, and the impact of this bidirectional relationship on the establishment of intestinal inflammation. Identification of these mechanisms may lead to new therapeutic strategies targeting iNKT cells for the treatment of IBD.

W106. IL-25 Regulates Host-Microbial Mutualism in the Intestine
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It has previously been shown that intestinal microbiota are critical for interleukin-25 (IL-25) production, yet the role of IL-25 in host-microbe interactions remains unknown. We have shown that IL-25 deficiency results in multiple defects in intestinal immune pathways that regulate microbial handling. Analysis of gut microbial communities revealed key differences in microbial composition in IL-$25^{-/-}$ mice. To examine the impact of this dysbiosis, intestinal inflammation was induced using dextran sulphate sodium (DSS). IL-$25^{-/-}$ mice displayed significantly increased intestinal inflammation as compared to C57BL/6 mice, and this dysregulated microbial homeostasis observed in IL-$25^{-/-}$ mice is transmissible to C57BL/6 mice following cross-fostering. Yet, the defects in intestinal immune homeostasis were still present in IL-$25^{-/-}$ mice even in the absence of the inflammation-associated gut microbiota, supporting the role of IL-25 in the regulation of these pathways. Inbreeding of cross-fostered C57BL/6 mice restored intestinal and microbial homeostasis, demonstrating that microbial dysbiosis can be rescued through IL-25-mediated maternal regulation of commensal microbiota. In support of this, analysis of breast milk revealed key differences in IL-$25^{-/-}$ mice. Taken together, these data demonstrate that IL-25 contributes to the maintenance of a healthy commensal microbiota via regulation of key microbial handling pathways.

W107. Gut Microbiota Composition Imprints Optimal Humoral Immunity
Hazel Poyntz[1], Marcus Robinson[1], Lucas Pitt[1], Charlotte Everitt[1], Catherine Plunkett[1], Lieke van den Elsen[1], Angela Jones[1], Karmella Naidoo[1], Jessica Jones[1], Markus Geuking[2], Kathy McCoy[2], Graham Le Gros[1] and Elizabeth Forbes-Blom[1]. [1]Malaghan Institute of Medical Research, Wellington, New Zealand; [3]University of Bern, Bern, Switzerland

The infant immune system co-evolves with the developing gut microbiota in a mutualistic relationship, providing signals that imprint immune health for life. Recent studies have demonstrated the immunomodulatory effects microbiota exert on humoral immune development using germ-free mice and antibiotic treatment. However, examining the impact that naturally divergent gut microbial communities have on the development of humoral immunity remains to be elucidated. We have developed a model system whereby two lines of BALB/c mice harbor naturally divergent gut microbiota, resulting in a prominent difference in the Firmicutes to Bacteroidetes ratio. These two lines of mice demonstrate disparate levels of immune activation, resulting in markedly different antibody responses. In turn, protective immunity to infectious disease and susceptibility to allergic disease is affected. Microbiota transfer in early life is able to alter the antibody phenotype in both lines of BALB/c mice; demonstrating microbial composition imprints the capability of the humoral response throughout life. Taken together, we have established a critical window

during the ontogeny of the immune system in which gut microbial composition may be therapeutically manipulated to promote optimal humoral immunity.

W108. Targeting Gut Microbial Composition to Enhance Vaccine Efficacy

Lieke van den Elsen, Hazel Poyntz, Angela Jones, Catherine Plunkettand and Elizabeth Forbes-Blom. Malaghan Institute of Medical Research, Wellington, New Zealand

Influenza remains a substantial public health burden with significant rates of morbidity, mortality and economic loss. The efficacy of influenza vaccination is not complete and could be improved. Toll like receptors (TLR) play an important role in promoting antibody responses and can act to adjuvant vaccine-induced antibody responses. Furthermore, recently has been demonstrated that TLR mediated sensing of gut microbiota is required for influenza-vaccine induced antibody responses. Therefore we investigated TLR responses in two lines of BALB/c mice with divergent gut microbial compositions that have marked differences in baseline antibody production. These mice showed significant differences in serum concentrations of IFN-γ, MCP-1 and IL-12p70, especially 6h following systemic LPS or CpG administration. Furthermore, symptoms of septic shock including body temperature were affected. Vaccination with trivalent inactivated influenza vaccine (TIV) resulted in markedly different primary and secondary (5 days post boost) immune responses measured as TIV-specific antibody titers. In addition, antibody responses to influenza infection varied significantly between these two lines of mice with different microbiota. Therefore we suggest modulation of the microbiota, e.g. with dietary components, as an effective approach to improve vaccine-mediated protection against influenza.

W109. Microbial Exposure During Dendritic Cell Maturation Significantly Impacts their Functional Phenotype and Activation of Autologous T Cells

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We have shown that the presence of two common colonizers of the neonatal intestine, lactobacilli and Staphylococcus (S.) aureus influences immune function and/or allergy development during childhood. Further, we have demonstrated that several different Lactobacillus (L.) strains regulate S. aureus-induced immune responses *in vitro*. Here, we assessed how L. reuteri and S. aureus supernatants (-sn) influence the *in vitro*-differentiation of dendritic cells (DC), their functional characteristics and how they stimulate autologous naïve T cells *in vitro*. Exposure to S. aureus-sn, but not L. reuteri-sn, enhanced DC maturation of both retinoic acid (RA)-DC and conventional (CO)-DC, as shown by increased expression of CD86, HLA-DR and CD83. On the other hand, IL-6 and IL-10 production was preferentially induced by L. reuteri-sn in both CO-DC and RA-DC. DC generated in the presence of S. aureus stimulated the production of IL-2, IL-10, IL-17 and IFN-γ in naïve T cells even without further T cell stimulation. This was not seen with DC matured in the presence of only L. reuteri. Our results show that microbial exposure during DC generation has a strong impact on subsequent T cell responses and provides support for a role of gut microbiota in early-life immune maturation.

W110. Smoking Induced Mucosal Inflammation is Negatively Regulated by the Interplay Between IL-10, IL-17 and the Gut Microbiota

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Cigarette smoking remains the major environmental risk factor for Crohn's disease and Chronic Obstructive Pulmonary disease through unknown regulatory mechanisms. Herein, we demonstrate that chronic exposure to cigarette smoke enhanced IL-10 production. Importantly, Il-10 deficiency triggered mucosal immune deviation toward Th1 and Th17-type responses to cigarette smoking. Vancomycin, but not colistin, prophylaxis reversed smoking-induced lung function decline and restricted pathogenic Th17 response in the ileum and the lung. Collectively, we identified a gene-plus-tobacco interaction that restricts mucosal immune deviation toward pathogenic Th17 inflammatory response to Gram positive bacteria. We propose that the mutualistic interplay of Il-10 and the gut microbiota intrinsically controls T cell mutualism in the lung and the intestine. Tobacco smoking may predispose to chronic intestinal inflammation in patients with IL10 defects or a failure to efficiently activate Treg cells.

W111. Using Zebrafish to Study Mucosal Immunity and Microbial Composition in the Presence and Absence of Adaptive Immunity

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Study of the innate and adaptive immune pathways controlling bacterial colonization and mucosal cytokine responses has proven difficult in rodents, considering the extensive cross-talk between bacteria and innate and adaptive immunity. Zebrafish lack a functional adaptive immune system in the first weeks of life, enabling study of the interaction between the microbiota and the innate immune system in the absence of adaptive immunity. Also, several transgenic zebrafish exist that enable *in vivo* tracking of innate immune cells. Here, we show that in larval zebrafish, lacking adaptive immunity, Vibrionales species (known pathobionts) are able to grow out, coinciding with very low expression levels of chemokine Cxcl8. Using cell transfer experiments, we show that adoptive transfer of T lymphocytes into Rag1-deficient recipients suppresses outgrowth of Vibrionales species and enhances epithelial Cxcl8 expression in the zebrafish intestines, showing that zebrafish T lymphocytes play an important role in intestinal homeostasis. Furthermore, we will illustrate the advantage of using transgenic zebrafish to study innate cells in the intestinal mucosal compartment by showing our latest imaging data on the recruitment of macrophages and neutrophils in response to the enterocolitis-inducing chemical DSS.

W112. A Single Viral Caspase-8 Inhibitor is able to Disrupt Intestinal Immune Homeostasis *in vivo*

Barbara Buchen[1], Claudia Günther[2], Vinay Murtadak[3], Michael Stürzl[3], Ethel Cesarman[4], Gianna Ballon[4], Markus F. Neurath[1] and Christoph Becker[1]. [1]Friedrich-Alexander-University, Erlangen, Germany; [2]Uniklinikum Erlangen, Erlangen, Germany; [3]University Hospital, Erlangen, Germany; [4]Cornell University, New York, NY

Recently it could be demonstrated that mice lacking caspase-8 expression in intestinal epithelial cells (IECs, $Casp8^{\Delta IEC}$) spontaneously developed inflammatory lesions in the terminal ileum and showed a high amount of Paneth cell death, indicating dysregulated antimicrobial immune cell functions in IECs (Nature, 2012). The caspase-8 activity can be tightly regulated by cellular FLIPs (cFLIPs). Interestingly certain viruses express a viral FLIP (vFLIP) which shares structural similarities with cFLIP. To elucidate the ability of vFLIP to influence the caspase-8 activity and the gut homeostasis, we analyzed mice, which express vFLIP only in IECs. These mice showed spontaneous development of inflammatory lesions and a high amount of immune cell infiltration, underlined by increased expression of pro-inflammatory markers. Moreover they showed a reduction in Paneth cell number and a high amount of cell death in the small intestine. Furthermore we could discover a dysregulation of the NFκB pathway in the intestinal epithelium. Taken together, they show a dramatic phenotype which shares similarities with the phenotype of $Casp8^{\Delta IEC}$ mice, indicating a dysregulation in the immune defense. The observation of the high amount of cell death suggests that vFLIP might control the caspase-8 activity and therefore interact with the cell death protein platform.

W113. The Influence of the Intestinal Microbiota on Salmonella Infection

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The intestine of humans and other mammals harbors a complex community of microorganisms that contribute to host's homeostasis. However, several questions remain unanswered about their influence on infection. Here we study the importance of the intestinal microbiota in oral Salmonella infection. We show that germfree mice have a higher bacterial burden in mesenteric lymph nodes (MLN) compared to conventional mice. In contrast, bacterial penetration into the lamina propria of the small intestine is unaltered. The recruitment of neutrophils and monocytes is not altered in Salmonella-infected germfree mice. However, infected germ free mice have a greater population of $CD11c^{+}MHC\text{-}II^{high}CD103^{+}CD11b^{+}$ dendritic cells. Germfree mice also show a higher frequency of IFN-γ-producing NK and $CD4^{+}$ T cells early during infection. Similarly, adult mice treated with antibiotics and orally infected with Salmonella also have greater bacterial burden and frequency of IFN-γ-producing cells in MLN. Overall, the data suggest that the severity of the infection is a consequence of the lack of competition between Salmonella and the microbiota, rather than alterations in the immune system attributed to the absence of commensals from birth in germfree mice.

W114. The Role of Epithelial Caspase-8 During Infection with Salmonella typhimurium

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Deficiency of caspase-8 in intestinal epithelial cells (IECs) of mice leads to spontaneous ileitis and increased sensibility towards DSS-induced colitis. We now investigated the role of epithelial caspase-8 in infectious colitis. Therefore we infected Caspase-$8^{\Delta IEC}$ mice with Salmonella typhimurium. In contrast to wild type mice, Caspase-$8^{\Delta IEC}$ mice showed a high lethality after infection. Excessive cell death and the following barrier breakdown enable the commensal gut microbiota to invade into subepithelial areas, resulting in a systemic infection. This might be caused by the absence of Paneth cells and a reduced number of goblet cells in the intestine, which both have a crucial role in the antimicrobial defense and therefore maintaining the mucosal barrier in the gut. RNA data did not show an altered expression of antimicrobial peptides in the colon of control versus Caspase-$8^{\Delta IEC}$ mice challenged with Salmonella typhimurium. This suggests that probably an altered intestinal microflora, which is present in Caspase-$8^{\Delta IEC}$ mice, might enable Salmonella typhimurium to replicate more extensively. Our data demonstrate a crucial role for caspase-8 in controlling intestinal homeostasis in response to infectious colitis.

W115. High Resolution Flow Cytometry of the Intestinal Microbiota

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The intestinal microbiota is an important component of the mammalian organism critical for nutrient metabolism and immune homeostasis. Pathological changes within the microbiota, known as dysbiosis, have been implicated in the pathogenesis of chronic inflammatory disorders like inflammatory bowel disease (IBD). Currently, analysis of the microbiota is done primarily by qPCR- or next generation sequencing approaches. These methods are not only laborious and time-consuming but are also biased towards more abundant bacterial strains. We present here a novel, fast and inexpensive flow cytometry-based approach to characterize the complexity of the intestinal microbiota. High resolution measurement of forward and side scatter of the microbes as well as their DNA content, allowed the discrimination of over 35 different bacterial subpopulations from formaldehyde-fixed murine stool samples. Using this method, we could discriminate the microbiota of mice derived from different facilities and could monitor the adaptation of the intestinal microbiota in individual mice upon co-housing. In addition, we tracked the dynamic changes of the microbiota in two mouse models for IBD, i.e. T cell transfer colitis and DSS-induced colitis. By our flow cytometry-based analysis we could confirm the previously described loss of microbial complexity upon intestinal inflammation. Taken together, we have established an inexpensive and fast, flow cytometry-based method to monitor changes in the intestinal microbiota. We will now translate this approach to the human intestinal microbiota to test the feasibility of defining specific microbiota changes as biomarkers for the diagnosis and prognosis of IBD.

W116. MiBC: The Mouse Intestinal Bacterial Collection: Host-Specific Insights into Cultivable Diversity and Genomic Novelty of the Mouse Gut Microbiome

Thomas Clavel[1], Birte Abt[2], Ruediger Pukall[2], Floor Hungenholtz[3], Sandrine Brugiroux[4], Thi Phuong Nam Bui[3], Caroline Plugge[3], Dirk Haller[1], Hauke Smid[3], Daniel Peterson[5] and Bärbel Stecher[4]. [1]Technische Universität München, Freising-Weihenstephan, Germany; [2] Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; [3]Wageningen University, Wageningen, Netherlands; [4]Ludwig Maximilian University, Munich, Germany; [5]Johns Hopkins University, Baltimore, MD

The use of molecular techniques generated major breakthroughs in microbial ecology of the mammalian gut. However, it became clear in recent years that more effort towards the isolation and thorough characterization of gut bacterial isolates is urgently needed for amendment of databases, improving thereby the interpretation of meta-omics datasets, and for the design of targeted functional studies in gnotobionts. By establishing the Mouse intestinal Bacterial Collection (MiBC), we aimed at providing the first exhaustive and state-of-the-art repository of bacterial strains and associated genomes from the mouse intestine. We isolated aerobic and strictly anaerobic bacteria from various gut location, mouse strains and facilities and selected 100 bacteria, including strains from wild and newborn mice, representing 74 species across 26 families that cover the majority of known phylogenetic diversity in the mouse gut. Via analysis of own and SRA-derived 16S rRNA gene sequence datasets from the mammalian gut, 12 species that are most prevalent in or specific to the mouse intestine were identified. Genome sequences from these and additional members of the collection were obtained, thereby providing novel mouse-derived bacterial genomic information. Novel diversity

was described via taxonomic characterization of 16 new bacteria within the Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria, including novel butyrate- and secondary bile acid-producing species. In summary, we demonstrate that cultivable bacteria represent a substantial part of the mouse gut ecosystem and we provide via MiBC a unique tool to the scientific community.

W117. Microbial-Signaling Pathways in Dendritic Cells in Inflammatory Bowel Disease
Jie Liang, Amelia Karlsson and Gianna Hammer. Duke University, Durham, NC

Although pathological T cells that react to microbial commensals drive pathologies of inflammatory bowel disease (IBD), it is well known that interaction between T cell and microbe is indirect. T cell functions are dependent on an intermediary cell termed the antigen-presenting cell (APC). Dendritic cells (DCs) are key APCs well recognized to have non-redundant functions in microbial-sensing and T cell activation. In IBD pathogenesis, we have recently identified that DCs lacking the NF-kB suppressor, A20, activate pathological T cells and cause IBD. A20 suppresses multiple IBD-linked signaling pathways, including toll-like receptor and other pathways that use the signaling adaptor MyD88. Several studies support a pathogenic, and potentially exclusive role for MyD88 in IBD pathogenesis. By contrast, we report here that in the absence of MyD88, dendritic cells lacking A20 retain potent APC function to activate pathological, anti-commensal T cells that cause IBD. Surprisingly, loss of MyD88 resulted in a skewed IFNg:IL-17 ratio, in favor of anti-commensal T cell production of IL-17. In the lamina propria, IL-17$^+$ T cells were preferentially expanded in the colon—this expansion occurred in a MyD88-independent fashion and resulted in almost overlapping abundance of cytokines in colonic tissue from mice whose DCs did or did not express MyD88. By contrast, MyD88 remarkably enhanced inflammatory cytokine expression in the small intestine, suggesting that signals that drive IBD pathogenesis could be distinct in different regions of intestine. These studies highlight a key role for MyD88-independent signals in the dynamic between host and commensal and pathogenesis of IBD.

W118. Antibiotic Manipulation of the Adult Murine Microbiota Has Long-Term Effects on the Mucosal Immune Response in NOD2$^{-/-}$ Mice
Ashleigh Goethel[1], Sandrine Rouquier[1], Galliano Zanello[1], Dana Philpott[1] and Ken Croitoru[1,2]. [1]University of Toronto, Toronto, ON, Canada; [2]Mount Sinai Hospital, Toronto, ON, Canada

Inflammatory bowel diseases (IBD) are multifactorial diseases, involving genetic mutations, alterations of the gut microbiota and environmental triggers. The strongest genetic association is with nucleotide-binding oligomerization domain-containing protein 2 (NOD2), a pattern recognition receptor that recognizes a component of the bacterial cell wall. During adulthood, environmental perturbations, such as antibiotics, induce transient shifts in the microbiota composition. We sought to determine whether antibiotic-induced dysbiosis in a NOD2 deficient mouse could lead to an altered immune response against the microbiota. Adult WT and NOD2$^{-/-}$ mice received amoxicillin [200mg/L] ad libitum in drinking water for 7 days, followed by control water for 4 weeks. Fecal samples were collected to monitor changes in microbiota. On day 35, acute polyclonal T cell activation was induced by i.p. anti-CD3. Bacterial load, measured using targeted qPCR for RNA polymerase B (rpoB), was significantly reduced at day 7; returning to pre-treatment levels by day 14 in both WT and NOD2$^{-/-}$ mice. At day 35, NOD2$^{-/-}$ mice had a significantly enhanced IL-17 response following anti-CD3 treatment, which was reduced with antibiotic treatment. This suggests that antibiotic alteration of murine adult microbiota in NOD2$^{-/-}$ mice leads to an altered immune response to the commensal gut microbiota.

W119. Perinatal Treatment with a Probiotic *Escherichia coli* Strain for the Prevention of Airway Inflammation in Mice
Christian Zwicker, Melanie R. Hassler, Alina Neunkirchner, Gerda Egger, Ursula Wiedermann and Irma Schabussova. Medical University of Vienna, Vienna, Austria

Clinical studies have demonstrated that application of the probiotic *Escherichia coli* (E. coli) strain O83 effectively prevented the development of allergic diseases in children with familial predispositions. The aim or our recent study is to investigate the impact of perinatal exposure to E. coli O83 on the development of allergic airway inflammation in a mouse model. In particular, we will test whether perinatal exposure to this probiotic strain induces epigenetic changes associated with allergy-protective effects in the offspring. Firstly, we tested the immunomodulatory properties of E. coli O83 *in vitro*. Incubation of mouse splenocytes and bone marrow-derived dendritic cells with E. coli O83 led to the induction of IFNγ and IL-12, respectively, suggesting Th1-biased immunomodulatory properties of this strain. In order to analyze potential histone modifications in T cells, we established the

technique of chromatin immunoprecipitation by using an *in vitro* model of $CD4^+$ T cell polarization. In the context of this study we wish to characterize potential cellular, molecular and epigenetic mechanism that might be involved in the prevention of allergy by probiotic bacteria.

W120. Early Life Antibiotics Alters the Development of the Gut Microbiota and Mucosal Immune T Cell Populations
Ashleigh Goethel[1], Sandrine Rouquier[1], Galliano Zanello[1], Dana Philpott[1] and Ken Croitoru[1,2]. [1]University of Toronto, Toronto, ON, Canada; [2]Mount Sinai Hospital, Toronto, ON, Canada

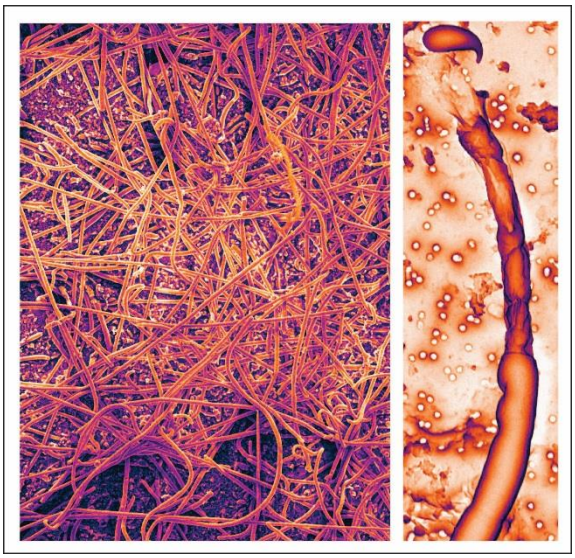
Antibiotic exposure during early life is associated with development of auto-inflammatory diseases, including asthma, multiple sclerosis and Crohn's disease, although the mechanism remains unclear. Exposure to antibiotics and the resultant changes in gut microbiota during early-life may lead to disruption of normal mucosal immune development, as this is strongly influenced by commensal microbes. We hypothesized that antibiotic disruption of the microbiota during early life would have a prolonged impact on both gut microbiota community structure and T cell function within the intestinal tract, resulting in defective immune tolerance to the commensal gut microbiota in a genetically susceptible host ($NOD2^{-/-}$), leading to increased susceptibility to colitis. Neonatal WT and $NOD2^{-/-}$ littermates received amoxicillin [200mg/L] in the drinking water from birth to weaning. Fecal samples collected at weaning were analyzed by targeted quantitative PCR (qPCR) of 16S ribosomal DNA for microbiota composition. Neonatal amoxicillin treatment resulted in a significant reduction of Bifidobacterium and Lactobacillus in WT and $NOD2^{-/-}$ littermates. Phenotypes of intraepithelial (IEL) and lamina propria lymphocyte (LPL) populations were not different in control-treated WT and $NOD2^{-/-}$ littermates. However, antibiotic-treated $NOD2^{-/-}$ littermates showed an enhanced IL-17 response. Together, this suggests that neonatal antibiotic perturbation of microbiota development alters NOD2 signaling in microbe-driven immune responses.

W121. Infection with Adherent-invasive *Escherichia coli* Results in an Imbalance in Apoptosis and Autophagy Responses and Microbiota Composition Worsening Experimental Colitis
Trevor Darby[1], Emma Smith[2], Siobhan McDonnell[2], Heather Byrne[2], Aine Fanning[1], Orla O'Sullivan[3], Paul Cotter[3], David Clarke[2], Fergus Shanahan[1], Ken Nally[1] and Silvia Melgar[1]. [1]Alimentary Pharmabiotic Centre, Cork, Ireland; [2]University College Cork, Cork, Ireland; [3]Teagasc Moorepark Food Research Centre, Fermoy, Ireland

Background: Adherent-invasive *Escherichia coli* (AIEC) are commonly found in the mucosa of Crohn's Disease (CD) patients. AIEC induces pro-inflammatory mediators in macrophages and intestinal epithelial cells (IECs) suggesting an important role in CD-immunopathology. Aim: To investigate the functional effects of AIEC infection on autophagy and apoptosis responses and microbiota composition in an experimental model and IECs. Methods: Streptomycin-treated mice were orally gavaged with $1x10^8$AIEC-HM605 challenged with 2.5%DSS or non-DSS-treatment for 3 days. Colons from these mice and IECs infected with HM605 were assayed for the expression of cytokine, apoptosis and autophagy genes by qRT-PCR and western blot. Analysis on fecal bacterial composition was performed using 16S rRNA amplicon pyrosequencing technology. Results: HM605 colonized the cecum and colons, induced a significantly higher *in vivo* permeability and expression of apoptosis and autophagy genes in the colons but did not induce colitis in non-DSS challenged mice. In contrast, HM605-infected and DSS-challenged mice presented significant symptoms of colitis, increased colonic IL-6 and mKC/CXCL1 levels and dysregulated expression of autophagy and apoptosis genes, and an imbalance in fecal Proteobacteria and Bacteriodetes spp, when compared to non-infected DSS-treated mice. HM605-infection of IECs induced significantly higher secretion of IL-8 and CCL20 levels, mounted a PI3K/AKT-dependent-mTOR/MAPK-independent autophagy response, and presented an imbalance in autophagy and apoptotic gene expression accompanied by a reduction in anti-CD95-induced caspase-3/-7 apoptosis. Conclusions: The collected data reveal new insights into AIEC survival mechanisms in the host and on effects on microbial composition providing a plausible explanation of AIEC contribution to CD-pathophysiology.

W122. The Gut Symbiont Segmented Filamentous Bacteria: Cultivation and Host Response in an *in vitro* Co-Culturing System

Pamela Schnupf[1], Valerie Gaboriau-Routhiau[2], Marine Gros[1,3], Robin Friedman[4], Moya-Nilges Moya-Nilges[4], Giulia Negro[4], Nadine Cerf-Bensussan[2] and Philippe Sansonetti[4,5]. [1]Institut Imagine, Paris, France; [2]INSERM 1163, Paris, France; [3]Universite´ Paris Descartes-Sorbonne Paris Cite´, Paris, France; [4]Institut Pasteur, Paris, France; [5]College de France, Paris, France



Colonization of the mouse small intestine by SFB leads to the post-natal maturation of the mucosal immune system and induces a healthy state of physiological inflammation, characterized by enhanced innate defenses, stimulation of both B and T cell responses, and a particular striking induction of Th17 cells. The broad immunostimulatory properties of SFB do not result in pathology but protect the host from enteric pathogens and can modulate disease severity in a range of murine autoimmune models, making SFB a key member of the intestinal microbiota and a critical microbe in both health and disease. Despite numerous efforts, SFB have resisted *in vitro* culturing for over 50 years, thereby preventing the characterization of its growth and the host-bacterial interaction. Here we successfully cultured mouse SFB *in vitro* in an SFB-host cell co-culturing system for the first time and provide novel insights into the growth requirements and replicative-cycle of SFB. In addition, we demonstrate attachment of SFB to host cells and analyses the host response to SFB challenge *in vitro*. The ability to now decipher the cross-talk between SFB and its host should greatly aid our understanding of this critical symbiosis, which, notably, is disrupted by antibiotics.

W123. Comparative Innate Immune Interactions of Human and Bovine Secretory IgA with Pathogenic and Non-Pathogenic Bacteria

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The gastrointestinal tract is colonized by a diverse population of commensal/symbiotic bacteria, which provide many metabolic functions for the host and play an important role in developing and maturing our immune system. Secretory IgA (SIgA) from milk is involved with early colonization and maintenance of these bacteria as well as providing defense against pathogens. SIgA may bind bacteria using specific antigenic sites or non-specifically via glycans attached to the α-heavy-chain and secretory component moieties of the SIgA complex. We studied bovine SIgA for its innate binding activity with bacteria that are commonly associated with the human gastrointestinal tract and compared this activity with human SIgA. Using human and bovine SIgA isolated from milk, we incubated the proteins with a broad range of commensal, pathogenic and probiotic bacteria and measured numbers of bacteria binding SIgA, using flow cytometry. We found that human and bovine SIgA both bound similar levels of bacteria; 80 to 90% of each commensal bacteria was bound by SIgA, while levels of pathogenic and probiotic bacteria binding SIgA varied from 30 to 90%. Overall, we showed that human and bovine SIgA interacted with bacteria in a comparable way.

W124. Altered Microbiota by Enhanced T Follicular Help and Hyper IgA Results in Impaired Glucose Metabolism

Lisa Perruzza[1,2], Michele Proietti[1], Caterina Elisa Faliti[1,2], Tanja Rezzonico-Jost[1], Anna Maria D'Erchia[1,3], Bruno Fosso[1], Graziano Pesole[1,3], Kathy D. McCoy[2] and Fabio Grassi[1,5]. [1]Institute for Research in Biomedicine, Bellinzona, Switzerland; [2]University of Bern, Bern, Switzerland; [3]University of Bari, Bari, Italy; [4]National Research Council, Bari, Italy; [5]University of Milan, Milan, Italy

The cross-talk between the epithelial component, gut associated immune system and microbiota ensures the physiological control of energy uptake, immune system activation and microbial commensalism. The P2X7 receptor is an ATP-gated nonselective cationic channel expressed in a variety of cell types. In T cells the dual gating property of the receptor can lead to pro-inflammatory signals or opening of a pore permeable to molecules up to 900 Da and cell death. Mice with deletion of p2rx7 show expansion of T follicular helper (Tfh) cells in Peyer's patches with increased IgA responses. In addition, P2rx7$^{-/-}$ mice housed in spf facility have increased body weight, blood glucose, insulin levels and fat accumulation. Analysis of gut microbiota revealed a 3-fold increase in

the ratio between Firmicutes and Bacteroidetes. We demonstrate that the phenotype of P2rx7$^{-/-}$ mice could be reproduced by reconstitution of Cd3e$^{-/-}$ mice with P2rx7$^{-/-}$ Tfh cells. Reconstituted mice with mutant Tfh cells showed enhanced germinal center reaction, higher concentrations of fecal IgA and impaired glucose metabolism. These observations indicate a causal role of P2rx7$^{-/-}$ Tfh cells in altered glucose metabolism. Parallel to Tfh cells role we also show that the phenotype of P2rx7$^{-/-}$ mice could be reproduced by fecal transplant into wild-type animals. Our results emphasize the role of Tfh cells and secretory IgA in the modulation of gut microbiota that ultimately regulates the metabolic balance of the organism.

W125. Understanding the Interactions of the Probiotic Yeast *Saccharomyces boulardii* with the Mucosal Immune System
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The probiotic yeast Saccharomyces boulardii is currently used for the treatment of numerous gastrointestinal disorders, including antibiotic associated diarrhea, inflammatory bowel disease, and recurrences of Clostridium difficile infection. The beneficial effect of this probiotic has been attributed to direct effects on other bacteria, trophic effects on the host intestine, and increases in IgA and anti-inflammatory cytokines. However, the exact mechanisms by which S. boulardii exerts its effects on the host immune system are still not fully understood. Better insight into S. boulardii's interactions with the mucosal immune system will enable a more tailored approach to clinical applications of this probiotic as well as adaptation of this organism for drug delivery in the treatment of gastrointestinal disorders. Here we characterize the interactions of S. boulardii with the murine mucosal immune system. We explore the nature of the increased antibody produced upon oral gavage with S. boulardii and identify the B cell populations contributing to this phenomenon. The data presented here will further elucidate the downstream immunological pathways induced by S. boulardii and provide the foundation for future experiments investigating drug delivery applications of this probiotic.

T1. Antimicrobial Food Additives Influence the Diversity of the Human Gut Microbiota: Studies in Germ-Free Mice
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The incidence of allergies and autoimmune diseases is increasing worldwide. Recent data suggest that gut microbiota have the capacity to modulate not only local, but also systemic immune responses and to the extent previously unthinkable. In this study, we focus on environmental factors, specifically food additives, which may modify the composition of gut microbiota and thus influence host's immune responses. To address this issue, we administered germ-free C57BL/6 mice colonized with human microbiota either sterile water or water supplemented with antimicrobial food additives. The daily intake of additives was calculated to match the maximum daily intake reached in human populations in Europe. The effect of additives on microbial diversity was analyzed by amplification and high-throughput sequencing of the hypervariable regions of the 16S rDNA genes. The resulting sequences were compared with RDP database and OTU assigned. Our preliminary data indicate a significant effect of antimicrobial food additives on the diversity of the human gut microbiota.

T2. Antimicrobial Food Additives Influence the Diversity of the Human Gut Microbiota: *in vitro* Studies
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The role of gut microbiota in health and disease is becoming increasingly more obvious, but environmental factors, such as food components, specifically antimicrobial food additives (AMFAs) which may influence its diversity and function are not well understood. To address this issue we have isolated culturable bacterial strains from human gut microbiota, identified them using 16S rDNA sequence analysis and determined the minimum inhibitory and fractional inhibitory concentrations in broth microdilution assay with the most widely used AMFAs. Our results from these *in vitro* studies indicate that some intestinal bacteria are highly susceptible to selected AMFAs while others are resistant. We have also observed a synergistic effect of some AMFA combinations which may further increase the impact of AMFAs on the gut microbiota diversity. We conclude that AMFAs have the capacity to modify the diversity of human gut microbiota even at very low concentrations especially when used in combinations.

T3. Increased Transcription of Occludin and MUC2 in Subjects with Metabolic Syndrome Following Modulation of the Gut Microbiota by a Diet Rich in Arabinoxylan and Resistant Starch

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Background: A dysbiotic gut microbiota and a weakened colonic defense barrier might contribute to the low-grade inflammation associated with the metabolic syndrome (MetS). Animal studies indicate that consumption of dietary fibers change the gut microbiota, enhance the colonic defense barrier, and reduce inflammation. We hypothesized that a diet rich in the two dietary fibers, arabinoxylan and resistant starch, would strengthen the colonic defense barrier evaluated by increased occludin and MUC2 transcription and decreased fecal calprotectin, a marker of gut neutrophil infiltration. Methods: Nineteen subjects with MetS completed a randomized crossover study with two 4-week diet interventions encompassing a healthy-carbohydrate diet (HCD) rich in arabinoxylan and resistant starch and a low-fiber western style diet. Before and after each intervention we performed endoscopy with biopsies and collected stool samples. Results: The healthy-carbohydrate diet changed the gut microbiome and most distinctly enhanced Bifidobacterium ($p<0.01$). Colonic transcription of occludin increased by 17% ($p=0.03$) and MUC2 by 21% ($p=0.02$) after HCD, whereas fecal calprotectin decreased by 30% ($p<0.05$). Conclusion: Consumption of a diet rich in arabinoxylan and resistant starch for four weeks modified the gut microbiome, increased colonic occludin and MUC2 transcription and decreased fecal calprotectin suggesting a reinforced colonic defense barrier in subjects with MetS.

T4. A Highly Differentiated T Follicular Helper (Tfh) Cell Population in Peyer's Patches Promotes Germinal Center and IgG1 Responses

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T follicular helper cells (Tfh) are essential for the germinal center reaction and the production of high affinity antibodies. Tfh cells produce the cytokine IL-21, which is important for germinal center B cell proliferation and survival and the formation of plasma cells. To study IL-21 producing cells we developed an IL-21eGFP BAC transgenic mouse strain in which a gene encoding a diphteria toxin receptor (DTR)-eGFP fusion protein was placed under IL-21 transcriptional control. Using the new reporter strain we identified a GFP^+ subpopulation of highly differentiated Tfh cells in Peyer's Patches of the gut. Differentiation of GFP^+ Tfh cells in Peyer's Patches is highly dependent on the bacteria microbiota, especially on gram-positive bacteria. DT ablation of GFP^+ T cells demonstrated that this Tfh subpopulation is necessary for optimal germinal center reaction and IgG1 responses in Peyer's Patches.

T5. A Member of Plant Rhizosphere Microbiota, *Stenotrophomonas maltophilia*, Creates MyD88/IL-10 Mediated Intracellular Co-Habitation Niche in Murine Colonic Macrophage

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We surprisingly noticed, by using 16S-rRNA-based metagenome analysis, the existence of Stenotrophomonas maltophilia, a member of plant rhizosphere microbiota, in the colonic resident macrophage in BALB/c mice. In order to clarify the molecular basis behind the symbiotic existence of the rhizosphere bacteria in macrophage, we initially performed confocal laser and transmission electron microscopic analyses of S. maltophilia in murine bone marrow (BM) derived macrophage. It was revealed that S. maltophilia was constitutively resident in not only endosome but also cytosol of BALB/c BM macrophage. The same analyses by using innate immunity-related gene deficient BM macrophage suggested that MyD88/IL-10 was absolutely required for cytosol and endosomal existence, while NLRP3/procaspase-1 exclusively required for cytosol habitation. This cytosol habitation should be triggered by a S. maltophilia secreting molecule designated as "smlt2713", because the smlt2713 gene-deficient S. maltophilia strain was not able to establish cytosol cohabitation, and instead made aberrantly giant S. maltophilia containing vacuoles in macrophage. We speculate the smlt2713 protein might constitutively activate NLRP3/procaspase-1 complex and create a symbiotic environment in tissue resident macrophage. Since the smlt2713 protein and/or the smlt2713 protein possessing S. maltophilia showed the ability to produce IL-10 by BM-macrophage in NLRP3/procaspase-1 as well as MyD88 dependent manner, the

rhizosphere-derived bacteria might create and maintain an immunologically symbiotic condition in the colon via a unusual cytosolic PRR (NLRP3/procaspase-1) and endosomal PRR (MyD88/TIRAP) signaling interaction.

T6. Differential Conditioning of Mucosal and Systemic DCs by Secretory IgA in Complex with the Commensal Bacterium *Lactobacillus rhamnosus*

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The intestinal cross-talk between commensal bacteria and dendritic cells (DCs), as well as local IgA production, are essential characteristics ensuring gut homeostasis. However, the effect of secretory IgA (SIgA) on commensal-DCs interaction has not been addressed yet. In this study, we analyzed the impact of the commensal representative Lactobacillus rhamnosus (LPR), alone or associated with SIgA, on mucosal and systemic DCs freshly recovered from mouse Peyer's patches (PPs), mesenteric lymph nodes (MLNs) and spleen. Exposure of LPR to mucosal DCs conditioned these latter to exhibit anti-inflammatory and regulatory profiles characterized by: low surface expression of co-stimulatory markers; higher anti-/pro-inflammatory cytokine production ratios; promotion of regulatory T cells priming; increased expression of vitamin A-metabolizing enzyme and of TLR pathway regulatory proteins. Association with SIgA further promoted the anti-inflammatory/regulatory LPR-induced conditioning of mucosal DCs, particularly in PPs. In contrast, splenic DCs were activated when exposed to LPR, a feature dampened with SIgA. These data suggest that in addition to DCs origin and bacterial stimuli, SIgA may contribute to the regulation of immune processes after association with commensal bacteria. We conclude that commensal-SIgA immune complexes found in PPs could modulate mucosal DC functions towards a tolerogenic status prone to maintenance of intestinal homeostasis.

T7. Fructose Might also be Detrimental for Mucosal Immunity: A Preliminary Experimental Study

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Additional usage of the fructose by processed-food manufacturers is one of the big risks in terms of metabolic diseases. This situation is a serious public health problem particularly in developed countries. Besides effects on carbohydrate metabolism, fructose has been shown to affect immune system. In this study we aimed to investigate effects of fructose on gut microbiota and mucosal immunity of rats. 18 sprague-dawley rats were included. Each 6 rats were given 15% fructose and 15% glucose, respectively (by drinking water) for 6 weeks. 6 rats were included to control group. Blood sugar levels and body weights were measured every week during study. After sacrification, interleukins in intestinal tissues, biochemical tests from blood, nitrite and nitrate in urine, pathological morphology of intestines and gut microbiota were examined. No significant difference was observed between groups in terms of weight and bacteria ratio. Fasting blood glucose, 2 Hour OGTT, T_3 and T_4 levels was found to be significant in glucose and fructose groups according to sham ($p<0,05$). Testosterone and IL-1β levels of caecum were significant in glucose group ($p<0,05$). IL-1β levels were higher in fructose group but not statistically significant. Our results indicate that inflammation exists in colon and deterioration of microbiota begins. Further and long durational studies are urgently needed.

T8. Dysbiosis and Anti-Commensal Immunity Following Acute Gastrointestinal Infection is Influenced by Host Genetics

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The gastrointestinal (GI) tract represents one of the primary sites for pathogen entry. During GI infection the inflamed mucosal tissue and pathogen virulence genes can cause changes in the diversity of the microbiota, allowing the outgrowth of opportunistic commensal species even after the pathogen is cleared. Here we show that TLR1-deficiency during acute GI infection by Yersinia enterocolitica shifts the host immune response from a TH17 to a neutrophil-dominant response. This shift is accompanied by an outgrowth of a sulfate-reducing Proteobacteria species, chronic inflammation and development of systemic anti-commensal immune responses. Yersinia, similar to Salmonella, can utilize tetrathionate generated by neutrophils for respiration. Both a Yersinia mutant that cannot utilize tetrathionate or depletion of neutrophils were able prevent the bloom of Proteobacteria and systemic anti-commensal antibody responses. These data indicate that the increased neutrophil response in TLR1-deficiency produce tetrathionate that is utilized by Yersinia thereby producing different metabolites and by-products which allow the

expansion of sulfate-reducing Proteobacteria. These data demonstrate that dysbiosis can be a consequence of pathogenic bacterial metabolism, which is affected by the genetic context of the host immune response.

T9. Regulation of Inflammation and Dysbiosis During Colon Carcinogenesis by a Commensal-Derived TLR6 Ligand
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Colorectal cancer (CRC) is the 3rd most common cancer in the US and a role for the microbiota has been suggested. Dysbiosis of the microbiota has been shown to involve the direct expansion of DNA-damaging bacteria or communities that aggravate inflammation, yet the molecular signals contributing to dysbiosis remain elusive. The proximity of the intestinal epithelium with the microbial world suggests that signaling via toll-like receptors (TLR), which sense conserved microbial motifs, may be likely players in regulating the composition of the microbiota. Recently, we observed that polymorphisms in TLR6 are associated with predictive outcomes in CRC patients and that Ulcerative colitis patients with the same polymorphism in TLR6 had worse inflammation and more extensive disease, two highest reported risk factors for later developing CRC. These human phenotypes lead us to hypothesize that TLR6 signaling regulates inflammation and CRC. Indeed, we found that TLR6 signaling in a mouse model of carcinogenesis limited tumor size, number and location compared to TLR6-deficient (6KO) mice. TLR6 signaling also had profound effects on commensal dysbiosis. Despite a more severe cancer phenotype, the microbiota from 6KO mice was protective when transferred to commensal-depleted WT but not 6KO mice. Further, co-housing of 6KO and WT mice resulted in protection from tumorigenesis in the TLR6-sufficient WT mice only. Our data indicates that TLR6 signaling is critical for restraining tumorigenesis, however the dysbiosis that occurs in the absence of TLR6 signals allows for the maintenance of protective commensal bacteria that mediate anti-tumor responses in a TLR6-dependent manner.

T10. Intestinal Expression of the Blood Group Gene b4galnt2 Influences Susceptibility to Intestinal Inflammation
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Glycans play important roles in host-microbe interactions. The glycosyltransferase gene b4galnt2 encodes a beta-1,4-N-acetylgalactosaminyltransferase known to catalyze the last step in the biosynthesis of the Sd(a) and Cad blood group antigens and is expressed in the GI tract of most mammals, including humans. Loss of B4galnt2 expression is associated with altered intestinal microbiota. We hypothesized that variation of B4galnt2 expression alters susceptibility to intestinal inflammation, induced by infection with Salmonella Typhimurium or in dextran sodium sulfate (DSS)-induced colitis. Here, we found B4galnt2 intestinal expression was strongly associated with increased susceptibility to Salmonella as evidenced by increased histopathological changes, intestinal inflammatory cytokines and infiltrating immune cells. Fecal transfer experiments demonstrated a crucial role of the B4galnt2 dependent microbiota in conferring susceptibility to Salmonella infection. Interestingly, the loss of B4galnt2 intestinal expression increased the susceptibility to chronic DSS treatment. A significantly enhanced pathological score was found in B4galnt2 deficient mice, although lower infiltration of CD68+ and CD3+ cells were found in those mice. These data support a critical role for B4galnt2 in intestinal inflammation. We speculate that B4galnt2-specific differences in host susceptibility to intestinal inflammation underlie the strong signatures of balancing selection observed at the B4galnt2 locus in wild mouse populations.

F129.The Impact of a Natural Ecology Model on Microbiota-Host Immune Homeostasis in the Mouse
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We have developed a model where inbred mice are raised in large indoor enclosures containing soil, vegetational elements and farm animal excrements, along with wild-caught mice that introduce natural murine microbes. This simulates one of the most common habitats for a house mouse: a barn floor. Influences of a natural lifestyle on microbiota and immunity can thus be studied under strictly controlled genetic conditions.
Following co-housing of C57BL/6 and wild mice for 2-3 months in this system, lab mice had developed a fecal microbiota converging with wild mice, along with a diversified IgA repertoire. We detected increased levels of induced regulatory T cells in

peripheral and gut lymph nodes, and effector and central memory CD8+ and CD4+ T-cells as well as terminal-stage (CD11c+/CD27-) NK cells were elevated in lymphatic organs. TGF-beta in serum was higher, and Peyer's Patches had enlarged germinal centers. These accumulated signs of immune activation, and increased activation of regulatory type of immunity, may result from continuous or repeated environmental challenges. Hence, immune and microbial homeostasis may stabilize at a more "experienced" state when mammals live in their natural habitat under realistic microbial conditions, with important implications for studies of immune regulation.

IMMUNE CELL MIGRATION

T11. Regulatory T Cell Trafficking to the Gut During Colitis-Associated Colon Cancer

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Chronic inflammation of the colon, as it occurs during ulcerative colitis (UC), prevalently predisposes the tissue to colitis-associated colon cancer (CAC) formation. Although the detailed etiology of UC is poorly understood, regulatory T cells (Tregs) are considered to play a key role in restoring homeostasis during inflammation. However, these suppressive properties might as well be beneficial for tumor progression. We recently identified that the development of CAC is associated with a significant increase in Treg numbers in colonic tumors. Furthermore, depletion of Tregs improved the cytotoxic CD8 T cell response, resulting in reduced tumor growth. Based on these results, this present study now focusses on the migration of Tregs during CAC. We identified a differential gene expression pattern of tumor-infiltrating Tregs and a mainly unmethylated foxp3-TSDR, providing evidence that tumor-infiltrating Tregs are mostly thymus-derived. Moreover, blocking the emigration of Tregs from the mesenteric lymph nodes, led to a reduced number of tumor-infiltrating Tregs, resulting in diminished tumor growth. Therefore we assume that Tregs are rather not induced during CAC formation, but are more likely to have tumor prone migration behavior. In future experiments we will dissect whether targeting tumor-specific homing molecules will allow us to modulate immune responses during CAC.

T12. IL-10-Dependent Down-Regulation of CXCR3 on Th1 Cells Abolishes Experimental Colitis in Mice

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Inflammatory bowel disease (IBD) is characterized by chronic, uncontrolled $CD4^+$ T cell-driven inflammation in the intestinal mucosa. Although the etiology of IBD is poorly understood, it is widely accepted that loss of tolerance to the enteric flora is involved in the development of the disease. Therefore, re-establishing tolerance or gut homeostasis is one of the key features in the development of new therapeutic strategies. Targeting antigen to DEC-205 on dendritic cells (DCs) has been shown to induce tolerance under steady-state conditions. However, whether this approach abrogates inflammatory responses mediated by differentiated Th1 cells is currently unknown. Here we demonstrate that antigen-targeting to DEC-205 protects mice against severe intestinal inflammation by down-regulation of the chemokine receptor CXCR3 on antigen-specific Th1 cells resulting in abrogated migration of these cells into the gut. Strikingly, this process depends on DC-derived IL-10, since neutralization of IL-10 abolishes protection against inflammation. Moreover, we show that in the inflamed gut mucosa of IBD patients the frequency of $CD4^+CXCR3^+$ T cells is highly elevated compared to non-inflamed tissue. Interference with this pathway may therefore be a promising approach for the treatment of IBD. In summary, we propose a hitherto undescribed mechanism of how antigen-targeting to DEC-205, and more importantly how IL-10 mediates anti-inflammatory properties towards Th1 cells, thereby providing new therapeutic options.

T13. Lymph Nodes and Peyer's Patches Harbor Resident $CD4^+$ T Cells That Accumulate After Prolonged Antigen Exposure

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$CD4^+$ T cells can acquire various migratory properties during immune responses to provide enhanced immuno-surveillance and protection. However, the comprehensive analysis of these migratory properties has been difficult due to highly dynamic nature of T cell circulation. We developed two independent long-term *in vivo* cell tracking methods to analyze the migration of

effector/memory $CD4^+$ T cells. We identified a resident population of effector/memory $CD4^+$ T cells that stays in lymph nodes and Peyer's patches (PPs) without circulation or proliferation. Resident $CD4^+$ T cells constitute up to 50% of all effector/memory cells, including, but not limited to, follicular helper T cells. This functionally heterogeneous population of resident cells expresses low levels of egress-promoting sphingosine-1-phosphate receptor 1 (S1pr1) and possesses a distinct TCR repertoire. Furthermore, resident cells constituted a significant portion of all $CD4^+$ T cells in PPs and accumulated in these organs after continuous oral antigen exposure. Our results define a previously unrecognized population of effector/memory $CD4^+$ T cells in lymphoid tissues which might perform similar functions as non-lymphoid tissue-resident memory T cells.

T14. Visualizing the Immune Response to Epithelial Injury in Mouse Small Intestinal Mucosa Reveals a Two-Phase Respond of Polymorphonuclear Leucocytes in LysM-GFP Mice

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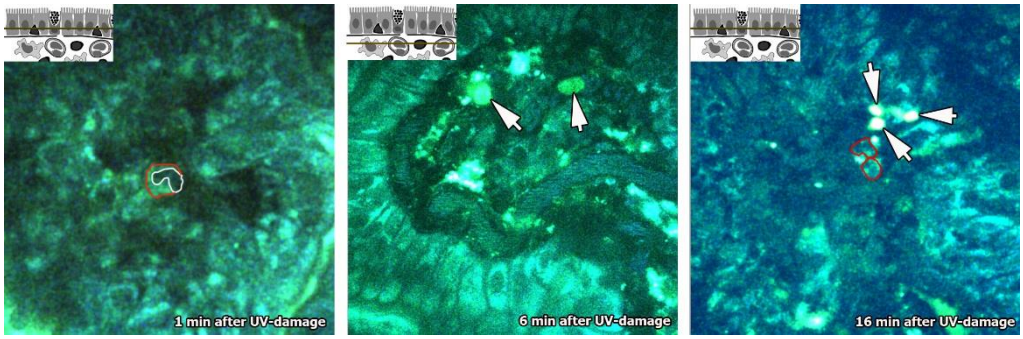

Intact intestinal barrier function is crucial for the maintenance of dietary nutrients resorption and restricts uptake of luminal antigens and toxins. Our previous studies have shown that larger epithelial defects (> 7 µm in size), induced by UV-laser application, leads to immigration of polymorphonuclear leucocytes, identified by their typical shaped nuclei and high motility of 25 µm/min. The lamina propria, underlying the epithelium, accommodates numerous eosinophils in physiological state but no neutrophils, which are believed to be the first immune cells at a site of tissue injury. We applied intravital autofluorescence 2-photon microscopy in LysM-GFP mice, in which neutrophils are brightly labeled, but eosinophils are identified by autofluorescence, highly fluorescent granules and their typical bi-lobular shaped nuclei. After epithelium injury with UV-laser application, we visualized the first unlabeled eosinophils after 1-3 minutes in the basal part of the epithelium, where they moved vigorously. Labeled neutrophils were visible crawling within the blood vessels in the lamina propria a few minutes after tissue damage but arrived at the site of epithelial damage after 15 minutes at the earliest. We show that eosinophils are the first immune cells to arrive at an epithelial damage in mouse small intestine, coming directly from the lamina propria, whereas neutrophils have to be recruited from the blood vessels.

T15. CCR9 is not Required for the Homing of Pro-Inflammatory Effector T cells, but is Crucial for Recruitment and Expansion of $FoxP3^+$ $CD8^+$ Tregs in the Small Intestine

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Chemokine receptor 9 (CCR9) is required for the homeostatic recruitment of T cells to the mucosa of the small intestine. Accordingly, CCR9 has been suggested as a potential target to inhibit the recruitment of pro-inflammatory effector T cells (T_{eff}) in inflammatory bowel disease (IBD). Since the contribution of CCR9 to the recruitment of T_{eff} in inflammation is not entirely clear, we aimed to address this question using IFABP-tOva mice. These mice express Ovalbumin (Ova) specifically in small intestinal epithelial cells, which allows triggering of acute inflammation following transfer of Ova-specific $CD8^+$ T cells (OT-I cells) and adjuvant treatment. Strikingly, intestinal inflammation in IFABP-tOva mice could also be triggered following transfer of CCR9-deficient OT-I cells, demonstrating that CCR9 is not required for homing of T_{eff} cells. Interestingly, OT-I cells transferred to IFABP-tOva mice did not only differentiate into T_{eff}, but also into $FoxP3^+$ $CD8^+$ Tregs, which in contrast to T_{eff} cells expressed high levels of CCR9. Indeed, recruitment and expansion of this regulatory subset in the small intestine was strongly dependent on CCR9. Hence, our data show that T_{eff} and regulatory T cell subsets use distinct mechanisms for migration to the small intestine and suggest that inhibition of CCR9 in IBD could be more harmful than useful.

T16. Stromal Cells as Trend-Setters for Cells Migrating into the Lymph Node

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Lymph node stromal cells are found to be part of immune response induction and tolerance. To do this efficiently the immune response has to be adapted to the lymph node location. Therefore, differences between peripheral lymph nodes and mesenteric lymph nodes were identified to induce an effective immune defense. Stromal cells were considered to be perfectly adapted to their draining area and not changeable concerning their expression pattern. Here we show that stromal cells can change their profile after isolation and transplantation into a different draining area. We generated new lymph nodes using freshly isolated lymph node stromal cells from different draining areas. Subsequently, these newly organized lymph nodes were able to induce not only a region-specific but also an antigen-specific immune response. Thus, stromal cells are trend-setters for immune cells in producing a microenvironment that allows an optimized immune defense.

T17. Identification of the Orphan Receptor GPR15 as a Potential Gut Homing Receptor on Human CD4 T Cells

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Immune responses in the intestine are tightly regulated to ensure host protective immunity in the absence of immune pathology. A permanent disturbance of this delicate balance can lead to the development of inflammatory bowel disease (IBD). Additionally, there is compelling evidence that dysregulated effector CD4 T cells accumulate in the inflamed intestine and contribute to the chronicity of disease. Homing of CD4 T cells to the intestine is thought to be a vital process in disease initiation and progression. G-protein coupled receptors guide lymphocytes towards the site of inflammation, including the G-protein coupled orphan receptor GPR15. This receptor has been recently described to regulate homing of mouse regulatory and effector CD4 T cells to the large intestine. Here we characterized the expression pattern of GPR15 and its putative role in regulating human T cell homing to the gastrointestinal tract. In healthy individuals and IBD patients, GPR15 was expressed on memory CD4 T cells in combination with several chemokine receptors regulating lymphocyte migration towards the gastrointestinal tract and the skin. $GPR15^+$ memory T cells were identified within circulating as well as tissue resident lymphocytes. GPR15 expression marked a unique subset of gut-homing CD4 T cells and was independent of integrin $\alpha4\beta7$ and CCR9 expression. $GPR15^+$ CD4 T cells produced the cytokines IFNγ, IL-22 and IL-17A. Finally, GPR15 expression was altered in mucosal tissue of Crohn's disease patients. Taken together, our findings provide new evidence to a putative tissue homing receptor, which regulates the migration of memory CD4 T cells to gut and skin.

T18. TRPM7 Kinase Activity Plays an Essential Role in T Cell Colonization of Small Intestine Epithelium

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The gene transient receptor potential-melastatin-like 7 (Trpm7) encodes a protein that functions as ion channel and serine/threonine kinase. TRPM7 is widely expressed in cells of both innate and adaptive immune system. Tissue-specific deletion of Trpm7 in the T cell lineage was shown to affect thymopoiesis, indicating that the channel and/or the associated kinase are important in T cell development. To specifically address the role of TRPM7 kinase activity in T cells, we used mice carrying an inactive TRPM7 kinase domain (TKI). In contrast to conditional Trpm7-/- mice, TKI mice are characterized by normal T cell development in the thymus, indicating that the kinase activity is not responsible for the thymic phenotype observed previously. Moreover, T cells in spleen and peripheral lymph nodes are normally distributed. However, TKI mice show hypotrophic Peyer's patches and reduced amount of secreted IgA in the small intestine. Notably, intraepithelial T cells (IEL) are particularly affected by lack of TRPM7 kinase activity and CD103 expression is strongly reduced. The lack of IEL in TKI mice results in significantly reduced expression of MHCII in intestinal epithelial cells. We found that the defect of IEL retention within small intestine epithelium is T cell intrinsic. In fact, adoptive transfer of lymphopenic host with naïve CD4 cells from TKI mice did not result in CD103 upregulation, reconstitution of IEL pool and MHCII expression in intestinal epithelial cells. Our results suggest that TRPM7 kinase activity plays a fundamental role in T cell colonization and patrolling of gut epithelium.

T19. Discrete Intestinal Stromal Cell Populations Differentially Express the Atypical Chemokine Receptor, ACKR4
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Intestinal immune responses are dependent on the action of chemokines and their receptors. CCL19 and CCL21 drive the CCR7-dependent migration of dendritic cells from the lamina propria (LP) to the mesenteric lymph nodes (MLN), whilst CCL25 recruits $CCR9^+$ gut-homing leukocytes from the circulation to the LP of the small intestine. CCL19, CCL21 and CCL25 also bind to the atypical chemokine receptor ACKR4 (CCRL1). Interestingly, rather than inducing cell migration, ACKR4 binds and internalizes its ligands, targeting them for intracellular degradation. ACKR4 is expressed by lymphatic endothelial cells in secondary lymphoid organs, including the MLN. However, if and where it is expressed in the mucosa is unknown. Using ACKR4-eGFP reporter mice, we show that ACKR4 expression in the intestinal LP is restricted to a discrete population of fibroblasts. This ACKR4-expressing fibroblast population has a unique transcriptional signature, thus differentiating them from other intestinal fibroblasts. To explore the biological and functional significance of ACKR4 expression on intestinal stromal cells, we have compared DC migration from the LP to the draining MLN in WT and ACKR4-deficient mice. These studies aim to establish the role of ACKR4 in intestinal immunity, and dissect the complexity of chemokine networks in the intestine.

T20. Plet1-Mediated Cell Detachment Controls Steady State Migration of Dendritic Cells in the Intestine
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Migration of dendritic cells (DC) from mucosal tissues to draining lymph nodes (LN) is a prerequisite for induction of normal and pathogenic T cell responses. DC migration is controlled by the chemokine receptor CCR7, which guides cells through the extracellular matrix (ECM) of the intestinal lamina propria (LP). However, the molecular mechanisms that control DC mobilization and detachment are incompletely understood. We recently identified the GPI-anchored surface protein Placenta-expressed transcript 1 (Plet1) as specifically expressed on $CD11c^+MHCII^{hi}$, $CCR7^+$ migratory DC in the gut-draining LN and LP. $Plet1^+$ DC subsets analysis revealed that Plet1 expression was highly enriched on $CD103^+CD11b^+$ DC, both at steady state, and during inflammation. Plet1 deficiency did not affect chemokine responses as $Plet1^{-/-}$ DC showed normal CCR7-mediated migration *in vitro*. Structure-prediction analyses of the Plet1 protein, and *in vitro* adhesion assays suggest a role of Plet1 in modulating ECM-integrin interactions by DC. This was confirmed *in vivo*, as migration of LP DC was severely impaired in the absence of Plet1: $Plet1^{-/-}$ DCs failed to migrate to the LN and accumulated within the LP. Taken together, these data unravel a previously unappreciated mechanism where Plet1 controls DC migration via their detachment from the ECM in the intestine.

IMMUNOLOGY OF ASTHMA: BASIC

OR.33. Functional Analysis of Protective IL1RL1 Variants Associated with Asthma Risk
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GWAS studies have identified polymorphisms in both IL33 and IL1RL1, the gene encoding ST2, the high affinity chain of the IL-33 receptor, that associate with asthma susceptibility. We identified amino acid changing variants in IL1RL1 associating with asthma incidence and found these SNPs to be protective from asthma risk in our study population. These variants result in coding changes to the intracellular region of ST2, which contains the TIR domain of the receptor that is critical for signaling downstream of IL-1 cytokine family and TLRs. Mutations or deletions to this region can inhibit ligand-induced responses. IL-33 responses were diminished in cell lines expressing all 4 IL1RL1 missense variants. To further elucidate how this haplotype could affect IL-33 activity, we compared IL-33 activity and ST2 expression between donors carrying either haplotype. We observed reduced IL-33 mediated IL-8 secretion from purified blood eosinophils derived from individuals carrying the protective haplotype. We also observed greater soluble ST2 expression in these individuals. Additionally, we have recently observed that expression of other IL1R family members may also be affected by this haplotype due to the tight linkage disequilibrium at this locus. Expression of IL18R is also elevated in these individuals, suggesting a link between IL33 and IL18 responses *in vivo*. Our results provide a link between the genetic

predisposition to asthma and IL-33 mediated responses. Given IL-33 promotes Th2 immunity, perturbations that diminish this response may provide protection from asthma risk.

OR.34. Aeroallergen-Induced IL-33 Predisposes to Respiratory Virus-Induced Asthma by Dampening Type I and III Interferon Production

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Frequent viral lower respiratory infections (vLRI) and allergic sensitization in early life are independent risk factors for asthma onset, yet together significantly increase the development of persistent asthma. We developed an experimental model of asthma to investigate this synergy. Neonatal BALB/c mice were inoculated with low dose pneumonia virus of mouse (PVM; 1pfu) then exposed to low dose (1µg) cockroach antigen or vehicle control at 3 days post infection (dpi). Some mice were re-infected 6 weeks later and exposed to weekly doses of allergen. Virus and allergen co-exposure was critical in both early and later life for disease onset and progression, including airway hyper-reactivity, airway remodeling and type 2 inflammation. Allergen exposure during primary vLRI increased IL-33 release and impaired antiviral cytokine production, leading to increased epithelial viral burden, Th2-type inflammation and airway smooth muscle growth. Neutralization of IL-33 in early life prevented type 2 inflammation, airway remodeling and reversed the dampened interferon response mediated by cockroach antigen. Substitution of allergen with exogenous IL-33 attenuated antiviral cytokines, elevated viral load and promoted airway remodeling. Mechanistically, we found that IL-33 degraded IRAK1 to dampen type I IFN production by plasmacytoid DC. In summary, we identify a novel role for IL-33 in regulating antiviral immunity and as a target to attenuate the synergistic interplay between two important environmental insults in the onset and progression of asthma.

OR.35. Inhibition of CD23-Mediated IgE Transcytosis Suppresses the Initiation and Development of Allergic Airway Inflammation

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The epithelial lining of the airway tract and IgE are considered essential controllers of inflammatory responses. CD23 is capable of transporting IgE or IgE-allergen complexes across the human airway epithelial cells (AEC) *in vitro*. However, it remains unknown whether the CD23-dependent IgE transfer pathway in AECs initiates and facilitates allergic inflammation *in vivo*, and whether inhibition of this pathway attenuates allergic inflammation. Here, we show that in wild-type (WT) mice, epithelial CD23 transcytosed both IgE and OVA-IgE complexes across the airway epithelial barrier, while neither type of transcytosis was observed in CD23 knockout (KO) mice. In chimeric mice, OVA sensitization and aerosol challenge of WT/WT (bone-marrow transfer from the WT to WT) or CD23KO/WT (CD23KO to WT) chimeric mice, which express CD23 on radioresistant airway structural cells (mainly epithelial cells) resulted in airway eosinophilia, including collagen deposition and a significant increase in goblet cells, and increased airway hyper-reactivity. In contrast, the absence of CD23 expression on airway structural or epithelial cells, but not on hematopoietic cells, in WT/CD23KO (the WT to CD23KO) chimeric mice significantly reduced OVA-driven allergic airway inflammation. In addition, inhalation of the CD23-blocking B3B4 antibody in sensitized WT mice before or during challenge suppressed the salient features of asthma, including bronchial hyper-reactivity. These results identify a previously unproven mechanism in which epithelial CD23 plays a central role in the development of allergic inflammation. Further, our study suggests that inhibition of CD23 in the airway is a potential therapeutic approach with which to inhibit the development of asthma.

OR.36. Notch Signaling in CD4⁺ T Cells is Required for the Induction of Allergic Asthma *in vivo*

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Introduction: Differentiation and activation of T helper 2 (Th2) cells is critical in allergic asthma. Binding of Notch ligands on dendritic cells (DCs) to Notch molecules on T cells can instruct differentiation of naïve T helper cells through direct induction of the key Th2 transcription factor Gata3, via the downstream protein RBPJ: Delta-like ligands and Jagged induce Th1 and Th2 differentiation, respectively. However, it remains unknown whether Notch signaling induced by DCs is critical for Th2-mediated allergic airway inflammation (AAI) *in vivo*. Materials & Methods: To analyze the role of Notch signaling *in vivo*, we employed mice with CD4-cre mediated T cell-specific deletion of the Rbpj gene or both Notch1 and Notch2 genes, as well as mice lacking Jagged1, Jagged2 or both Jagged molecules in DCs (CD11c-cre mediated) in a house-dust mite-mediated asthma model. Results: In contrast to wild-type mice, RBPJ-deficient mice failed to develop AAI, characterized by eosinophilic inflammation, Th2 cytokines, Gata3 induction in T cells and serum IgE. Likewise, Notch1 and 2 deficient mice failed to develop any signs of Th2 inflammation. Remarkably, conditional Jagged1, Jagged2 or Jagged1 and 2 KO mice developed AAI symptoms that were similar to those in WT mice. We are currently investigating which cells induce Th2 inflammation via Notch signaling in a house-dust mite-mediated asthma model. Conclusion: Although future experiments should establish which cells express crucial Th2-inducing Notch ligands, we conclude that Notch signaling in T cells is required for AAI induction in mice.

T21. PDC Expansion of Natural TREG Cells Limits Severe-Pneumovirus-Induced Bronchiolitis and Attenuates the Onset of Virus-Provoked Asthma

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Circulating plasmacytoid dendritic cells (pDC) are inversely associated with infant wheeze, bronchiolitis and childhood asthma. Here we addressed whether inducible depletion of pDC in early-life alone increases the severity of Pneumovirus infection and predisposes toward virus-provoked asthma. Temporary depletion of pDC (using pDC-DTR neonatal mice) attenuated interferon alpha and lambda production in the lung, increased viral load in the airway epithelium and promoted severe bronchiolitis (neutrophilia, sloughing and weight loss) during low dose pneumonia virus of mice infection (10 plaque forming units, intranasal route) in early-life. Heightened inflammation was associated with significantly lower numbers of neuropilin-1+ natural regulatory T cells (nTreg), but not peripherally-induced Treg, and diminished IL-10 and TGF-β production in the lung. Relative to other antigen presenting cells, the neuropilin-1 ligand semaphorin-4a was highly expressed on pDC and ligation of neuropilin-1, but not the interferon alpha receptor, was necessary for pDC mediated expansion of nTreg. Viral challenge of pDC-DTR but not WT mice in later-life (6 weeks later) induced the hallmark features of asthma, including airway hyper-reactivity, eosinophilia and type-2 inflammation. Adoptive transfer of naïve nTreg during primary infection of pDC-depleted mice prevented the development of both severe bronchiolitis and post-viral asthma. These data are the first to demonstrate that pDC-mediated support of nTreg is necessary to limit severe virus-induced bronchiolitis, and is sufficient to halt the onset of childhood asthma. We propose that boosting the tolerogenic function of pDC in early-life represents a novel preventative strategy for the treatment of both bronchiolitis and asthma.

T22. Hepatocyte-Specific Allergen Expression by Adeno-Associated Virus-Mediated Gene Transfer Suppresses Allergic Airway Inflammation

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Allergic asthma is a chronic airway inflammatory disease characterized with elevated serum antigen-specific IgE, lung eosinophilia and airway hyper-responsiveness (AHR). Th2 cell-mediated immunity is mainly considered to regulate the pathogenesis of asthma. T regulatory (Treg) cell-maintained tolerance was demonstrated to benefit several T cell-mediated diseases including autoimmune

diseases and allergy. Immune tolerance induction by DNA vaccine or allergen-specific immunotherapy might offer an effective treatment for asthma. Some studies proposed that hepatic gene transfer can trigger transgene-specific immune tolerance via generating Foxp3$^+$ Treg cells. Thus, we examine whether hepatocyte-specific allergen expression could induce immune tolerance to suppress asthmatic responses in ovalbumin (OVA)-sensitized mice. Before sensitization, mice were pre-treated once with pseudo-typed adeno-associated virus (AAV) 2/8 vector carrying membrane-bound OVA (mbOVA) cDNA that is controlled by hepatocyte-specific alpha 1 antitrypsin promoter via intravenous injection. AAV2/8-mbOVA virus significantly suppressed AHR, lung eosinophilia, and Th2-typed cytokines in lungs and from cells of secondary lymphoid organs of OVA-sensitized mice. However, serum levels of OVA-specific IgE were not influenced. Foxp3 expression of $CD4^+CD25^+$ T cells was prominently increased in liver and lung tissues from AAV2/8-mbOVA virus-treated mice. The results suggest that immune tolerance induction by hepatic gene transfer is potentially applied to modulate airway inflammatory responses.

T23. Lung B Cells Promote Ongoing Allergic Inflammation in a Mouse Model of Asthma via B Cell Receptor-Independent Antigen Presentation

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The importance of B cells to present antigens for antibody production is well documented. In contrast, very little is known about their capacity to influence T cell response in ongoing allergic inflammation. Using a mouse model of allergic asthma, we observed that lung B cells upregulated expression of MHC-II, CD86 and OX40L upon house dust mite (HDM) challenge and efficiently presented antigen to T cells *in vitro*. B cell depletion during challenge severely impaired expansion of activated T cells and their capacity to secrete Th2-type cytokines. Interestingly, efficient HDM presentation was a property not limited to B cells carrying surface Ig specific for HDM, since naïve, memory and B cells of unrelated specificities were equally efficient. Preliminary data suggest the existence of receptor-mediated uptake in the process. Collectively, we highlight the existence of a novel mechanism that may lead to exacerbation of the allergic response.

T24. Activation of Lung $CD103^+$ Conventional DCs Reduces Allergen Driven Asthma

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Asthma is a Th2-mediated inflammatory lung disorder in response to inhaled antigens, such as house dust mite (HDM). Dendritic cells (DCs) are crucial for both induction ($CD11b^+$cDCs) and maintenance of asthmatic immune responses (moDCs), whereas pDCs and $CD103^+$cDCs are described to be tolerogonic. DCs can be activated upon TLR stimulation, initiating the NF-κB pathway, which is negatively regulated by A20. Absence of A20 in DCs leads to their overt activation. In this study, we investigated whether activation of $CD103^+$cDCs further increases their tolerogenic function in asthma. $A20^{fl/fl}$xLangerin-cre (Langerin-A20 mice) mice were exposed to a HDM-driven model for asthma. Sorted DC subsets from MLN of Langerin-A20 mice were also co-cultured with OT-II cells to determine DC specific Th cell differentiation and proliferation. HDM-sensitized langerin-$A20^{WT}$ mice displayed an asthmatic phenotype in broncho-alveolar lavage, with increased eosinophils, and Th2 cells/cytokines. Surprisingly, asthmatic characteristics were reduced in langerin-$A20^{KO}$ mice, while Tregs and IL-10$^+$ CD8 T cell numbers were increased compared to langerin-$A20^{WT}$ mice. Co-culturing OT-II cells with sorted $CD103^+$cDCs from Langerin-$A20^{KO}$ mice specifically induced a increased proliferation of Tregs, compared to $CD103^+$cDCs from Langerin-$A20^{WT}$ mice. Lung $CD103^+$cDCs of Langerin-$A20^{KO}$mice showed increased PD-L1 expression, while no differences were observed in ICOSL, IL-10 and TGF-β. Activated $CD103^+$cDCs dampen asthmatic inflammation through induction of Tregs and tolerogenic $CD8^+$ T cells, probably mediated by increased PD-L1 expression. These results offer new insights in the pathogenesis of asthma, and could contribute to new potential strategies to treat asthma patients.

T25. Analysis of Interleukin-33 Producing Cells After Infection with Cryptococcus neoformans in an Experimental Mouse Model

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The opportunistic fungal pathogen Cryptococcus neoformans causes an asymptomatic respiratory infection in immunocompetent individuals resulting in growth control. In contrast, immuno-suppression, e.g. in AIDS, promotes fungal proliferation and haematogenous dissemination, ultimately leading to a life-threatening meningitis. Haematogenous dissemination of Cryptococcus is favored by a type 2 immune response characterized by key cytokines interleukin (IL)-4, -5 and -13, non-protective alternative activation of pulmonary macrophages and eosinophil recruitment, thus closely resembling features of allergic airway inflammation (AAI). A type 2-biased immune response may even occur in immunocompetent individuals and therefore predispose for the development of airway inflammation and finally allergic asthma. Yet, the precise mechanism of type 2 cytokine induction is not known in pulmonary cryptococcosis. In our study we focus on the cytokine IL-33 which can be an early inducer of type 2 immunity. Therefore, we performed a kinetic analysis of intra-nasally infected IL-33 citrine reporter mice with several time points of analysis up to 70 days post infection (dpi). We identified different cell types in the lung that became citrine positive, indicating il33 expression, during cryptococcal infection using flow cytometry and immunofluorescence microscopy. In our study lung epithelial cells represent the main citrine positive population. The lung epithelium is the first contact site for lower respiratory tract infections in general. A detailed understanding how respiratory epithelium contribute to Th2 immune response during an infection with Cryptococcus could provide new opportunities for early, effective therapy not only for pulmonary cryptococcosis but hopefully AAI in general.

IMMUNOLOGY OF THE EYE

T26. Vitamin A Deficiency Alters Ocular Immune System

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Vitamin A has been shown to regulate the growth and differentiation of epithelial cells in many tissues, and it has both positive and negative regulatory functions in the immune system. To study the effects of depletion of retinoic acid on ocular surface and lacrimal immune system, we generated vitamin A-deficient (VAD) mice by continuous feeding of a VAD diet beginning in gestation. We found that muc5AC concentration was significantly reduced in tear of VAD mice without alteration of other antimicrobial peptide. Accordingly, total amount of bacteria was significantly increased in the ocular surface of vitamin A-deficient (VAD) diet – fed mice. Furthermore, in the lacrimal gland of VAD diet-fed mice, IFN-g-secreting CD4+ T cells had significantly been increased than those of the control diet-fed mice. Taken together, these data indicate that vitamin A deficiency interferes with the integrity of the ocular mucosal barrier.

T27. Visualization of Inoculated Eyedrop Draining Passages and Identification of Eyedrop Draining Lymph Nodes and Antigen Presenting Cells in Eyedrop Vaccination

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In our previous study, IN vaccination showed significantly stronger level of immune induction compared to the eye drop vaccination. However, the eye drop vaccination activates both tear-associated lymphoid tissues (TALT) and nasal-associated lymphoid tissues (NALT). Moreover, there has been only T cell proliferation assay for the detection of draining lymph nodes of eye drop vaccination. Therefore, the clarification of the draining passageway of eye drop vaccination and the comparison of the draining lymph nodes between those two different entry sites are needed. In here, we firstly visualized the route of drainage of the eye drop vaccine and showed that the draining lymph nodes for eye drop vaccination are different from those of intranasal vaccination in mice. The visualizing materials administered by eye drop drained out through tear duct and nasal cavity. After the FITC solution was injected by eye drop, superficial parotid lymph nodes (SPLN), mandibular lymph nodes (MdLN), inguinal lymph nodes (InLN) and spleen (SPL) were all FITC positive. However, 2 days after FITC-beads inoculation by eye drop, superficial parotid lymph nodes (SPLN) were bead positive only in the presence of poly(I:C) and little mandibular lymph nodes (MdLN) were bead positive. In contrast, intra-nasally administered beads were detected in both SPLN and MdLN. Among FITC-beads positive cells in

SPLN, they were CD11c$^+$CD86$^+$ or F4/80$^+$. These results suggest that inoculated eye drop vaccine antigens are captured by CD11c+ DCs and possibly participate in the presentation of the antigens to T cells in SPLN, rarely in MdLN, which a pattern is distinguished from that of IN vaccination.

IMMUNOLOGY OF THE UG TRACT

OR.25. The Role of Gap Junction Mediated Antigen Transport in Immunopathogenesis of Mucosal Intracellular Pathogens
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We have shown previously using mouse models that Chlamydia-specific CD8$^+$ T cells do not significantly affect bacterial clearance but cause pathological sequelae such as fluid-filled oviduct dilatation (hydrosalpinx), which also occurs in humans. We recently found that chlamydial antigens can be detected in uninfected cells neighboring infected genital epithelial cells. Based on this, we hypothesized that gap junction mediated antigen transport (GMAT) via channels formed by connexin (Cx) proteins, predominantly Cx43 expressed on oviduct epithelium, plays a role in activation of antigen-specific CD8$^+$ T cells. Using Cre-Lox technology, we generated mice (Foxj1Cre-Cx43flox mice) with a conditional deficiency of Cx43 only in ciliated columnar epithelial cells specifically the oviduct, but not uterine horn, epithelium in the genital tract. Resolution of chlamydial infection, splenic antigen-specific total cellular cytokine response and serum antibody response were comparable between Foxj1Cre-Cx43flox and WT mice. However, the frequency of Chlamydia-specific CD8$^+$ T cells in spleens of Foxj1Cre-Cx43flox mice was significantly reduced compared to WT mice. Moreover, the incidence and severity of oviduct pathology in Foxj1Cre-Cx43flox mice was significantly reduced compared to WT animals, whereas uterine horn pathology was comparable on day 80 after intravaginal chlamydial infection. Furthermore, HeLa cells engineered to express Cx43, not those without, efficaciously transferred $Ova_{257-264}$ peptide to co-cultured mouse APC, which subsequently activated CD8$^+$ T cells derived from Ova primed OT-1 mice. These results suggest that efficient transfer of microbial peptides at mucosal surfaces from infected to uninfected cells via Cx43 GMAT plays an important role in activation of pathogen-specific CD8$^+$ T cells that induce immunopathology.

OR.26. The Molecular Basis of Acute Cystitis; IL-1ß and Inflammasome Dysregulation
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Despite its prevalence and deleterious effects, acute cystitis has remained a molecular enigma. We now define acute cystitis as a hyper-inflammatory condition of the urinary bladder, driven by IL-1b over-activation in inflammasome-deficient hosts. IL-1b was selected for study as >85% of acute cystitis isolates triggered an IL-1b response in human bladder epithelial cells. IL-1b was identified as an essential innate immune response arbitrator, as IL-1b$^{-/-}$ mice were unresponsive to infection, in contrast to Asc$^{-/-}$ and Nlrp3$^{-/-}$ mice, where inflammasome dysregulation caused a dramatic acute cystitis phenotype, with hyper-activation of IL-1b and IL-1b-dependent gene expression. The IL-1b dependence of acute cystitis was confirmed by IL-1b receptor antagonist therapy, which prevented acute cystitis in susceptible mice. In the absence of a functional inflammasome, IL-1b was processed via an alternative mechanism, involving the Mmp7 metalloproteinase, which was strongly upregulated in pathological bladder epithelium. MMP-7 was shown to cleave IL-1b *in vitro* and an MMP inhibitor attenuated disease in Asc$^{-/-}$ mice. Clinical relevance was demonstrated by increased urine IL-1b and Mmp7 levels in patients with acute cystitis, compared to patients with asymptomatic bacteriuria or healthy controls. These results provide, for the first time, a comprehensive molecular framework for the pathogenesis of acute cystitis, raising the possibility that IL-1b immunotherapy might be used to prevent pathology in patients prone to acute cystitis. As effects of inflammasome dysregulation were bladder specific, effects on acute pyelonephritis susceptibility would not be expected to occur.

OR.27. *Ex vivo* Analysis of Herpes Simplex Virus Type 2 (HSV-2) Specific T Cells in the Human Female Reproductive Tract

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Local mucosal cellular immunity is critical in providing protection to HSV-2 and vaccine strategies that target protective T cell responses to the genital mucosa are urgently needed. In order to characterize and quantitate HSV-2 reactive mucosal T cells, lymphocytes were isolated from endocervical cytobrushes and biopsies from 10 HSV-2 infected women and examined *ex vivo* for the expression of markers associated with tissue residency as well as functional memory T cell responses to HSV-2. Cervical biopsies yielded higher total numbers of $CD3^+$ lymphocytes compared to cytobrushes, total CD3+ lymphocytes from biopsies and cytobrushes were comprised predominantly of $CD4^+$ T cells and in contrast to their circulating counterparts, cervix-derived $CD4^+$ and $CD8^+$ T cells expressed the tissue-specific markers CD69 and CD103. Cervix-derived T cells were analyzed *ex vivo* for HSV-2 reactivity: 9 of the 11 cervical samples yielded sufficient cell numbers for analysis and of those, 8 contained HSV-2 specific $CD4^+$/IFN-γ^+ T cells (median 6.79%). In contrast, only 5 of the cervical samples yielded sufficient numbers of $CD8^+$ T cells for analysis and of those, only one contained HSV-2 specific $CD8^+$/IFN-γ^+ cells (1.08%). Cervix-derived HSV-2 specific $CD4^+$ T cells also expressed IL-2 and CD103 and were enriched in the cervix compared to the blood. The study of these mucosal T cells will be central to the elucidation of immune correlates of protection to HSV-2 and to the design and development of effective immune strategies preventing HSV-2 acquisition and reactivation.

T28. An *ex vivo* System of Human Cervical Tissue to Study the Immunoregulatory Effect of Seminal Plasma on the Female Genital Mucosa

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Background. Semen deposition on the female genital mucosa (FGM) induces a local inflammatory response. Although this phenomenon has long been reported, its underlying mechanisms and implications for susceptibility to infections remain largely unknown due to the lack of comprehensive experimental systems. To this purpose, we developed and validated an *ex vivo* model of coitus. Methods. Polarized explants of human ectocervical mucosa were exposed to seminal plasma (SP) diluted 1:1 or 1:3, or culture medium, for 2, 4 or 12 hours. In situ apoptosis was evaluated using a TUNEL assay. Protein and gene expression of pro-inflammatory/growth factors were measured in culture supernatant and tissue respectively. Attraction of peripheral blood leukocyte by supernatants was assessed with a transwell migration assay. Explants exposed to SP were infected with $HIV\text{-}1_{BaL}$ and viral replication was measured as $p24_{gag}$ antigen release in supernatant. Results. In comparison to explants incubated with culture medium, exposure to SP resulted in: equal fraction of apoptotic cells; increased expression of IL-1α, IL-6, TNF-α, CXCL1, CXCL8, CCL20, CSF2, IL-7, and cyclooxygenase 2; higher number of migrating leukocytes, mostly neutrophils and monocytes. Treatment of explants with the cyclooxygenase inhibitor indomethacin did not affect SP-induced changes in protein and gene expression. Finally, explants exposed to SP supported productive HIV-1 replication. Conclusion**s**. Exposure of human cervical explants to SP recapitulates the main features of the response occurring in the FGM upon coitus. Our system will be implemented to further characterize this response and understand its genesis, and potential contribution to HIV-1 transmission to the FGM.

T29. Properties and Mechanisms of the Innate and Adaptive Mucus Barrier Against Pathogens

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Viruses and intracellular obligate bacteria must penetrate across mucus to establish infections; thus, mucus represents the body's first line of defense. Nevertheless, the precise molecular and biophysical mechanisms by which mucus can block the translocation of pathogens in mucus remains not well understood. I will present our recent discovery that antibodies possess a glycan-dependent "muco-trapping" effector function that provides an exceptionally potent yet largely unrecognized mechanism of immune protection at mucosal surfaces of the female reproductive, gastrointestinal and respiratory tracts. Specifically, mucosal antibodies can help immobilize both viral and motile bacterial pathogens through interactions between N-glycans on Fc domain and mucins at sub-neutralizing antibody levels, and trapping viruses in mucus alone is sufficient to block viral transmission *in vivo*. I will present unpublished findings on the precise influence of sugar patterns on IgG-Fc, as well as antibody-mucin interactions across the menstrual cycle and in the presence of different commensal microbial communities. I will also present unpublished work on how

specific strains of commensal Lactobacilli can critically compromise the innate barrier properties of mucins against HIV and other enveloped viruses. Together, these results contribute to an emerging understanding of the complex interplay between the host immune system, pathogen and commensals.

T30. Immunoglobulin-Associated Glycans: Functions in Mucosal Defenses and Immunopathology
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Glycans of immunoglobulins of all isotypes play an essential role in the immunoglobulin Fc-mediated effector functions. These include the half-life in the circulation, activation of the complement cascade by the lectin pathway, inhibition of adherence of bacteria to epithelial cells, and reactivity with receptors expressed on epithelial cells, NK cells, monocytes, and macrophages. Altered structures of glycan moieties of IgG have been reported in a number of human autoimmune and infectious diseases (e.g., rheumatoid arthritis, periodontitis, HIV and Mycobacterium tuberculosis infections). Deficient galactosylation of the hinge-region O-glycans of human IgA1 is involved in the most common glomerulonephritis, IgA nephropathy. As the result of disbalance of specific glycosyltransferases, some O-glycans became deficient in galactose. When the exposed terminal N-acetylgalactosamine residues become recognized by naturally occurring antibodies, immune complexes are formed in the circulation. Some of these large immune complexes with nephritogenic properties deposit in the glomerular mesangium and initiate the pathological changes. In the absence of causal therapy, approaches that prevent the formation of large complexes are currently explored. In summary, structural and functional studies of immunoglobulin-associated glycan deserve due appreciation as important participants in mucosal defense mechanisms, inhibitors of several immunologic processes, and, conversely, mediators of pathogenic processes.

INFLAMMATORY BOWEL DISEASE: CLINICAL

T112. Alterations of Intestinal Microbiota in Ulcerative Colitis Patients Treated with Sequential Antibiotic Combination and Faecal Microbiota Transplantation
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Background: Fecal microbiota transplantation (FMT) is emerging as a new therapeutic approach to restore normal function in the intestinal microbiota. In this study, we demonstrate alternations of intestinal microbiota and immune response in ulcerative colitis (UC) patients treated with a sequential therapy involving FMT following a combination of antibiotics. Methods: An antibiotic combination therapy with oral amoxicillin 1500mg/day, fosfomycin 3000mg/day and metronidazole 750mg/day was administered to UC patients for two weeks prior to FMT. Faecal microbiota of the donors and the patients after or before treatment (8 samples from each group, total 32 samples) were processed by sequencing and analysis of the 16S rRNA gene using a Next-generation sequencer MIseq (Illmina). Cytokine and chemokine in their blood samples were analyzed by Multiplex Immunoassays (Bioplex). Results: After a two-week-antibiotics therapy, the proportion of phylum Bacteroidetes significantly decreased ($P<0.01$), while the proportion of phylum Proteobacteria significantly increased ($P<0.001$). In half of the post-FMT patients, the proportion of phylum Bacteroidetes increased up to the level of donor. Further, along with the recovery of the Bacteroidetes strains after FMT, a trend toward an improvement in patients' clinical symptoms score was noted. Conclusion: To our knowledge, this is the first clinical study of a sequential therapy involving FMT following a combination of antibiotics. We hope that the strategy we have applied may serve as the basis for further progress in understanding how alterations of the intestinal microbiota and immune response may become an effective therapeutic strategy for UC patients.

T113. Hierarchical Inflammasomes Activation in the Inflammatory Bowel Diseases

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Background and Aims: Inappropriate innate immune responses to invading bacteria and/or tissue damage contribute to disease development in IBD. The inflammasome complex plays a key role in regulating the host's immune response by providing a platform for the activation of caspase-1 and the maturation of IL-1β and IL-18. It is hypothesized that inflammasome activation is a key initiating event in the pathogenesis of IBD and hence this study aims to identify the pathways which lead to specific inflammasome complex activation and development of IBD. Methods: Paired active and quiescent mucosal biopsies were obtained from UC and Crohn's disease patients at a private hospital in Tasmania, Australia. Inflammasome expression profiles were established relative to healthy controls using qRT-PCR, targeted RNA-sequencing and inflammasome localization was determined using immunohistochemistry. Results: Evidence suggests a hierarchical inflammasome expression profile exists for both UC and CD and differences in the cellular localization of inflammasome proteins, especially NLRP 3 and 6. Furthermore, RNA sequencing has identified SNPs within targeted inflammasome genes which are specific to this Tasmanian cohort. Significance: Determining inflammasome activators and downstream pathways will direct possible treatment options and establish the therapeutic benefits of targeting specific inflammasomes in the inflammatory bowel diseases.

T114. Heterogeneity in the IL-13 Receptor Pathway and Activity in Active Ulcerative Colitis

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Introduction: **I**L-13 has been shown to be elevated in ulcerative colitis (UC) and be pathogenic in animal models of UC. However trials of anti-IL-13 antibodies or agents blocking IL-13 have shown efficacy only in subsets of patients. We studied UC patients with active disease for heterogeneity in IL-13 activity and receptor expression to identify subsets of patients who could be more responsive to anti-IL-13 strategies. Methods: Whole endoscopic colon biopsies and isolated epithelial cells and LPMCs from active(n=8) and remission(n=10) UC patients were studied. Epithelial cell SOCS-1 expression following *in vitro* IL-13 exposure and RT-PCR and RNASeq on RNA from un-stimulated cells were performed. Results: Active UC patients versus remission were significantly different by CRP (40.8mg/dL±17.6v1.8±0.6,p<0.03), Hgb (12.2g/dL±0.9v14.1±0.4,p<0.05), and Alb (3.4g/dL±0.3v4.2±0.1,p=0.02). Active UC patients also expressed significantly more ALOX12 and IL13RA2, but had similar *in vitro* IL-13-induced epithelial cell SOCS1 and basal IL13RA1, tissue claudin-2 and eoxtaxin-3 expression. In both active and remission patients IL-13-induced SOCS1 had significant inverse correlation with epithelial IL13RA1 expression (r^2=0.63) and did not correlate with IL13RA2 expression. When active UC patients were stratified by presence or absence of IL13RA2 expression, those patients positive for IL13RA2 had significantly more eotaxin-3 gene expression (11.3-fold±6.1v3.9±0.9,p<0.02). This was not seen in remission UC patients. Conclusions: These data suggest that there are identifiable subsets of active UC patients that differ in local effects of IL-13. The active UC patients with IL13RA2 and eotaxin-3 expression (both IL-13-onduced) may represent an IL-13-skewed inflammation that is more responsive to targeted anti-IL-13 agents.

T115. Alterations of Fecal Microbiota and Metabolite Landscape After Oral or Intravenous Iron Replacement Therapy in Patients with Inflammatory Bowel Diseases

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Iron deficiency is a common complication in patients with inflammatory bowel diseases (IBD) and oral iron therapy is suggested to change the gut microbial ecosystem and to exacerbate IBD symptoms. We performed an open-labelled clinical trial to compare the effects of oral (PO) vs. intravenous (IV) iron replacement therapy (IRT) in patients with Crohn's disease (CD; N = 31), ulcerative colitis (UC; N = 22) and iron deficient controls (NI = 19). After 3 months, changes in disease activity were independent of iron sulphate (PO) or iron sucrose (IV) treatments. However, high-throughput 16S rRNA gene sequencing of fecal samples identified marked inter-individual differences, lower phylotype richness and proportions of Clostridiales in IBD patients. Major shifts in bacterial diversity occurred in approximately half of all participants after IRT, but CD patients were most susceptible. High-resolution mass spectrometry showed clear separations of both UC and CD from anemic controls. IV- and PO-specific fingerprints

were evident at the level of metabolomes, with changes affecting cholesterol-derived host substrates. In conclusion, bacterial diversity and composition associated with iron therapy are altered in IBD participants. Oral iron administration affects bacterial phylotypes and fecal metabolites compared to IV therapy independent of the clinical outcome.

T116. Specific Serum IgG Subclass Antibodies Against Food and Microbial Antigens in Inflammatory Bowel Diseases Patients
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Background: Inflammatory bowel disease (IBD), particularly Crohn's disease (CD), is associated with increased microbial-specific IgG and IgA antibodies, whereas alterations of anti-food antibodies are still disputed. The knowledge about IgG subclass antibodies in IBD is limited. Methods: Serum IgG1, IgG2, IgG3 and IgG4 specific for nutritional and microfloral antigens (wheat and milk extracts; purified ovalbumin; Escherichia coli and Bacteroides fragilis lysates; mannan from Saccharomyces cerevisiae) were analyzed by ELISA in patients with CD (n = 56), ulcerative colitis (UC; n = 29), acute gastroenteritis/colitis (n = 12) as well as non-inflammatory controls (n = 62). Results: Anti-Saccharomyces cerevisiae antibodies (ASCA) of all IgG subclasses and anti-B. fragilis IgG1 levels were increased in CD patients compared to UC patients and controls. The discriminant validity of ASCA IgG2 and IgG4 was comparable to that of ASCA pan-IgG and IgA, whereas it was inferior for ASCA IgG1/IgG3 and anti-B. fragilis IgG1. Stricturing/penetrating disease behavior in CD patients was significantly associated with increased ASCA IgG1/IgG3/IgG4, anti-B. fragilis IgG1 and anti-E. coli IgG1 levels. Conversely, anti-TNF-α treatment in CD patients was associated with reduced ASCA IgG1/IgG2/IgG4, anti-B. fragilis IgG1 and anti-E. coliIgG1. Several anti-food IgG subclass concentrations were decreased in IBD patients with arthropathy compared to those without joint involvement. Anti-food IgG subclass levels did not correlate with food intolerance. Conclusion: Several microbial-specific but not food-specific IgG subclasses are increased in CD patients. The relevance of decreased anti-food IgG subclass levels in IBD patients with arthropathy remains unclear and requires further investigation.

T127. Fecal Micro-RNA Screening in Crohn's Disease Patients
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Background: micro-RNA (miRNA) are small non-coding RNAs that regulate numerous intracellular functions. Owing to relatively long persistence in extracellular environments and tightly regulated expressions, miRNA are good clinical biomarkers. In this study, we measured the expression of 800 different human miRNA in stool samples from controls and Crohn's disease patients. Material/Methods: Stool samples were obtained from 6 active Crohn's disease patients with ileal involvement and 6 healthy age- and sex-matched controls. Stool samples were weighted, diluted in distilled water and homogenized in Trizol®, and total RNA was extracted by the phenol/chloroform method followed by precipitation and purification with phenol-free columns. A_{260}/A_{280} and A_{260}/A_{230} RNA purity ratio were 2,02(± 0,05) and 1,86(±0,21), respectively. miRNA screening was performed using the human miRNA Expression Assay kit (Nanostring) following the recommended protocol. Results: Of the 800 measured miRNA, the analysis was restricted to 95 miRNA with levels higher than the background (4 times higher in all samples or 8 times higher in any sample). Cluster analyses indicate the existence of distinct groups of fecal miRNA depending on their abundance in patients as compared to controls (more, less, or equally abundant). Patients with the most severe diseases displayed distinct miRNA profiles, and the most highly detectable fecal miRNA in Crohn's disease patients vs. controls was miR-223. Conclusions: To our knowledge, this is the first fecal miRNA screen performed in IBD. Further investigation will aim at confirming these findings in a bigger cohort, and at understanding the biological function and cellular sources of the detected miRNA.

INFLAMMATORY BOWEL DISEASE: BASIC

PS.1. Integrin αvβ8-Mediated TGFβ Activation by Effector Regulatory T Cells is Essential for Suppression of T Cell-Mediated Intestinal Inflammation

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Foxp3$^+$ regulatory T cells (Tregs) play a pivotal role in suppressing self-harmful T cell responses to prevent inflammatory bowel disease. An important cytokine in the development and function of Tregs is TGFβ, a latent cytokine that must be activated to function. Evidence suggests that T cells need to respond to TGFβ in order for Treg-mediated suppression to occur in the intestine. Yet, how TGFβ is regulated to promote this suppression is poorly understood. We now show that both mouse and human Tregs have high expression of the TGFβ-activating integrin αvβ8, which enables these cells to activate high levels of latent TGFβ. However, deletion of integrin αvβ8 expression from Tregs resulted in no overt inflammatory phenotype at rest, indicating that the integrin plays little role in controlling immunity during homeostasis. Strikingly, we find that integrin αvβ8 expression and function is highly upregulated on an activated/effector Treg subset marked by KLRG1, and that Tregs lacking expression of integrin αvβ8 were unable to function as suppressive cells during models of colitis *in vivo*. Thus, we have uncovered an essential mechanism by which effector Tregs suppress T cells during intestinal inflammation, highlighting a key role for Treg-mediated activation of latent-TGF-beta in suppression of self-harmful T cell responses. Our work therefore highlights a novel pathway that could be therapeutically targeted to treat inflammatory bowel disease.

OR.49. The DNA Methylation Machinery Regulates Treg Homeostasis in the Intestine

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Colonization of newborn mice with gut microbiota induces vigorous proliferation of colonic regulatory T (Treg) cells, which critically contribute to establish intestinal symbiosis by suppressing inflammatory response to the microbiota. However, the molecular machinery controlling colonic Treg homeostasis remains largely unknown. Here we report that a DNA methylation adaptor Uhrf1/Np95 is essential for Treg expansion particularly during early life. Microbial colonization upregulated Uhrf1 in colonic Treg but not conventional T cells. Mice with T cell-specific deletion of Uhrf1 ($Cd4^{cre}Uhrf1^{fl/fl}$) mice showed a defect in proliferation and functional maturation of colonic Treg cells. As a consequence, $Cd4^{cre}Uhrf1^{fl/fl}$ mice spontaneously developed severe colitis. Such pathological changes are attributed to the impaired regulation of T cell-intrinsic DNA methylation, because $Cd4^{cre}Dnmt1^{fl/fl}$ mice also displayed the similar phenotype. DNA methylome analysis revealed that Uhrf1 deficiency de-repressed the cyclin-dependent kinase inhibitor Cdkn1a due to hypomethylation of its promoter region, leading to cell-cycle arrest of Treg cells. The knockdown or genetic deletion of Cdkn1a in Uhrf1-deficient Treg cells at least partially rescued the cell cycle arrest. Thus, DNA methylation-dependent epigenetic silencing of Cdkn1a is required for the maintenance of Treg homeostasis in the colon. Collectively, our findings demonstrated that the Uhrf1-Dnmt1 axis activated by microbial colonization is essential for the maintenance of gut immunological homeostasis.

OR.50. Foxp3$^+$ T Cells Expressing RORγt Represent a Stable Regulatory T Cell Effector Lineage with Enhanced Suppressive Capacity During Intestinal Inflammation

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Foxp3-expressing regulatory T cells (Treg) are essential for immunological tolerance, best illustrated by uncontrolled effector T cell responses and clinical presentation of systemic autoimmunity in mice and humans upon loss of Foxp3 expression. The vast majority of Foxp3$^+$ Treg is already generated within the thymus, however, Treg can adopt specific effector phenotypes upon activation in the periphery, reflecting the diversity of functional demands in the different tissues of the body. Here, we report that Foxp3$^+$CD4$^+$ T

cells coexpressing RORγt, the master transcription factor for inflammatory Th17 cells, represent a stable effector Treg lineage that is specifically enriched in the gut of mice possessing a complex microbial microflora. Transcriptomic and epigenetic profiling revealed that $Foxp3^+RORγt^+$ T cells display signatures of both Treg and Th17 cells, albeit the degree of similarity was higher to $Foxp3^+RORγt^-$ Treg than to $Foxp3^-RORγt^+$ T cells. Importantly, $Foxp3^+RORγt^+$ T cells were significantly demethylated at Treg-specific epigenetic signature genes such as Foxp3, Ctla-4, Gitr, Eos and Helios, suggesting that these cells have a stable regulatory, rather than inflammatory function. Indeed, adoptive transfer of $Foxp3^+RORγt^+$ T cells in the T cell transfer colitis model confirmed their Treg function and lineage stability *in vivo*, and in addition revealed a significantly enhanced suppressive capacity as compared to $Foxp3^+RORγt^-$ Treg. Thus, our data suggest that RORγt expression in Treg contributes to an optimal suppressive capacity during gut-specific immune responses, which renders $Foxp3^+RORγt^+$ T cells an important effector Treg subset in the intestinal system.

OR.51. Mitochondrial Dysfunction in the Epithelium Activates Paracrine WNT-Related Signaling and Hyperproliferation of the Intestinal Stem Cell Compartment

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Mitochondrial dysfunction contributes to metabolic and inflammatory pathologies. Heat shock protein 60 (HSP60), a mitochondrial unfolded protein response (mtUPR)-associated chaperone, is highly expressed in the intestinal epithelium under conditions of chronic inflammation. This study uses tissue-specific HSP60 knockout mouse models to investigate the impact of mitochondrial function on epithelial cell homeostasis. Postnatal deletion of Hsp60 in the epithelium ($Hsp60^{flox/flox}$XVillinCreER^{T2}) activated hallmarks of MT-UPR, including the induction of Chop, mitochondrial protease ClpP and chaperone Grp75. Consistent with decreased expression levels of mitochondrial cytochrome c oxidase subunit I (mtCoxI) in the epithelium of $Hsp60^{\Delta IEC}$ mice, mitochondrial respiration and ATP production were diminished in small intestinal crypt organoids upon tamoxifen-induced deletion of Hsp60. Mitochondrial dysfunction and MT-UPR induction in $Hsp60^{\Delta IEC}$ mice was associated with a significant loss of epithelial stemness and proliferative capacity in the transit amplifying zone. In parallel, HSP60-deficient epithelial cells released WNT10a, WNT2b and RSPO1 and induced hyperproliferative responses in stem cell compartments with sporadic failure of Cre-mediated Hsp60 deletion. This stem cell hyperproliferation led to a massive alteration in epithelial morphology. In conclusion, tissue-specific deletion of Hsp60 triggers mitochondrial dysfunction and loss of stemness. In response to the disruption of epithelial cell homeostasis, paracrine secretion of WNT-related signals activate hyperproliferation of residual stem cells that have escaped Hsp60 deletion.

OR.52. Restoration of Suppressive Activity of Regulatory T Cells from Crohn's Disease Patients Following *ex vivo* Expansion with Rapamycin: One Step Closer to Novel Cell Based Therapy?

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Cell-based therapy with $CD4^+CD25^{hi}CD127^{lo}FOXP3^+$ regulatory T cells (Tregs) is conceptually attractive to treat chronic mucosal inflammation in Crohn's disease (CD). We recently showed that Tregs expanded (with rapamycin) from FACS-sorted CD PB $CD4^+CD25^{hi}CD127^{lo}CD45RA^+$ precursors, yield an epigenetically stable $FOXP3^+$ cell population that is resistant to pro-inflammatory cytokine expression and homes to human gut in a murine human xenograft model. However, $CD4^+CD25^{lo\text{-}int}$ conventional T cells (Tcons) from inflamed CD mucosa are resistant to Treg-mediated suppression *in vitro*. To determine if culture with rapamycin enhanced the suppressive ability of $CD45RA^+$ Tregs from CD PB, Tcon suppression by expanded and freshly-isolated Tregs from the same donor was compared. Expansion significantly improved the suppressive ability of $CD45RA^+$ Tregs (97.5%±1.5% vs. 55.9%±10.8% at 4:1 Tcon:Treg ratio;<0.05,n=3). Next, LPMCs and MLN mononuclear cells (MLNMCs) were obtained from inflamed CD resection specimens. Expanded $CD45RA^+$ Tregs significantly suppressed activation of MLN and LP $CD3^+$ cells (CD154 expression at 7h), and significantly impaired MLN $CD3^+$ proliferation (CFSE dilution at 96h). These data show that *in vitro* expansion of CD PB $CD45RA^+$ Tregs enhances their suppressive ability and that these cells may modulate immune responses in niches relevant to the pathogenesis of CD, supporting their use in forthcoming clinical trials.

OR.69. Resident Viruses in the Gut Control Inflammation Through TLR3/7-Mediated cAMP Activation

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Metagenomic analysis has shown that resident viruses inhabit in the healthy gut; however, little is known about how resident viruses are involved in the maintenance of gut homeostasis. Here we have tried to clarify how resident viruses control gut homeostasis in mice with DSS-induced experimental colitis and patients with colitis. We analyzed inflammation in TLR3/7 double knockout mice and wild-type mice after treatment with agonists for TLR3 and TLR7 or inactivated rotavirus. We investigated the associations of human Tlr3 and Tlr7 variants and susceptibility to the colitis using direct sequencing and subsequent genotying of the two gene variants. Mice deficient in both TLR3 and TLR7, which lack the ability to recognize viral single- and double-stranded RNAs, were more susceptible to DSS-induced experimental colitis. Conversely, when WT mice were reconstituted with TLR3/7 agonists or inactivated rotavirus, colitis symptoms were significantly ameliorated. Furthermore, cAMP was accumulated after ligation of resident viruses to TLR3/7 and this accumulated cAMP regulated DSS-induced colitis by inhibiting NF-kB activation. Importantly, combined TLR3/7 genetic variations significantly influenced the severity of ulcerative colitis in humans. These results imply that the recognition of resident viruses by TLR3/7 might be required for gut protection and homeostasis.

OR.70. Loss of IL-10 Receptor Signaling in Patients with Infantile IBD Results in Aberrant Th17 Responses and Enhanced T Cell Proliferation

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Background: Loss of function mutations in the IL10 receptor (IL10R) genes cause severe infantile IBD. We have recently reported that innate immune IL10R signaling is a key regulator of intestinal immune response and anti-inflammatory macrophage generation in mice and humans. Other groups have demonstrated in mice that IL10R signaling is required for both normal suppressive function of T regulatory cells (Tregs) and for prevention of T effector cell-mediated colitis. The goal of the current study was to define whether IL10R signaling modulates T cells responses in humans. Results: Analysis of seven IL10R-deficient patients demonstrated similar frequencies of peripheral blood and colonic lamina propria $FoxP3^+$ Tregs, compared to control healthy subjects. Moreover, IL10R-deficient peripheral blood Tregs suppressed the proliferation of T naïves, comparable to control Tregs. The generation of Tregs from control and IL10R-deficient T naïve cells was also similar, suggesting that the generation and in-vitro function of Tregs is normal in IL10R-deficient patients. However, IL10R-deficient T naïves exhibited significantly higher proliferative capacity, and a marked increase in the generation of Th17 cells, compared to T naïves from control subjects. Moreover, IL10R-deficient patients had increased numbers of Th17 cells in the intestinal lamina propria. Conclusions: IL10R signaling regulates Th17 generation and T cell proliferation in humans, while it does not appear to regulate the generation of Tregs in blood and mucosal compartments. Furthermore, in-vitro Treg suppression is not dependent on IL10R signaling. Therapies targeting the Th17 axis might be beneficial for IL10R-deficient patients as a bridge to stem cell transplantation.

OR.71. Spontaneous Innate Immune Mediated Colitis

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TNFAIP3 is an anti-inflammatory enzyme inhibiting NF-κB through a negative feedback loop. In intestinal epithelial cells (IEC), TNFAIP3 prevents IEC apoptosis and increases barrier function. To assess the role of IEC expression of TNFAIP3 on intestinal innate immune function, we crossed RAG-$1^{-/-}$ mice and villin-TNFAIP3 (v-TNFAIP3) transgenic mice, which constitutively TNFAIP3 in IEC. Neither RAG-$1^{-/-}$ nor v-TNFAIP3 mice spontaneously develop colitis, however, all v-TNFAIP3 x RAG-$1^{-/-}$ mice exhibited early onset spontaneous colitis by 4 weeks of age. This colitis was prevented by antibiotic treatment, implicating microbes in the pathology of this model. Gene arrays and immunohistochemistry revealed that v-TNFAIP3 x RAG-$1^{-/-}$ mice had altered anti-microbial peptide (AMP) expression in the colon, including decreased Ang4 and increased Reg3b expression. RAG-$1^{-/-}$ mice maintained the largely

sterile inner-mucus layer of the gut but the inner mucus was infiltrated by microbes in v-TNFAIP3 x RAG-1$^{-/-}$ mice. Cytokine immunoarrays of colonic tissue from v-TNFAIP3 x RAG-1$^{-/-}$ mice showed an elevation of IL-1α, but not other cytokines typically elevated in other models of colitis. Thus, our model is that expression of TNFAIP3 in IEC leads to inhibition of NF-kB dependent AMP production, resulting in invasion of the inner mucus layer by a subset of microbes that drive IL-1a production leading to innate-immune mediated colitis. These findings may be particularly relevant to understanding the pathology of inflammatory bowel disease in immunocompromised individuals.

OR.72. A Critical Role for Cellular Inhibitor of Protein 2 (cIAP2) in Colitis-Associated Colorectal Cancer and Intestinal Homeostasis Mediated by the Inflammasome and Survival Pathways

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Cellular inhibitors of apoptosis proteins (cIAPs) are critical arbiters of cell death and key mediators of inflammation and innate immunity. cIAP2 is frequently overexpressed in colorectal cancer and in regenerating crypts of ulcerative colitis patients. However, its corresponding functions in intestinal homeostasis and underlying mechanisms in disease pathogenesis are poorly understood. We found that mice deficient in cIAP2 exhibited reduced colitis-associated colorectal cancer tumor burden but, surprisingly, enhanced susceptibility to acute and chronic colitis. The exacerbated colitis phenotype of cIAP2-deficient mice was mediated by increased cell death and impaired activation of the regenerative inflammasome-IL-18 pathway required for tissue repair following injury. Accordingly, administration of recombinant IL-18 or pharmacological inhibition of caspases or the kinase RIPK1 protected cIAP2-deficient mice from colitis and restored intestinal epithelial barrier architecture. Thus, cIAP2 orchestrates intestinal homeostasis by exerting a dual function in suppressing cell death and promoting intestinal epithelial cell proliferation and crypt regeneration.

W126. CD14 is a Modifier of IBD Development by Influencing the Intestinal Barrier Function

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Inflammatory bowel disease (IBD) is characterized by relapsing inflammation of the gut. The pathogenesis of disease still remains unknown. However, intestinal barrier disruption likely plays a key role in IBD development. In a mouse model based on Interleukin-10 (Il10) deficiency Cd14 was suggested as a genetic modifier of colitis susceptibility. Barrier function was analyzed in C57BL/6J.129S1-Cd14^{tm1Smg} (B6-Cd14$^{-/-}$) mice as well as in B6.129S1P2-Il10^{tm1Cgn}Cd14^{tm1Smg} (B6-Il10$^{-/-}$Cd14$^{-/-}$) mice, a model of chronic colitis. Intestinal permeability was investigated *in vivo* by intestinal FITC-dextran uptake and *in vitro* by qRT-PCR of Tight-Junction-Proteins (TJ) and immunohistological staining of Occludin, Ki67 and TUNEL apoptosis assay. Severity of intestinal inflammation was evaluated histologically and TNFα and IFNγ gene expressions were quantified by qRT-PCR. B6-Cd14$^{-/-}$ mice showed no differences in this experimental setup compared to wildtype controls. However, FITC-dextran uptake was increased and TJ expression was decreased in B6-Il10$^{-/-}$Cd14$^{-/-}$mice compared to Il10-deficient mice. Likewise immunohistology indicated a barrier disruption of the B6-Il10$^{-/-}$Cd14$^{-/-}$ mice. Histology and inflammatory cytokine expression revealed increased intestinal inflammation in B6-Il10$^{-/-}$Cd14$^{-/-}$mice. Cd14 deficiency seems to have no influence on epithelial tightness under steady state conditions but it presumably untightens the intestinal barrier under inflammatory conditions. In conclusion Cd14 influences IBD development by modulating the intestinal barrier function.

T31. Interleukin-36 Induces the Inflammatory Mediators from Human Colonic Subepithelial Myofibroblasts

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Background: Interleukin (IL) -36 is a new member of IL-1 family. It has been reported that IL-36 was associated with chronic inflammatory disorders, such as psoriasis and rheumatoid arthritis. There are few reports about the role of IL-36 in intestinal inflammation, including inflammatory bowel disease (IBD). In the present study, we investigated IL-36 expression in the inflamed mucosa of patients with IBD and characterized biological activities of IL-36 in human colonic subepithelial myofibroblasts (SEMFs). Methods: SEMFs were stimulated with IL-36 cytokines. The expression of mRNA and protein in the samples were analyzed by real-time PCR and ELISA, respectively. Western blots were performed to evaluate the signal transduction of IL-36. Results: IL-36γ

mRNA expression significantly increased in active UC samples as compared to in healthy and inactive UC samples. IL-36α and IL-36γ, but not IL-36β, significantly enhanced the secretion of inflammatory mediators from SEMFs. The inductions of these mediators by IL-36α and IL-36γ were mediated by mitogen activated protein kinases (MAPKs), leading to the activation of nucleic factor kappa-B (NFκB). Conclusions: IL-36α and IL-36γ induced inflammatory mediators in SEMFs through the activation of MAPKs and NFκB. We suggest that IL-36 plays an important role in intestinal inflammation and may contribute to the pathophysiology of IBD.

T32. Epithelial Type 2 TNF Receptor-Specific Signaling in the Development of Colitis-Associated Carcinogenesis
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Background & Aim: We previously reported up-regulation of type 2 receptor for TNF (TNFR2) in the inflamed colonic epithelia and further up-regulation in colitis-associated cancer (CAC). However, the role of TNFR2 expression in the setting of CAC has not been elucidated. We therefore analyzed TNFR2 signaling in the colonic epithelial cells. Methods & Results: TNFR2 up-regulation was observed in a murine colonic epithelial cell line, MOC1, when stimulated with recombinant (r) IFN-γ. Epithelial NFκB-induced expression of myosin light chain kinase (MLCK) was associated with disrupted tight junction (TJ) in a rTNF dose- dependent fashion. Such MLCK up-regulation and TJ disruption in MOC1 cells were abrogated by anti-TNF mAb (MP6-XT22), TNFR2-specific siRNA or even MLCK inhibitor (ML-7). Using an animal model of CAC involving azoxymethane and dextran sodium sulfate, the colonic lamina propria was found to have pro-tumorigenic cytokine production such as IL-1β, IL-6 and MIP-2 in association with epithelial NFκB activation, TNFR2 and MLCK up-regulations and TJ disruption. Treatment with either MP6-XT22 or ML-7 restored TJ, decreased pro-tumorigenic cytokine production and reduced CAC development. Conclusions: Epithelial TNFR2 signaling in the context of IBD may be involved in epithelial permeabilization and pro-tumorigenic cytokine production that promote CAC development.

T33. Efficacy of Phytotherapeutics in a Refined Chronic DSS-Induced Colitis Model
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The clinical forms of inflammatory bowel disease (IBD), ulcerative colitis and Crohn's disease, are chronic-relapsing inflammatory disorders of the gastrointestinal tract. The model of dextran sulfate sodium (DSS)-induced acute colitis is one of the most widely used models in IBD research. Although it reflects certain aspects of clinical symptoms, a caveat is the lack of chronicity and a chronic inflammatory response. We aimed to develop a refined chronic DSS model that reflects signs of chronic inflammation for evaluating the effects of phytopharmaceuticals based on Citrullus colocynthis and Salvia officinalis.
To monitor the induction and persistence of colitis, body weight, stool consistency, and colonic hemorrhage were assessed daily. To evaluate morphological changes and the extent of local inflammatory response in the colon, we determined a histological score based on tissue sections. Chronification of inflammatory processes was evaluated by analyzing pro-inflammatory cytokines. We propose a refined model of DSS-induced chronic colitis that reflects important features of IBD without excessive weight loss. Pathology is mainly characterized by diarrhea and blood in stool and can be significantly reduced by 6-thioguanine as an approved therapeutic for IBD. The therapeutic efficacy and mode of action of several phytopharmaceuticals is tested in the established model.

T34. High Fat Diet and the Colonic Mucus Barrier: Implications for Obesity and Inflammatory Bowel Disease
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The prevalence of obesity is increasing at an alarming rate worldwide. Prolonged high fat diets (HFDs) induce low-grade chronic intestinal inflammation in mice, and HFDs are a risk factor for the development of human inflammatory bowel diseases. We hypothesized that during HFD-induced obesity, endoplasmic reticulum (ER) and oxidative stress occurs in intestinal secretory cells, which triggers inflammatory signaling and reduces synthesis and secretion of proteins forming the protective mucus barrier. We comprehensively analyzed changes in mucus barrier components, ER/oxidative stress, and inflammation in mice fed HFDs for 3, 11 and 22 weeks. We also examined the effect of suppressing ER stress during a HFD with IL-22 (a potent suppressor of oxidative

stress) and whether HFDs exacerbated acute and chronic murine colitis. HFD modulated the differentiation and function of intestinal goblet cells via Kruppel-like Factor (KLF)-4 and down-regulation of Muc2 intestinal mucin. Long-term HFDs cause intestinal inflammation, ER stress and protein misfolding, which was resolved by IL-22 (2wk treatment). HFDs had no effect on the severity of acute DSS colitis, however, there was an increase in the rate of prolapse in chronic ER-stress-driven Winnie colitis. The effect of diet significantly alters mucosal immunity and influences gut barrier function.

T35. Induction of Chronic Gut Inflammation By Infection with *Salmonella enterica*

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Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a chronic-relapsing disorder of the gastro-intestinal tract. Apart from genetic predisposition and environmental factors, an inappropriate immune response to the commensal flora is discussed as a major driving factor. Chemically-induced models reflect certain aspects of the disease very well, but their potential to study pathophysiology of IBD is limited. Our aim was to establish a model of bacteria-induced chronic gut inflammation to investigate the role of commensal versus pathogenic bacteria in the development of IBD. C57BL/6 mice were infected with Salmonella enterica in combination with antibiotic treatment, resulting in a persistent infection and chronic inflammation in the gut. A clinical score was assessed and fecal bacterial load was evaluated systematically. Histological changes in colon tissue were assessed, and expression of inflammatory markers was analyzed in colon tissue sections and homogenates. We observed a chronic infection in mice represented by a significantly elevated clinical score and persistent bacterial load in fecal pellets. Signs of chronic inflammation were also evident in sections of the colon tissue. In summary, we propose a potential model of IBD that better reflects the natural etiology and the clinical features of the disease.

T36. Commensal House Dust Mite Allergen in Human Gut: A Contributor to Gut Inflammatory Disease in Humans?

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Introduction: Recently we have demonstrated that neonatal gut can be directly exposed to the respiratory allergen house-dust-mite (HDM) allergen (Dermatophagoides pteronyssinus, Der p1) through breast-milk. Currently, there is no evidence for presence of a respiratory allergen in the human gut or its possible impact on gut inflammation. Aim: To explore the direct effect of HDM on healthy gut mucosal function. Methods: Colonic biopsies (n=30) and duodenal fluid (n=28) were taken from healthy adults undergoing routine colonoscopy. Stool was obtained from healthy volunteers (n=10). Der p1 was measured in duodenal and stool samples by ELISA. Direct effect of HDM (0.1-100 ng/ml) was assessed on tissue resistance, *ex vivo* paracellular permeability, ZO-1 and occludin tight-junction protein expression by immunostaining, and cytokine production in cultured colon (TNF-alpha and IL-10). Permeability and cytokine production was examined in presence or absence of cysteine- (E-64, 10 mM), serine- (AEBSF, 1 mM) or non-selective (chymostatin, 10 mg/ml) protease-inhibitors. Results: Derp1 was present in duodenal and stool samples. HDM induced a dose-dependent increase in gut paracellular permeability, TNF-alpha and IL-10 production and a reduction in tissue resistance ($P<0.001$). This was associated with reduced expression of ZO-1 and occludin immunostaining in colonic sections ($P<0.01$). HDM-induced permeability and cytokine production was significantly abolished by chymostatin and E-64 ($P<0.001$) but not AEBSF. Conclusion: HDM is present in human gut mucosa. It directly impairs the gut barrier function. These effects are cysteine-protease dependent. These novel results warrant further studies to explore the potential contribution of HDM to pathophysiology of gut inflammatory diseases.

T37. The Importance of the Mucosal Antimicrobial Peptide Expression and Gut Microbiota in Anti-TNF Therapy Response in Ulcerative Colitis

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Anti-TNF therapy is a treatment for ulcerative colitis (UC) patients but only 50-70% responds to treatment. Our aim was to determine antimicrobial peptides (AMP) and microbiota profiles in UC patients before start of anti-TNF therapy and correlate this to therapy outcome evaluated after 14 weeks of therapy. Proteomic analysis of biopsies taken before therapy start, showed that therapy responders, but not non-responders, expressed Defensin 5 (Def5) and eosinophilic cationic protein (ECP). Def5, ECP and 9

other AMPs were analyzed by rtPCR in mucosal biopsies. Multivariate factor analysis discriminated responders and non-responders according to the expression of the 11 genes. The most important nominators for response were increased expression of Def5 (p=0.006) and ECP (p=0.03), whereas non-responders were defined by increased expression of cathelicidin (p=0.05). Responders also had higher serum levels of ECP than non-responders (p=0.03). Microbiota analysis (GA-map™ Dysbiosis Test) revealed higher dysbiosis indexes and lower levels of Faecalibacterium prausnitzii in non-responders compared to responders. In conclusion, anti-TNF therapy responders and non-responders display different patterns of mucosal AMP expression and gut microbiota before start of therapy. This indicates that anti-TNF treatment response might be predicted by antimicrobial peptides and the gut microbiota.

T38. CCR9 Regulates Colitis Development by Inhibiting Regulatory T Cell (Treg) Development

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T cells reactive to microbiota regulate the pathogenesis of inflammatory bowel disease (IBD). As T cells trafficking to intestines is regulated through interactions between highly specific chemokine-chemokine receptors, great efforts have been made to develop intestinal specific immunosuppression based on blocking these key processes. CCR9, a gut-trophic chemokine receptor expressed by lymphocytes and dendritic cells, has been implicated in regulation of IBD through mediating recruitment of T cells to inflamed sites. However, the role of CCR9 in inducing and sustaining inflammation in the context of IBD is poorly understood. In the current study, we demonstrate that CCR9 inhibits Treg cell development, which contributes to its regulation of intestinal inflammation. While $CCR9^{-/-}$ mice are more resistant to disease compared to wild type (WT) mice upon DSS insults, CCR9 deficiency does not affect effector T cell induction of colitis in a microbiota antigen specific T cell-mediated model. Interestingly, $CCR9^{-/-}$ mice demonstrate a high level of $Foxp3^{+}$ Tregs, and ligation of CCR9 by its ligand CCL25 inhibited Treg cell differentiation *in vitro*. Furthermore, partial depletion of Tregs in $CCR9^{-/-}$ mice increased the susceptibility to DSS insults to the level similar to WT mice. Collectively, our data indicates that CCR9 signaling inhibits Treg cell development, which contributes to its regulation of colitis development, in addition to acting as a gut-homing molecule.

T39. Methionine-Choline Deficient Diet Relieves Dextran Sodium Sulfate-Induced Colitis via Maintenance of Non Type Ⅰ NKT Cells

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Dietary choline is important for the prevention of tissue oxidative damage and ER stress. However, little is known about the role of choline in intestinal homeostasis. In the present study, we investigated the effect of choline deficiency in intestinal immunity in a mouse model of dextran sodium sulfate (DSS)-induced colitis. C57BL/6(B6) mice and Jα18 deficient (KO) mice were fed methionine-choline deficiency diet (MCD) or control food (CF) for 4 weeks before the induction of DSS colitis. Body weight change, colon length, and histological damage in the colon were examined. Characterization of hepatic $NK1.1^{+}$ T cells was assessed by fluorescence-activated cell sorting analysis (FACS) analysis. Oral administration of MCD facilitates the body weight loss and shortening of colon length associated with DSS-induced colitis on B6 mice. Histological analysis also revealed that the colonic inflammation was exacerbated by MCD. However, the colitis on KO mice was milder with MCD administration. FACS analysis revealed that NK 1.1^{+} T cells in the liver were significantly decreased in MCD-fed B6 mice. On the other hand, there are no significant differences in the number of $NK1.1^{+}$ T cells between CF and MCD-fed KO mice. Even 1-week feeding with MCD exaggerated colitis in B6, but not KO mice. These results suggest that choline deficiency may maintain non type Ⅰ NKT cells which improve colitis.

T40. Epithelial Notch-1: A Central Regulator of Intestinal Homeostasis

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Mounting evidence points to paramount importance of Notch-1 signaling in the maintenance of intestinal architecture and barrier function. We have recently shown that epithelial Notch-1 is indispensable in bridging innate and adaptive immunity in the gut and is required for supporting protective epithelial pro-inflammatory responses (Matthern, Laitman et al., Mucosal Immunology 2014).

Thus, we hypothesized that intestinal epithelial Notch-1 contributes to mucosal homeostasis and protects against tissue injury during colitis. We have generated colonocyte specific conditional Notch-1 heterozygote mice (CDX2-Notch-1$^{+/-}$), as complete deletion of Notch-1 in these cells caused embryonic lethality. We found that CDX2-Notch-1$^{+/-}$ mice exhibited spontaneous goblet cell hyperplasia and serrated lesions. This phenotype was accompanied with increased colonocyte proliferation and enhanced production of pro-inflammatory mediators in the colon. Moreover, we observed a nuclear redistribution of adherens junction proteins. In experimental models of DSS-induced acute/recovery and chronic colitis, CDX2-Notch-1$^{+/-}$ mice displayed severe chronic injuries and high susceptibility. Mechanistically, TNFα-stimulated Notch-1 knockdown Caco-2 cells proliferated faster than controls, confirming a defect in epithelial maturation and barrier function. Overall, these results emphasize the key role of Notch-1 signaling in protecting from intestinal inflammation, and exhibit potential for the development of therapeutics targeting Notch-1 signaling in intestinal disorders.

T41. Critical Role for Signal Transducer and Activator of Transcription 6 (STAT6) During Colitis Associated Colorectal Cancer
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Whereas a causal association between chronic inflammation and tumor development has been well established and supported in both, animal and epidemiological studies, the role of transcription factors, in particular those related with cytokine signaling, during colitis-associated colorectal cancer (CAC) development is almost unknown. Here we have analyzed the pathophysiologic implication of STAT6 signaling pathway on the establishment and progression of CAC. CAC was induced in both STAT6-KO and BALB/c (wild-type, WT) mice by injection of 12.5 mg/kg azoxymethane followed by 2% dextran sodium sulfate exposure to elicit colitis. On days 20, 40 and 68 after CAC induction, mice were sacrificed to evaluate colonic inflammation, proliferation and tumorigenesis. STAT6-KO mice did not show any bleeding or diarrhea throughout all the experiment, besides; more than 60% of these mice were free of tumor at day 68, whereas WT mice developed tumors as early as 20 days after CAC induction and by the end of the experiment 100% of these mice displayed tumors. Tumor numbers per mouse (0.8 versus 11.6), and bowel weight (0.3g versus 0.6g) were significantly decreased in STAT6-KO mice compared to WT mice. Both inflammation and proliferation scores in colon from STAT6-KO mice were significantly lower than WT mice. An intense apoptotic process early in CAC progression was recorded, as well as lower expression of COX2 and β-catenin in colonic areas of STAT6-KO mice, these data were associated with a strong mRNA expression of IFN-γ. Our data suggest a main role for STAT6 in favoring establishment and progression of CAC.

T42. Modulation of Intestinal Inflammatory Processes by Specific Knockdown of Cytokines with siRNA-Functionalized Calcium Phosphate Nanoparticles
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Inflammatory bowel disease is a group of chronic inflammatory conditions of the small and large intestine. The available therapies are only symptomatic and currently used drugs often lead to severe side effects or show no clinical improvements in some patients; therefore the development of new medications is reasonable. The interference of cytokine signaling mediated by siRNA might be a promising new therapeutic approach. In this project we analyze the potency of biodegradable calcium phosphate nanoparticles for the specific delivery of siRNA to the large intestine. We showed that the rectal application of these nanoparticles functionalized with siRNA directed against TNF-α, KC or IP-10 to mice suffering from DSS-induced colitis led to decreased expression with reduced protein levels of the target genes in colonic biopsies and mesenteric lymph nodes. These findings were accompanied by an amelioration of intestinal inflammation. In conclusion, these experiments imply that the specific and local modulation of the inflammatory response by a nanoparticle-based siRNA delivery system could be a promising approach for the treatment of intestinal inflammation.

T43. Differential Effects of Anti-TNF α Therapy on the Immune Cell Compartment in Crohn's Disease Patients
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Anti-TNFα antibody treatment has revolutionized the management of Crohn's disease (CD). Compared to treatment with corticosteroids or other commonly used anti-inflammatory agents in CD patients, the anti-TNFα agents are substantially more

potent in inducing mucosal healing. The reasons for this are unknown and, somewhat surprisingly, also the exact anti-inflammatory mechanisms of anti-TNFα therapy remain elusive. The aim of the present study was to investigate a potential change in the frequencies of immune cells in peripheral blood and the intestinal mucosa in CD as a result of three months anti-TNFα (adalimumab) treatment. All patients responded to the adalimumab therapy, as evaluated by the Harvey-Bradshaw Index, and other clinical markers after three months of treatment. The flow cytometry results showed significant decreases in the percentages of Th1, Th17, Th17/Th1 cells as well as $CD8^+$ $IFN\gamma^+$ T cells in both peripheral blood and the gut mucosa. In contrast, the frequency of $FoxP3^+$ regulatory T cells remained unchanged throughout the treatment period. We also observed an increase in epithelial γδ T cells frequencies as a result of the adalimumab treatment. Interestingly, macroscopically non-inflamed intestinal segments showed similar trends or changes in immune cell composition as inflamed segments. This study demonstrates that adalimumab treatment has considerable effects on the frequencies of pro-inflammatory T cell subsets, whereas regulatory T cell percentage is not significantly affected. Since the changes in immune cell composition correlated with the event of mucosal healing, it is possible that these changes are directly involved in the induction of wound healing.

T44. Gastric Control of the Intestinal Microbiome and IBD

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Gastrokine-1 (Gkn1) is a small, protease resistant protein that is expressed exclusively and abundantly in the stomach. It is secreted into the stomach mucus and is not found in the circulation. We have found that Gkn1 travels the length of the gastrointestinal tract intact, and binds to a subset of microbes within the gut lumen. $Gkn1^{-/-}$ mice display subtle changes in gene expression in colonic tissue, suggesting that Gkn1 impacts colonic physiology. $Gkn1^{-/-}$ mice have markedly reduced body fat and do not gain weight on a high fat diet, compared to $Gkn1^{+/-}$ littermates. The microbiome of $Gkn1^{-/-}$ mice is resistant to high fat diet-induced changes in the microbiome and maintains the typical ratio of Bacteroidetes:Firmicutes seen in $Gkn1^{+/-}$ or $Gkn1^{-/-}$ mice on a regular chow diet. $Gkn1^{-/-}$ mice are exquisitely sensitive to DSS-induced colitis. Gkn1 is anti-amyloidogenic and, consistent with this, we find that Gkn1 prevents bacterial biofilm formation *in vitro*. We hypothesize that the $Gkn1^{-/-}$ microbiome is resistant to diet induced modification due to the community stability provided by biofilm formation in the gut. Thus, a highly stable protein made in the stomach impacts the colonic microbiome and intestinal health. This suggests that eating Gkn1 could provide a benefit for patients with intestinal mucosal injury, or that eating anti-Gkn1 antibody could promote weight loss or prevent weight gain. These findings have implications for patients with iatrogenic lumenal discontinuity between the intestine and stomach.

T45. Expression and Distribution of Claudins in Crohn's Disease

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Aim: There is an impaired intestinal barrier function in patients with Crohn's disease. Recent reports suggest that this may be attributed to the altered expression of tight junction proteins. The aim of our current research is to investigate expression levels of several tight junction proteins from claudin family in colon tissue resections of patients with Crohn's disease. Patients and Methods: Colonic tissue specimens of patients with active Crohn's disease were used for the RT-qPCR and immunohistochemical analysis. Resection borders from tumor colectomy were used as controls. Total RNA was isolated from formalin-fixed paraffin-embedded tissue samples. RNA was reverse transcribed using random hexamers and SuperScript III First-Strand synthesis system. Gene expression was analyzed by RT-qPCR using the TaqMan method and glycerin-aldehyde-3-phosphate (GAPDH) as a housekeeping gene. Results were expressed as relative fold change using averaged control samples as calibrator. Expression and localization of claudins were also evaluated by immunohistochemical analysis. In addition, claudin expression was correlated with Th1 (e.g. IFNg and IL-12p40) and Th17 (e.g. IL-17) cytokine expression and clinical data. Results: Enhanced expression of Claudin-1 and -2 was observed in patients with Crohn's disease. On the other hand, expression of Claudin-4 and -8 was not statistically different between the two groups of patients. Conclusion: The results of this study point to difference in selective expression and localization of claudins in colonic tissue of patient's with Crohn's disease and controls, in relation to different expression of pro-inflammatory cytokines.

T46. Characterization of Flat Colonic Dysplasia in Mice with Spontaneous Colitis

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Patients with ulcerative colitis are at increased risk of developing colorectal cancer. Colitis-associated colorectal cancer (CACC) progresses through similar stepwise gene mutations observed in sporadic cancers, but the sequence in which they occur is reversed. Understanding the transition from colitis to tumor requires relevant animal models. Colonic carcinogenesis has been modelled using genotoxic carcinogens such as azoxymethane. We hypothesized that exacerbation of pre-existing chronic colitis in a Muc2 mutant (Winnie) mouse strain with dextran sulphate sodium (DSS) would induce colorectal tumourigenesis. Winnie mice received 1% w/v DSS orally and drinking water interchangeably, each for seven days for a total of 42 days. Colonic segments were collected for histological, immunohistochemical and gene expression evaluation. Winnies given DSS displayed flat, multifocal dysplasia of the mid-distal colon with 100% penetrance, and progression to adenocarcinoma in 17% of animals. Nuclear accumulation of β-catenin was absent in dysplasia. Preliminary analysis identified differentially expressed genes including Cox2 and neutrophil chemo-attractant Cxcl5. Exacerbation of colitis in Winnie resulted in exclusively flat colonic dysplasia after 42 days, without a genotoxic carcinogen. This experimental model will be useful for exploring the early changes in the colitis-dysplasia-carcinoma sequence in CACC.

T47. Innate Immune IL1β Production is Critical in Mediating Intestinal Inflammation in IL10 Receptor Deficiency in Mice and Humans

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Background: IL10 receptor (IL10R) mutations cause severe infantile IBD. We have recently reported that intact IL10R signaling is required for generation and function of anti-inflammatory macrophages (Mφ), and that adoptive transfer of WT $CD4^+$ T cells into $Il10rb^{-/-}Rag1^{-/-}$ mice leads to severe colitis. Our current objective was to determine the role of Mφ-induced IL1β in mediating the inflammatory process in IL10R deficiency in mice and humans. Results: LPS-treated bone-marrow derived Mφ from IL10R-deficient mice and monocyte-derived Mφ from four patients with loss of function mutations in IL10R genes produced significantly higher levels of IL1β and caspase-1, compared to Mφ from WT mice and healthy human controls, respectively. Moreover, pre-treatment of these Mφ with IL10 prior to LPS stimulation led to a decrease in IL1β production only in WT murine and human control Mφ, but not in IL10R-deficient murine or patient Mφ. A role for innate immune IL1β in mediating intestinal inflammation was also shown *in vivo*, since transfer of $Il1r^{-/-}$ $CD4^+$ T cells, but not WT $CD4^+$ T cells, into $Il10rb^{-/-}Rag1^{-/-}$ mice led to attenuated colitis. Finally, Anakinra (an IL1 receptor antagonist) treatment of a toddler with severe infantile IBD due to loss of function IL10RA mutation led to a marked clinical, laboratory and histological improvement enabling a significant decrease in steroid dose, prior to stem cell transplantation (SCT). Conclusion: Innate immune cell-derived IL1β is critical in mediating exaggerated pro-inflammatory responses in IL10R deficiency. The value of IL1 neutralization as a therapeutic bridge to SCT in patients with IL10R deficiency warrants further investigation.

T48. $Nlrp3^{-R258W}$ Mutation Protects Mice from Animal Models of Colitis and Colorectal Cancer

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Inflammatory bowel disease (IBD) has long been a prominent threat to human health. Moreover, uncontrolled progression of IBD can lead to increased risk for colorectal cancer. As an important gene in the innate immune system, NLRP3 has been linked with IBD and colorectal cancer, however the research results are controversial, which prompted us to study the role of NLRP3 in these diseases further. Using mice carrying the $Nlrp3^{-R258W}$ mutation which acquires an autoactivation of the NLRP3 inflammasome, we discovered that this mutation provided the mice with significant protection from DSS induced colitis. It was found that the $Nlrp3^{-R258W}$ mice exhibited dramatically less weight loss and mortality compared with wildtype controls. Our data also showed that the $Nlrp3^{-R258W}$ mice generated less pro-inflammatory cytokines/chemokines such as IL-6 and COX2, but produced more protective cytokine such as IL-18. More surprisingly, cohousing of $Nlrp3^{-R258W}$ mice with wildtype mice reduced their resistance to DSS colitis,

which indicated that microbiota played a key role during the regulation of IBD by the Nlrp3^{-R258W} mutation. In addition, from AOM/DSS induced colorectal cancer model, we found a similar protective effect in the Nlrp3^{-R258W} mice, which was also dependent on the microbiota from the mutation mice as evidenced by cohousing experiments. In summary, we have demonstrated that the Nlrp3^{-R258W} mutation led to a shift in the microbiota of the mice, which turned out to be a key causative factor to mediate the resistance to colitis and colorectal cancer.

T49. Tumor Suppressor Death-Associated Protein Kinase Regulates Intestinal Homeostasis and Colorectal Carcinogenesis upon Injury Through Inflammasome-Mediated IL-18 Maturation

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Death-associated protein kinase (DAP-kinase, DAPk, or DAPK) is a well-known tumor suppressor. Diminished DAPK expression is found in various types of cancer including B lymphoma and chronic lymphocytic leukemia. We observed that DAPK mRNA was significantly down-regulated in CRC tissues compare with adjacent normal tissues in 55 colorectal patients, suggesting a strong correlation between DAPK expression and the tumorigenesis of CRC. DAPK has also been found as a positive modulator in NLRP3 inflammasome signaling, which plays a critical role in the development of colitis and colitis-associated carcinogenesis (CAC). Therefore, we sought to assess the role of DAPK in inflammation-driven colon carcinogenesis of gastrointestinal inflammation. We found that DAPK-deficient mice showed increased susceptibility to acute and recurring DSS-induced colitis. DSS treatment in DAPK-deficient mice led to a significant reduction in body weight compared with DSS-treated wild-type mice. The colon length was significantly shorter in DAPK-deficient mice than that in control mice. Furthermore, mature IL-18 was significantly decreased in DSS-treated DAPK-deficient colon homogenates. In colonic epithelial cells, over-expressing DAPK increased the IL-18 maturation by triggering inflammasome activation, while knockdown DAPK decreased the IL-18 maturation, suggesting DAPK is required for inflammasome-mediated IL-18 production in colonic epithelial cells. Based on these results, we demonstrated that DAPK may regulate IL-18 maturation through the epithelial inflammasome. Upon injury, DAPK-deficiency resulted in defective regeneration of the colonic mucosa, leading to increased intestinal inflammation and accelerated colitis-associated tumorigenesis. This finding may provide insights into the development of new therapeutic targets and approaches to treat IBD and colitis-associated CRC.

T50. Impact of Interleukin-10 During Bacterial Induced Colitis

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Besides the fundamental role of IL-10 in intestinal homeostasis IL-10 is described as a key regulator of pathogen-specific immune responses. However, it is unclear whether intestinal infection interferes with IL-10-mediated homeostasis. In the present study we dissected the function of IL-10 in a mouse model of infectious colitis. BALB/c mice were infected with Citrobacter rodentium – an enteric pathogen that induces colitis with similarities to inflammatory bowel disease. Infected mice showed typical signs of bacterial-induced inflammation including increased colon weight-to-length ratios as well as higher histopathological scores with elevated crypt hyperplasia. Of note, IL-10 expression was upregulated in colonic tissue after infection, especially in $CD4^+$ T cells, macrophages, and dendritic cells with a peak 10-14 days post-infection. We first focused on the impact of $CD4^+$ T cell-derived IL-10 and infected CD4-Cre/IL10$^{fl/fl}$ mice with C. rodentium. Interestingly, mice deficient in $CD4^+$ T cell-derived IL-10 showed a faster clearance of the bacterial burden, but developed a more severe colitis with increased pathology and crypt hyperplasia compared to infected wild-type mice. Thus, we conclude that $CD4^+$ T cell-derived IL-10 is essential for the control of C. rodentium induced colitis. In future experiments we will examine the impact of macrophage- and dendritic cell-derived IL-10 in infectious colitis.

T51. Volcanic Ash Exacerbates Inflammation in a Mouse Model of Colitis

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Air pollution containing particulate matter and industrial pollutants has been related with Inflammatory Bowel Disease (IBD) early debut and hospitalization. Volcanic pollution has not been studied in IBD, even in highly exposed regions with high prevalence of IBD. We hypothesized that during the prolonged Puyehue eruptions (2011), volcanic ash (VA) exerted a pro-inflammatory effect promoting IBD refractoriness. We here studied the inflammatory effect of VA added to the drinking water in a colitis model. BALB/c

mice receiving drinking water with or without VA, (grouped w/ or w/o VA) were intrarectally administered with inflammatory stimuli (TNBS or flagellin) or ethanol (as control) at day 7. Weight was daily monitored, and at day 14 animals were sacrificed. Colon was removed and studied: weight, length, histology, ZO-1 distribution (confocal microscopy), cytokine and chemokine expression (qPCR) and, acetylcholine-induced colon contractility. Animals w/VA plus stimuli showed a greater weight loss, colonic weight/length ratio, DAI, HAI (with cellular infiltration, haemorrhage/vascularisation), TNF-α, Ccl20, T-bet, IFN-γ mRNA levels significantly increased (p<0,05), diminished ZO-1 (reflecting barrier impairment), and significantly-increased colon contractile activity. In conclusion, oral VA administration exerted a pro-inflammatory effect, exacerbating mice colitis. These findings could be related with the observed IBD worsening during volcanic particulate matter exposure.

T52. The Polysaccharides Derived from *Ganoderma lucidum* Fungus Mycelia (Designated as MAK) Ameliorate Indomethacin-Induced Small Intestinal Injuries through GM-CSF

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Introduction: Non-steroidal anti-inflammatory drugs often cause ulcers in the human small intestine, but few effective agents exist to treat such injury. "Ganoderma lucidum" Karst, also known as "Reishi", is a mushroom. We previously reported that a water-soluble extract from G.lucidumfungus mycelia (MAK) has anti-inflammatory effects in murine colitis. However, its effects on indomethacin-induced small intestinal injuries are unknown. The present study investigated the preventative effects of the polysaccharides derived from MAK on indomethacin-induced small intestinal injuries in mice. Methods: Peritoneal macrophages (PMs) were stimulated *in vitro* with MAK, and adoptively transferred to C57BL/6 mice intraperitoneally, which were then given indomethacin. Intestinal inflammation was evaluated after 24 hours. We performed *in vivo* antibody blockade to investigate the preventive role of GM-CSF, which derived from PMs stimulated with MAK. We then used PMs stimulated with MAK pre-treated by pectinase in an adoptive transfer assay to investigate the preventive role of polysaccharides. Results: Indomethacin-induced small intestinal injury was inhibited by adoptive transfer of PMs stimulated *in vitro* with MAK. In this transfer model, pre-treatment with anti-GM-CSF antibody reversed the improvement of small intestinal inflammation by indomethacin. PMs stimulated with pectinase-pretreated MAK impaired the anti-inflammatory effect of MAK. Conclusion: PMs stimulated by MAK appear to contribute to the anti-inflammatory response through GM-CSF in small intestinal injury induced by indomethacin. The polysaccharides from G.lucidum may elicit anti-inflammatory effect.

T53. Reduced *in vitro* Polarization of Salmonella-Specific IL-17 Secreting $CD4^+$ T (Th17) Cells by Salmonella-Infected Dendritic Cells Derived from Crohn's Disease Patients

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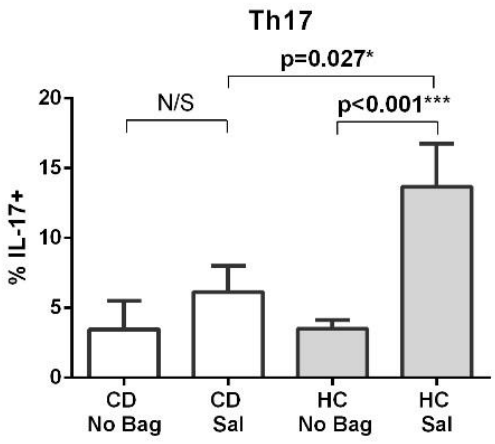

Introduction: Aberrant dendritic cell (DC) presentation of bacterial antigens may contribute to inflammatory bowel disease (IBD) pathogenesis. The balance between effector Th17 cells and $FoxP3^+$ regulatory T cells (Treg) is required the gut homeostasis and depends on DC cues. We reported the increased prevalence of circulating Treg and Th17 in Crohn's disease (CD) patients compared to healthy controls (HC), yet the role of DC-bacterial interactions are still unclear. Our aim was to assess T cell profiles responding to *in vitro* DC infection with salmonella in CD patients. Methods: Monocyte-derived dendritic cells (Mo-DC) were differentiated from peripheral blood of CD (n=13) and HC (n=16) using IL-4/GM-CSF and infected with salmonella Typhimuriumfor 24 hours. The infected Mo-DC were co-cultured with autologous naïve $CD4^+$ T cells for an additional 7-days and T cell polarization assessed using FACS. Results and conclusions: Mo-DC from CD patients were impaired in their ability to polarize $CD4^+$ T cells towards Th17 and Th1 following salmonella infection compared to that from HC. This suggests an impaired ability to generate robust antibacterial responses in CD patients. Impaired generation of salmonella-specific Th17 by infected DC does not contribute to the increased circulating pan-Th17 in CD patients.

T54. Differential Effects of Cigarette Smoke Extract upon *in vitro* Polarization of Salmonella-Specific IFN-gamma Secreting $CD4^+$ T (Th1) Cells by Dendritic Cells from Crohn's Disease Patients and Healthy Controls

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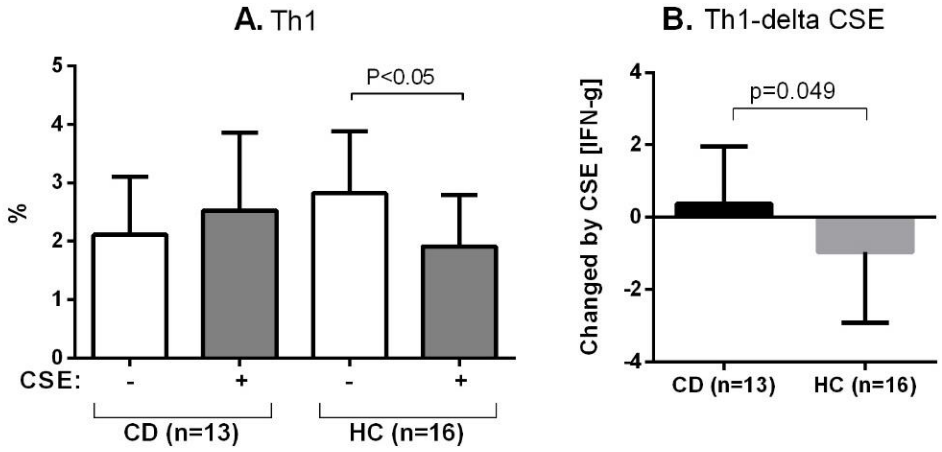

Introduction: Cigarette smoking worsens Crohn's disease (CD) but little is known regarding the mechanisms. Aberrant dendritic cell (DC) recognition and presentation of bacterial antigens may contribute to inflammatory bowel disease (IBD) pathogenesis. We reported that *in vitro* cigarette smoke extract (CSE) exposure of monocyte-derived DC (MoDC) prior to stimulation of autologous T cells resulted in Th1 skewing in CD samples, however, how this may impact bacterial-DC interactions and subsequent T cell polarization remains unclear. We hypothesize that CSE alters DC responses upon bacterial infection in CD patients compared to healthy control (HC). T cell profiles were used to assess DC responses to *in vitro* salmonella infection following CSE exposure. Methods: Mo-DC were differentiated from peripheral blood of CD (n=13) and HC (n=16) using IL-4/GM-CSF and exposed to freshly prepared CSE for 24 hours, followed by infection with salmonella Typhimurium for 24 hours. The infected Mo-DC were co-cultured with autologous T cells for an additional 7-days and T cell activation assessed using FACS. Results and conclusions: CSE exposure to Mo-DC significantly reduced salmonella-specific Th1 cell polarization in HC samples, but this was not seen in CD samples (Fig.A), leading to CSE-driven changes between the two cohorts were statistically significant (Fig.B). Therefore CSE differentially influences polarization of IFN-gamma secreting Th1 cells by DC in CD patients compared to HC.

T55. Preclinical Characterization of PTG-100, an Oral $\alpha_4\beta_7$ Integrin Peptide Antagonist for Ulcerative Colitis

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PTG-100 is a selective oral peptide antagonist of $\alpha_4\beta_7$ integrin with minimal systemic exposure, and is effective in blocking T cell homing and preventing mucosal damage in murine models of IBD. PTG-100 and the clinically validated anti-$\alpha_4\beta_7$ antibody vedolizumab have comparable potency and selectivity in a variety of assays including cell adhesion and binding to human $CD4^+$ memory T cells. PK studies in normal or dextran sodium sulfate (DSS) treated mice and rats show that oral dosing results in marked drug exposure in the small intestine, Peyer's Patches, colon, and mesenteric lymph nodes (MLN), but no significant measurable levels in the blood and urine. To measure the effect of oral dosing on trafficking of endogenous memory T cells, DSS mice were orally dosed daily with PTG-100 for 9 days, and harvested tissues were analyzed by FACS. FACS analysis showed a dose dependent reduction of $CD4^+$ $CD44^{high}$ $CD45RB^{low}$ β_7^+ T cells in the MLN and Peyer's Patches, and a concomitant increase in the spleen and blood. There was also a dose-dependent reduction in body weight loss and mucosal injury as assessed by endoscopy. These results support clinical advancement of PTG-100.

T56. Methanol Extract of *Juliania adstringens* Attenuates Dextran-Sodium-Sulfate Induced Colitis

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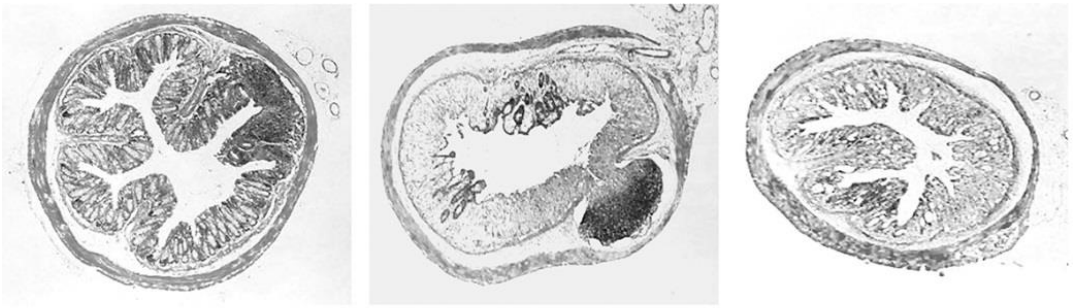

Inflammatory bowel disease (IBD) is a chronic condition of the intestine with unknown etiology involving multiple immune genetic and environmental factors. In the present study we were interested in examining the protective effect of Juliania adstringens Schltdl. methanolic extract (JAME), an Mexican folk medicinal plant, on inflammatory mediators in 3% dextran sulfate sodium (DSS)-induced ulcerative colitis (UC) in mice (BALB/c strain). Treatment with JAME at a dose of 200 mg/kg/day for 10 days improved colon shortening, body weight, the disease activity index (DAI), and histopathological score of DSS-induced colitis mice. The level of antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT), and nitric oxide (NO) as a marker of nitrosative stress and TNF-α, IL-1β and IFN-γ were measured in the colon homogenate. Treatment by DSS increased bowel SOD, CAT, NO, TNF-α, IL-1β and IFN-γ. All measured parameters were improved by Juliania adstringens treatment and reached close to normal levels. The present study further supports the role of oxidative/nitrosative stresses and pro-inflammatory cytokines (TNF-α, IL-1β and IFN-γ) in the pathogenesis of colitis and protective effects of this herb. Our results provide direct evidences that JAME have a therapeutic potential for alleviating inflammatory colitis in mice.

T57. TL1A Signaling Enhances the Differentiation of T_H9 Cells and T_H9-Driven Pathologies via STAT6 Signaling Pathways

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The TNF family member TL1A plays an important role in the development of inflammatory bowel diseases (IBD), experimental autoimmune encephalomyelitis, and allergic lung inflammation by modulating T_H1, T_H17, and T_H2 responses. TL1A polymorphisms have been identified through genome-wide association studies to confer susceptibility to IBD and have been associated with disease severity. IBD patients with TL1A risk haplotypes have elevated expression of TL1A in peripheral blood monocytes and transgenic mice overexpressing TL1A develop spontaneous small intestinal inflammation. However, the effects of TL1A on other T_H subsets remain unknown. Recently, T_H9 cells have been identified as an independent T_H cell subset and have been implicated in allergic lung inflammation, and IBD. In this study, we identified TL1A as a strong inducer of T_H9 cell differentiation *in vitro*. Mechanistically, TL1A enhanced STAT6 activation via NF-kB signaling pathway that lead to enhanced binding of the transcription factor $IRF4$ to the $Il9$ promoter. Utilizing an adoptive T cell transfer model of colitis we demonstrated that T_H9 cells differentiated *ex vivo* in the presence of TL1A are highly pro-inflammatory *in vivo* and lead to more severe intestinal and lung inflammation compared to T_H9 cells as characterized by increased histoscores, cell numbers in mesenteric lymph nodes and spleens, enhanced proliferation of transferred cells, and increased IL-9, IL-13, and IL-17 production. Using blocking anti-IL-9 antibodies attenuated TL1A-driven mucosal inflammation. Our results demonstrate that TL1A promotes T_H9 cell differentiation and function and define a role for IL-9 in TL1A-induced mucosal inflammation and potential therapeutic target in inflammatory diseases.

T58. Cumulative Risk Variant SNPs in ATG16L1 Locus are Associated with Reduced Ability of Salmonella Infected Dendritic Cells to Generate Autologous Bacterial-Specific Foxp3⁺ Regulatory T (Treg) Cells

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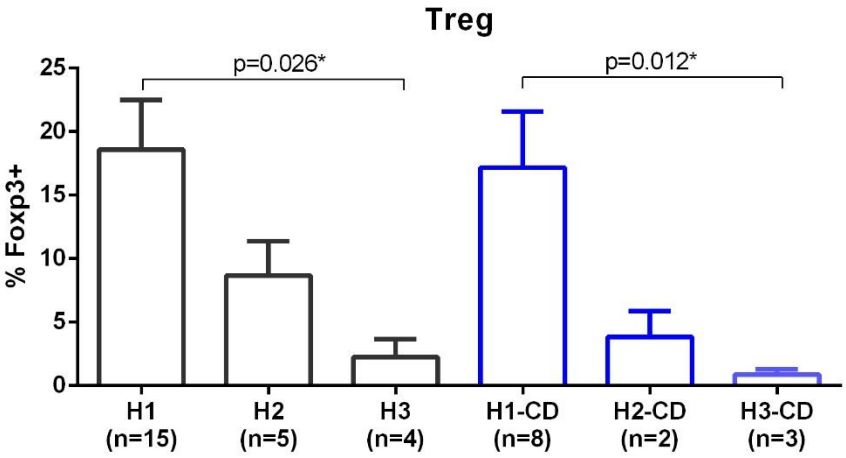

Atg16l1 SNPs may associate with Crohn's disease (CD) via impaired autophagy and bacterial clearance resulting in compromised bacterial antigen presentation by dendritic cells (DC). We hypothesize that immune-function modulation results from the cumulative effects of several SNP variants rather than by a single SNP of atg16l1. Our aim was to assess whether cumulative SNPs are associated with changes in the ability of DC to generate bacterial-specific T cell subsets upon *in vitro* bacterial infection. Genotypes of CD and healthy controls (HC) were analyzed using the Goldengate® assay. Individuals(13 CD and 11 HC) with homozygous in IL-23R(rs11209026,R381G) and wildtype in NOD2, PTPN22 and IRGM were enrolled in this study. Monocyte-derived DC were differentiated from peripheral blood of the study participants using IL-4/GM-CSF and infected with Salmonella Typhimurium for 24 hours. The infected DCs were co-cultured with autologous T cells for an additional 7-days and T cell polarization assessed using FACS. Participants were categorized into 3 groups based on genotype at atg16l1 locus: H1-all heterozygous, H2-homozygous in 2 SNPs(rs12994997 and s2241880,T300A) and wildtype in other 3 SNPs (rs13391356, rs2289472, and rs10192702), and H3-the reversed case of H2. No individuals with all homozygous nor all wildtype were found in this study. Among T cell subsets, Foxp3⁺ Treg were significantly decreased in the total cohort with H3 and CD patients with H3 compared to those with H1. This suggests that reduced bacterial-specific Treg generation is not disease specific, but cumulative risk variants in atg16l1 locus are associated with reduced ability to generate Foxp3⁺ (Treg) cells.

T59. CCR6 Deficiency Aggravates Colitis in a Spontaneous Mouse Model of Colitis

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Introduction/background: Chemokine receptor 6 (CCR6) is expressed by both pro-inflammatory cells and regulatory T cells and its ligand CCL20 is predominantly expressed by intestinal epithelial cells. CCR6/CCL20 axis is implicated to play a major role in the pathogenesis of IBD. The CCR6/CCL20 axis is paradoxical as it is involved in both immune regulation and activation. The contradictory role of CCR6/CCL20 in inflammation/intestinal homeostasis requires further investigation in a relevant spontaneous model of colitis. Aim: To assess the role of CCR6 in an inflammatory environment of spontaneous colitis model Winnie, with established colitis. Methods: CCR6 deficient Winnies were generated and assessed with clinical, histological& immunological parameters. Results: Clinical parameters showed significant increase in the colon weight/body weight in CCR6 deficient Winnie mice compared to sex and age matched controls. Histological examinations revealed the aggravated colitis specifically in mid to proximal colon region. Immuno-phenotyping of colonic lymphocytes indicated reduction in the Fox P3+ Tregs and increased Th17 cells. Discussion/conclusion: Our studies indicate that CCR6 deficiency aggravates colitis in an established chronic colitis setting. Further detailed mechanism of CCR6 function will provide an insight in understanding of CCR6/CCL20 in the pathogenesis of IBD.

T60. Microbiota Sensing by Nod2 Regulates Mucosal Damage in Small Intestinal Crypt Following Acute T Cell Activation

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The high numbers of T cells in the inflamed intestinal mucosa and the requirement for T cells in various animal models of chronic intestinal inflammation suggest a key role of T cells in the pathogenesis of Inflammatory Bowel Diseases. Loss-of-function mutations in the NOD2 gene were the first defined genetic risk factors identified for Crohn's Disease. In this study, we used a murine model of T cell-induced enteropathy to examine the role of Nod2 in regulating immune activation and T cell-induced small intestine mucosal damage. We found that acute T cell activation induced more severe damage in crypt architecture, epithelial apoptosis and delayed mucosal healing in $NOD2^{-/-}$ mice. Immune and inflammatory responses were also up-regulated as observed by the accumulation of Th17 cells and notably IFN-γ expressing Th17 cells. Small intestine mucosal damage required accumulation of microbiota dependent T cells as shown by the absence of mucosal damage in germ free mice. The delay in mucosal healing was prevented by antibiotic treatment indicating that microbiota sensing by Nod2 was important to control healing of mucosal damage in response to acute T cell activation. Our results demonstrate that Nod2 is an important regulator of mucosal damage following acute T cell activation.

T61. PI3 Kinase p85α Subunit-Deficient Macrophages Protect Mice from DSS-Induced Acute Colitis Due to the Enhancement of Anti-Inflammatory Cytokine Production

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We have recently demonstrated that phosphoinositide 3-kinase (PI3K) p85α hetero deficient ($p85\alpha^{+/-}$) mice exhibit reduced susceptibility to dextran sulfate sodium (DSS)-induced acute colitis. In the present study, we examined the role of PI3K p85α subunit in the development of acute colitis with a focus on macrophage (MΦ) functions. Experimental acute colitis was induced by giving 3% DSS in drinking water for 7 days. The severity of DSS-induced acute colitis was significantly attenuated in $p85\alpha^{+/-}$ mice compared with wild-type (WT) mice. The proportion of $F4/80^{+}CD11b^{+}$MΦs in the colonic mucosa was increased equivalently in both WT and $p85\alpha^{+/-}$ colitis mice. The LPS-induced mRNA expression of pro-inflammatory cytokines in $F4/80^{+}CD11b^{+}$MΦs isolated from the inflamed colonic mucosa was significantly suppressed in $p85\alpha^{+/-}$ colitis mice compared with WT colitis mice. Interestingly, we found that bone marrow-derived MΦs (BMMΦs) from $p85\alpha^{+/-}$ mice produced significantly higher amount of IL-10 in a response to LPS stimulation than BMMΦs from WT mice. Furthermore, the adoptive transfer of BMMΦs from $p85\alpha^{+/-}$ mice significantly improved the severity in WT colitis mice. These results suggest that the deficiency of PI3K p85α enhances the production of IL-10 in colonic MΦs, thereby suppressing the development of DSS-induced acute colitis.

T62. Conditional Epithelial Deletion of the Pathogen Recognition Receptor Nod2 Fails to Alter the Regulation of T Cell-Induced Enteropathy

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The pathogen recognition receptor Nod2 plays an important role in intestinal barrier maintenance and host defense against bacterial microbes. Inflammatory Bowel Diseases are thought to result from a loss of tolerance towards the normal intestinal microbiota due to an inappropriate and continuing inflammatory response and/or an unstable or leaky mucosal barrier. Loss-of-function mutations in the NOD2 gene are significantly associated with higher risks of developing Crohn's disease. We showed that acute T cell activation induced stronger damage in crypt architecture, epithelial apoptosis and delayed epithelial regeneration in $NOD2^{-/-}$ mice. To assess the role of Nod2 function in epithelial cells versus T cells, we created a conditional deletion of Nod2 in the intestinal epithelium. Anti-CD3 induced enteropathy in $NOD2^{\Delta IEC}$ mice and mucosal healing was not different from that in WT mice. In contrast, transfer of Nod2 deficient $CD4^{+}$ T cells into $Rag1^{-/-}$ mice led to a dramatic mortality rate after *in vivo* T cell activation. Our results indicate that Nod2 is an important regulator of small intestine mucosal damage and its expression in $CD4^{+}$ T cells is important to control the pathophysiological response to acute T cell activation in the gut.

T63. Evidence for the Differential Subordination of the IL-18 / IFNY Axis to Caspase-1 Among Patients with Crohn's Disease
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In Crohn's disease (CD), hierarchical architecture of the inflammatory network, including subordination of IL-18, an IFNY-inducing cytokine, to the inflammasome, have remained undeciphered. Heterogeneity among patients of such a subordination cannot be evaluated by animal models, monofactorial in their etiology and homogenous in disease progression. To address these issues, we setup an *ex vivo* model of inflamed mucosa explant cultures from full-blown CD patients. Th1 cytokine production, especially IFNY and IL-18, was assessed in relation with inflammation intensity. Subordination of the Th1 response to caspase-1, effector of the inflammasome, was determined in explant cultures subjected to pharmacological inhibition of caspase-1. We showed a correlation between secreted IFNY / IL-18 levels, and caspase-1 activation, with inflammation intensity of intestinal CD mucosa. Inhibition of caspase-1 activation using the specific inhibitor YVAD identified a homogenous non responder group featuring a caspase-1-independent IL-18 / IFNY response, and a heterogeneous responder group, in which both IL-18 and IFNg responses were caspase-1-dependent (40% - 70% inhibition by YVAD). These findings bring out the concept of heterogeneity of subordination of the Th1 response to inflammasome activation among CD patients. This *ex vivo* model should have therapeutic relevance in allowing to determine eligibility of CD patients for new targeted therapies.

T64. Cigarette Smoke Exposure Alters the Development of Inflammation in Experimental Crohn-Like Colitis
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Introduction: Human Crohn's disease (CD) is usually characterized by a relapsing and remitting course (flare-ups followed by clinical remission) in the early phases of the disease. Cigarette smoking is the best known environmental risk factor for CD. We studied the influence of four weeks cigarette smoke exposure on the disease course of TNBS-induced murine colitis, modeling distal active Crohn-like colitis. Methods & Results: 10 mice were exposed to the tobacco smoke of five cigarettes four times a day with 30 min. smoke-free intervals, five days per week for four weeks, after which colitis was induced by intrarectal TNBS treatment. At day two and three post-colitis induction, mice were sacrificed. We observed a strong induction of histologic inflammation by TNBS after two days, but not anymore after three days. In addition, we demonstrated an increase of Kc, Cxcl-2, Il-1β, Ccl19 and Ccr6 in smoke-exposed TNBS-challenged mice after two days. Cxcl-2 increased both at two and three days post-TNBS-enema. Kc, Il-1β, Ccl19 and Ccr6 increased only after two days post-TNBS-enema. Discussion: Here, we show an altered histology of TNBS colitis after four weeks of CS exposure. We found a further induction of Cxcl2 and Il-1β mRNA and protein due to smoke exposure, suggesting increased neutrophil attraction and increased macrophage activity in the inflamed distal colon. We speculate that CS boosts inflammation in the initiation phase towards an IBD-like flare-up. Knowledge of the molecular targets of smoke exposure might pave the way for adaptation of current treatment strategies in IBD patients.

T65. Interleukin-37 mRNA Expression in Microscopic and Ulcerative Colitis
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Interleukin-37 (IL-37) is a newly described mediator down-regulating pro-inflammatory cytokines following TLR stimulation. Furthermore, IL-37 mediates protective anti-inflammatory effects in a murine DSS-induced colitis model and in a myocardial ischaemia model. Since no previous study has reported mucosal IL-37 expression in microscopic colitis (MC) or ulcerative colitis (UC) patients, we analyzed IL-37 mRNA expression in collagenous colitis (CC), lymphocytic colitis (LC), UC patients and controls. IL-37 mRNA was significantly decreased (2-fold), in MC patients versus controls. However, there was no difference between patients with active MC compared to those in histopathological remission. Opposite to the active disease/remission results for MC, we found a five-fold significantly increased IL-37 mRNA expression in UC patients in remission as compared to those with active disease. TLR stimulation of the colon epithelial cell line T84 resulted in significantly increased IL-37 mRNA expression, suggesting the importance of further IL-37 functional studies. Having in mind the anti-inflammatory properties of IL-37, a constantly decreased mucosal expression of IL-37 in MC patients might be one of the reasons for development of inflammation in those patients. The increased IL-37 mRNA in UC remission patients indicates either a direct role for IL-37 in inducing remission, or simply being an indirect marker of remission.

T66. Response to Corticosteroids in Ulcerative Colitis may be Related to Modulation of mTOR Signaling Pathway Genes by MicroRNAs

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Background: Mechanisms of resistance to corticosteroids (CS) in ulcerative colitis (UC) are not well understood. Little is known about the influence of microRNAs (miR) in the response to CS in UC. Objective: To compare the transcriptomic profile in rectal mucosa of patients with active UC responding and non-responding to CS. Methods: Rectal biopsies were obtained from UC patients before and after three days of CS treatment. Patients were grouped in responders and non-responders according to Montreal's classification. miR were identified by means of a sequencing method (Illumina) and RNAm were study by microarrays method (Illumina) on those rectal biopsies with high integrity. Those miR and RNAm with a fold change ≥ 1.5 and adjusted p-value ≤ 0.05 were further studied. Results: 32 out of 48 tissue samples reached an integrity that allowed miR sequencing or microarrays study. Comparison between groups showed a differential miR expression of miR-1246, miR-1291, miR-5701 and miR-625-3p, miR-183-5p, miR-3607-3p, miR-4770, miR-449, miR-145-3p. The only gene with differential expression after microarrays study was DDIT4. In silico study reveals that DDIT4 is a potential target of three of the differential expressed miR (miR-183-5p, miR-625-3p, miR-3607-3p) and also that this gene is linked to the mTOR pathway (and indirectly with autophagy). Conclusion: There is a different profile of rectal microRNAs between responders and non-responders to CS. Our findings suggest that regulation of mTOR and autophagy pathways by miR might be involved in the response to CS in active UC.

T67. Constitutive Type 1 Interferon Selectively Promotes STAT1-Associated IL-10 Production by Human Intestinal T Cells in Health, but not IBD

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Immunoregulatory roles for Type 1 Interferon (T1IFN) are increasingly recognized. T1IFN signaling ameliorates murine colitis by promoting regulatory T cell function. In humans, T1IFN has been used as a treatment of IBD with variable efficacy. To test the hypothesis that T1IFN contributes to immune regulation in the human intestine, we assessed its expression in the colon, effect on Signal Transduction and Activator of Transcription 1 (STAT1) signaling and impact on the function of human intestinal T cells. Type 1 IFN (IFNβ) was detected in the human colon by immunohistochemistry and T cells isolated from colonic biopsies responded to exogenous T1IFN by phosphorylation of (p)STAT1. Colonic T cells from IBD patients showed increased responsiveness to T1IFN by more pSTAT1$^+$ T cells and enhanced expression of Interferon Stimulated Genes (MxA and 2'5'OAS). The effect of endogenous T1IFN was assessed by addition of neutralizing anti-IFNβ antibody to *ex vivo* biopsy organ cultures. In controls, neutralization of IFNβ reduced the frequency of IL-10 producing T cells, and increased IFNγ production. In contrast, neutralization of IFNβ in cultures of IBD biopsies had no selective effect on IL-10 but increased the frequency of T cells producing a broad range of cytokines (IL-10, IFNg, TNFa and IL-17). Therefore T1IFN, present in the human gut, impacts upon STAT1 signaling and T cell function differently in health and in IBD. In health, selective promotion of a regulatory IL-10 response in T cells to T1IFN may be important in maintaining mucosal integrity in response to viral or other pathogens.

T68. Colonic Microbiome and Barrier Dysfunction Contribute to Susceptibility to Colitis in NHE3xRag2 Double Knockout Mice

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Intestinal Na^+/H^+ exchanger NHE3 is functionally and/or transcriptionally repressed in IBD. $NHE3^{-/-}$ mice develop chronic colitis, IBD-like microbial dysbiosis, and are highly susceptible to mucosal injury. Here, we show a dramatic susceptibility of Rag2/NHE3 knockout (DKO) to colitis in the naïve T cell transfer model. Compared to $Rag2^{-/-}$, DKO mice develop early onset severe colitis, T cell and neutrophil infiltration, elevated mucosal inflammatory cytokines, and increased epithelial permeability. This response was suppressed by broad-spectrum antibiotics. In fecal 16S rRNA amplicon profiling, genotype and antibiotic use were the predominant

determinants of microbial diversity. The composition of the Firmicutes phylum was most significantly altered in DKO mice (Rag2 vs. DKO), with changes in among Lactobacillales (Lactobacillus, 24.1 vs. 15.6%; Enterococcus, 0.3 vs. 2.5%), Turicibacterales (1.7 vs. 4.8%), Erisipelotrichales (1.7 vs. 30.8%), and a dramatic decrease in Clostridiales (23.1 vs. 5.6%). Fecal transplant from T cell-transferred DKO mice into Abx-pretreated Rag2$^{-/-}$ mice was not sufficient to fully recapitulate the donors' severity of colitis, with the exception of significant difference in weight gain and a significantly elevated colonic neutrophil infiltration. Our data suggest that impaired epithelial Na^+/H^+ exchange contributes to the susceptibility to T cell mediated colitis via both altered colonic microbiota and an epithelial barrier defect.

T70. Intestinal Endothelial MyD88 Signaling is Protective During Salmonella-Induced Colitis

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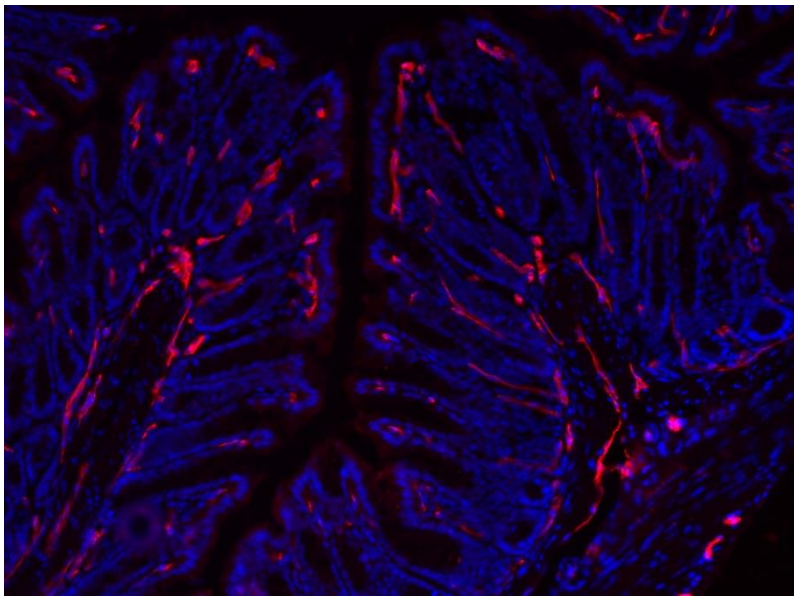

In recent years, mutations in several innate immune signaling pathways have been linked to an increased susceptibility of developing Inflammatory Bowel Disease (IBD). Germline defects in several of these innate pathways (MyD88, NOD2) have been shown to lead to increased susceptibility to disease[1,2], however the intricacies of this susceptibility require further investigation. Currently, it is not completely understood which cell types contribute to the protective effects of MyD88 signaling, though hematopoietic and non-hematopoietic linages are likely involved[3]. Here we aim to examine the role of MyD88 signaling in one such non-hematopoietic cell type: endothelial cells during Salmonella-induced colitis utilizing cell type specific knockout mice (EC-MyD88$^{-/-}$). The role of MyD88 signaling within endothelial cells is of interest to clarify as IBD patients often exhibit altered microvasculature in inflamed regions of the intestine[4]. In addition, germ-free mice do not develop the same extensive intestinal vascular networks as their conventional counterparts[5], suggesting a potential role for MyD88-dependant bacterial sensing in its regulation. Overall, we aim to determine the role of endothelial-MyD88 signaling during intestinal inflammation. EC-MyD88$^{-/-}$mice exhibited significantly accelerated macroscopic and histological tissue damage at early time-points (D1-3) post-infection compared to wildtype, even though similar intestinal pathogen burdens were found. Gene expression analysis showed significant induction of several chemokines in EC-MyD88$^{-/-}$ mice; with immunostaining revealing increased immune cell recruitment along with significant cell death (TUNEL+ cells) within the mucosal tissue. [1]Rakoff-Nahoum 2004 Cell 118:229-241 [2]Couturier-Maillard 2013 J Clin Invest 123:700-11 [3]Brandl 2010 PNAS 107:19967-19972 [4]Hatoum 2003 Gastroenterology 125:58-69 [5]Stappenbeck 2002 PNAS 99:15451-15455

T71. Human Feces-Derived *Fusicatenibacter Saccharivorans* Reduced in IBD Patients Induces IL-10 to Suppress Murine Model of Colitis

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Experimental and clinical findings indicate association between inflammatory bowel disease (IBD) and intestinal microbiota, and Honda's group demonstrated that Clostridium (C.) species, clusters IV and XIVa, induce IL-10-producing regulatory T cells, and protect the development of colitis in mice. Furthermore, we previously demonstrated that a single strain of C. butyricum induces IL-10-producing regulatory macrophages via PAMP-TLRs pathway, resulting in the suppression of colitis (Cell Host Microbe 2013). In this study, we analyzed the bacterial composition of human fecal samples from patients with ulcerative colitis (UC), Crohn's disease

(CD), and healthy subjects. First, we confirmed that the number of the total fecal bacteria in active UC was less than quiescent UC. The alteration of bacterial number was not observed in CD. Furthermore, we found that the prevalence of Fusicatenibacter saccharivorans (FS), which belong to C. cluster XIVa, was strikingly lower in active UC than quiescent UC. Administration of FS derived from human healthy subject suppressed the development of acute DSS-colitis in mice. To explore the mechanism, we stimulated colitic lamina propria mononuclear cells (LPMC) from murine IBD models with heat-killed FS. LPMC incubated with FS produced higher amounts of IL-10 compared to those stimulated with Enterococcus faecalis (EF), which was used as a control of gram-positive bacteria. FS also induced IL-10 production from human LPMC isolated from UC patients. The results suggest human-derived FS suppress intestinal inflammation, probably through IL-10 induction, and would be a future strategy of IBD bacteriotherapy to colonize in the intestine of IBD patients.

T72. Lupeol Inhibits LPS Induced NF-kappa B Signaling Pathways in Intestinal Epithelial Cells, Macrophages and Attenuates Experimental Colitis in Mice

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Background and aims: Lupeol, a natural pentacyclic triterpene, is shown to anti-inflammatory effect but its role in colitis has not been investigated. This study evaluated the effect of lupeol on the NF-κB signaling pathways and on experimental colitis. Methods: The human intestinal epithelial cell (IEC)-line COLO 205, the murine macrophage cell-line RAW264.7 were prepared and subsequently stimulated with lipopolysaccharide (LPS) alone or LPS plus various dose of lupeol. The production of cytokines (IL-8 from COLO 205; TNF-α, IL-6, IL-12 and IL-10 from RAW 264.7) was qualified by ELISA. The effect of lupeol on NF-κB signaling was examined by western blotting and an electrophoretic mobility shift assay (EMSA) to assess the DNA binding activity of NF-κB. In *in vivo* studies, dextran sulfate sodium (DSS)-induced acute colitis in wild-type mice and chronic colitis in IL-10$^{-/-}$ mice were treated with or without lupeol. Colitis was quantified by histologic scoring, and the phosphorylation of IκBα in the colonic mucosa was assessed using immunohistochemistry. Results: Lupeol significantly inhibited LPS-induced IκBα phosphorylation, NF-κB binding activity, and pro-inflammatory cytokine production in both IEC and macrophages. The administration of lupeol significantly reduced the severity of colitis, as assessed based on histology in the both murine colitis models. Furthermore, in colon tissue, the up-regulations of IκBα phosphorylation with colitis induction were attenuated in lupeol-treated mice. Conclusions: Lupeol may block the NF-κB signaling pathways, inhibit the activation of IECs and macrophages, and attenuate experimental murine colitis. These findings suggest that lupeol is a potential therapeutic agent for inflammatory bowel diseases.

T73. Prophylactic Potential of Recombinant Taenia Solium Calreticulin in Mice with TNBS-Induced Colitis

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Inflammatory bowel diseases (IBD) have been rising in developed countries. Multiple studies have shown that gastrointestinal helminths induce a regulatory environment, thus have been used to control IBD. Nonetheless treatment with living parasites is disadvantageous; therefore identification of parasitic immunomodulatory proteins is compulsory. Calreticulin is a calcium binding protein with key functions in the cell. Our group identified, cloned and generated Taenia solium calreticulin as a recombinant protein (rTsCRT). It induces a predominant Th2 response after oral immunization. Therefore, we investigated the prophylactic potential of rTsCRT in an experimental model of colitis. Mice were immunized with purified rTsCRT weekly for four weeks and colitis was induced by intra-rectal administration of trinitrobenzene sulfonic acid (TNBS). Three days later clinical disease activity, colonic inflammation, as well as cytokine production were assessed. Mice that did not receive TNBS showed no signs of disease or inflammation, which were increased in mice with colitis. rTsCRT immunization significantly decreased the clinical score index, the extent of colonic inflammation and the expression of IL-1β, IL-6 and TNFα. Colons of immunized mice overexpressed IL-13 and peritoneal macrophages from rTsCRT-treated mice produced more IL-10 as compared to cells from mice with colitis. rTsCRT also decreased MHCII expression in LPS-treated dendritic cells and induced expression of TGF-b. Additionally, we showed that rTsCRT reduces the genotoxicity produced by TNBS. We conclude that oral immunization with rTsCRT ameliorates TNBS-induced colitis in mice, suggesting that rTsCRT has immunomodulatory properties and prophylactic potential for the treatment of IBD.

T74. MicroRNA-424 May Regulate Transcripts Involved in the Pathogenesis of Inflammatory Bowel Disease (IBD)
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Recently we showed by massive sequencing analysis of ileal biopsies that increased expression of multiple microRNAs (miRs) occurs in ulcerative colitis patients after total proctocolectomy and pouch surgery, specifically in those developing pouchitis and Crohn's-like disease of the pouch (CLDP). Thus, we hypothesized that miRs may have a role in down-regulation of mRNA in IBD. Based on our in silico data mir-424 was selected to define the interaction with its potential targets solute carrier family 6 member 4 (SLC6A4) and solute carrier family 36 member 1 (SLC36A1) and the mechanisms modifying miRs expression in intestinal inflammation. The expression of miR-424 significantly increased (3.3 fold, $p<0.001$), while SLC6A4 mRNA expression significantly decreased (5.8 fold, $p<0.01$) and that of SLC36A1 was lower in ileal biopsies of CLDP patients compared to normal controls. Primary to mature miR-424 expression ratios were higher in the normal pouch compared to pouchitis (4 fold increase). Similarly, intestinal epithelial cell lines (HCT-116) stimulated with inflammatory cytokines expressed 4-fold more miR-424 ($p<0.05$) and less SLC6A4 (2.8 fold decrease, $p<0.05$) and SLC36A1 (1.3 fold, $p<0.01$) compared to no treatment. Transfection of miR-424 mimic into HCT-116 cells resulted in a decrease in SLC6A4 and SLC36A1 expression (1.3 fold, $p<0.05$) whereas transfection of miR-424 inhibitor resulted in an increase in SLC6A4 and SLC36A1 expression (2 fold, $p<0.01$ and 1.3 fold, $p<0.05$, respectively). miR-424 expression correlates with intestinal inflammation and it modifies its target transcripts. Thus miR-424 behavior may model the role of miRs in regulating intestinal inflammation in IBD.

T75. IL-22BP is Produced by Eosinophils in Human Gut and Inhibits the Protective Actions of IL-22 During Experimental Colitis
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Crohn's disease and ulcerative colitis, the two major forms of inflammatory bowel diseases (IBDs), are characterized by high levels of IL-22 production. Rodent studies revealed that this cytokine is protective during colitis but whether this is true in IBDs is unclear. In addition, IL-22 is regulated by a potent, specific and soluble inhibitor called interleukin 22-binding protein (IL-22BP), whose regulation has never been assessed in human gut. We show here that levels of IL-22BP are significantly enhanced in the inflamed mucosa of IBDs patients. This was explained by increased numbers of IL-22BP-producing eosinophils that we unexpectedly identify as the most abundant source of IL-22BP protein in both healthy and inflammatory human gut. In addition, using IL-22BP-deficient rats, we confirm that endogenous IL-22BP is effective at blocking protective actions of IL-22 during acute colitis. In conclusion, our study provides new important insights regarding the biology of IL-22 and IL-22BP in the gut and indicates that protective actions of IL-22 are likely to be suboptimal in IBDs thus making IL-22BP a new relevant therapeutic target.

T76. Involvement of IRF4 Dependent Dendritic Cells in T Cell Dependent Colitis
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Inflammatory Bowel Disease (IBD) is a chronic non-curable inflammatory disease of the intestine that affects as many as 1.4 million persons in the United States and 2.2 million persons in Europe. IBD results from abnormal immune response to bacterial components of the commensal microflora in genetically susceptible individuals and pathogenic $CD4^+$ T cells, which accumulate in the inflamed mucosa, are believed to be key drivers of the disease. While dendritic cells (DCs) are important in the priming of intestinal adaptive immunity and tolerance their role in the initiation and perpetuation of chronic intestinal inflammation remains unclear. In the current study we used the $CD45RB^{hi}$ T cell transfer model of colitis to determine the role of IRF4 dependent DCs in intestinal inflammation. In this model naïve CD4+ T cells when transferred into $RAG^{-/-}$ mice, proliferate and expand in response to bacterial derived luminal antigen, localize to the intestinal mucosa and induce colitis. Adoptive transfer of naïve T cells into $CD11cCre.IRF4^{fl/fl}.RAG\text{-}1^{-/-}$ mice resulted in reduced monocyte recruitment to the intestine and mesenteric lymph nodes (MLN) compared to Cre^- controls. Inflammatory cytokines including IFNγ, TNFα and IL-6 also were reduced in the serum and intestinal tissues of these mice. Additionally $CD11cCre.IRF4^{fl/fl}.RAG\text{-}1^{-/-}$ mice displayed significantly reduced numbers of $CD4^+$ T cells in intestinal draining mesenteric lymph nodes and spleen but not the colonic lamina propria. Collectively these results suggest an important role for Irf4 dependent DCs in T cell driven colitis.

T77. Establishment of a Human Colon Epithelial Cell Culture System to Study their Regulating Effects on Resident Lamina Propria Immune Cell Activation

Anne-Kristin Heninger, Laura Kummer, Judith Gras, Mohammed Al-Saeedi, Stefan Meuer and Jutta Schröder-Braunstein. University Hospital Heidelberg, Heidelberg, Germany

The intestinal epithelial layer represents an important barrier between luminal microbes, toxins and antigens on one side, and lamina propria immune cells on the other side. We have shown that loss of the epithelium leads to the initiation of an inflammatory response in resident lamina propria immune cells. To study heterotypic cell interactions between epithelium and lamina propria in the intestine, we began to establish an *ex-vivo* co-culture system. To this end, crypts, which include the epithelial stem cells, were isolated from healthy human primary colonic mucosa and embedded in Matrigel according to the previously published method of Sato et al. 2009. Individual organoid structures grew in size and developed several extending buds representing a multi-crypt structure. Within these buds the proliferation marker Ki67 was detected. The intestinal stem cells in the buds gave rise to differentiated crypts showing the upregulation of markers representative of epithelial cell differentiation. For the co-culture of epithelial cells and lamina propria immune cells, a Transwell system will be established based on a collagen-matrix obtained from decellularized porcine small intestine as generated in the laboratory of H. Walles. The system will be used to study the role of the epithelium in the control of initial inflammatory responses of lamina propria immune cells.

T78. High Sodium Chloride Enhances Inflammatory Cytokine Production in Human and Colitis in Mice

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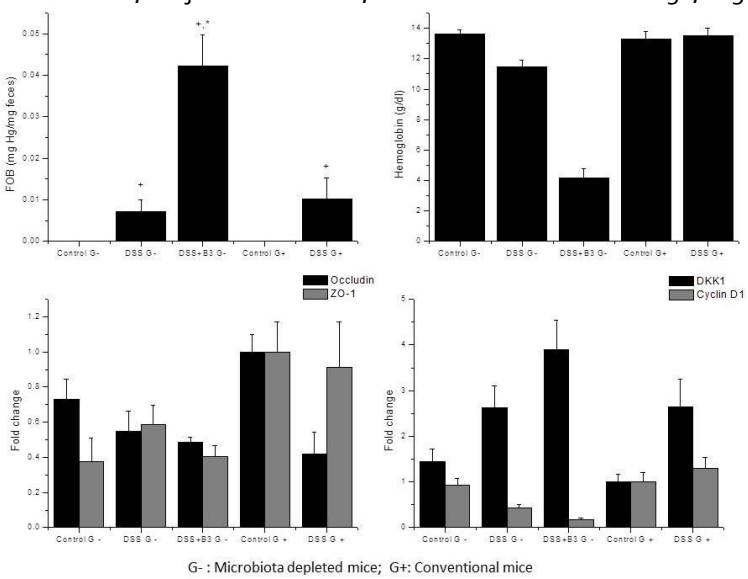
Background: Environmental factors are supposed to play a decisive role in the pathogenesis of inflammatory bowel diseases (IBD).Increased dietary salt intake has been linked with the development of autoimmune diseases, but the impact that such an environmental factor has on the course of IBD remains unknown. In this study we investigated the cytokine response of intestinal immune cells to high concentrations of salt. Methods: Normal intestinal lamina propria mononuclear cells(LPMC) were activated with anti-CD3/CD28 with or without increasing concentrations (20-80 mM) of NaCL.In parallel, LPMC were treated as above in the presence or absence of SB202190, a specific inhibitor of P38 activation. Transcription factor and effector cytokines were evaluated by flow-cytometry and real-time PCR. To examine if dietary NaCl intake influences the *in vivo* gut inflammation, high dose of NaCL (4% of the normal diet) was administered to mice 7 days before the induction of trinitrobenzene-sulfonic acid(TNBS)-colitis. Mice were then sacrificed at day 5 and LPMC and colon tissues were analyzed for inflammatory and anti-inflammatory molecules. Results: IL-17A and TNF-a were significantly increased in human LPMC following NaCl exposure while there was no significant change in IFN-g, IL-6, T-bet, RORgt and Foxp3. Pharmacologic inhibition of P38 abrogated the inducing effect of NaCL on LPMC-driven IL-17A and TNF-a production. Mice receiving high salt diet developed a more severe colitis following TNBS administration. Conclusions: High salt concentration enhances the production of inflammatory cytokine in the gut. Data provide novel insights into IBD pathogenesis and explain how high salt diet triggers/expands gut immune response.

T79. Effect of Budesonide on Dextran Sulfate Sodium Colitis in Mice Under Microbiota Depletion

Carlos J. Aranda, Borja Ocon Moreno, Maria Dolores Suarez Ortega, Olga Martinez Augustin and Fermin Sanchez De Medina López-Huertas. University of Granada, Granada, Spain



G- : Microbiota depleted mice; G+: Conventional mice

Budesonide is widely used as anti-inflammatory drug in inflammatory bowel disease (IBD). We have shown that in mice with colitis induced by dextran sulfate sodium (DSS), treatment with budesonide ameliorates colitis but enhances bacterial and LPS translocation, resulting in systemic deterioration, suggesting an impairment of mucosal barrier function. In order to evaluate the importance of translocation, C57BL6/J pseudogerm free (PGF) mice with antibiotic induced depletion of the microbiota were used. Mice received DSS in drinking water and budesonide by gavage (3 micrograms/day) for

6 days. Surprisingly, the budesonide group showed increased body weight loss, rectal bleeding and disease activity index compared to the vehicle group. Hemoglobin and red blood cell were also decreased. Tight junctions were altered by budesonide, showing a decrease in ZO-1 and occludin by RT-qPCR. In addition, cyclin D1 was reduced and DKK1 was increased, suggesting a deleterious effect of the glucocorticoid on epithelial dynamics and restitution. Because the latter are modulated by the microbiota, we conclude that epithelial compliance is decreased by the absence of bacteria and this negative effect is enhanced by budesonide.

T80. Activation of the Unfolded Protein Response in Resident Lamina Propria Cells During the Initiation of Intestinal Inflammation

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Mucosal lamina propria cells of IBD patients are highly activated in contrast to the hyporesponsive phenotype of these cells under homeostatic conditions. Given, that human studies are based on patient biopsies taken years after disease onset, the molecular events initially causing this inflammation are still largely unknown. In this study, we aimed to characterize early activation mechanisms of resident lamina propria cells by utilizing an *ex vivo* human intestinal organ culture model which was recently described by us. In this model, healthy human colonic mucosa was depleted of epithelial cells by EDTA treatment, which resulted in the activation of lamina propria immune cells and their emigration out of the tissue. Bioinformatic analysis of in situ gene expression profiles revealed the induction of signaling pathways not yet associated with the activation of lamina propria cells under inflammatory conditions, e.g. the response to unfolded protein. The upregulation of known target genes of this pathway such as GADD153, C/EBPβ, and HSPA5 in the latter cell population was subsequently confirmed in situ by qRT-PCR and/ or immunofluorescence. These findings provide insight into molecular events underlying the change from a hyporesponsive to a highly activated phenotype in lamina propria cells at the very onset of inflammation.

T81. Gut Microbial-Specific $CD4^+$ T cells from Crohn's Disease Patients Exhibit a Pro-Inflammatory Th17 Phenotype

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Experimental models have led to the theory that intestinal inflammation, as seen in Crohn's disease (CD), results from a loss of tolerance towards commensal microorganisms. In fact, about half of CD patients develop antibodies towards gut microbial antigens. There is however little evidence supporting the existence of a microbial-specific T cell response in CD. In testing reactivity to several gut microbial antigens in peripheral blood mononuclear cells, we detected T cell reactivity towards some antigens including FlaX, Fla2, and YidX in healthy individuals and CD patients; T cell proliferation was however higher among CD patients. Intracellular cytokine staining revealed the presence of Th17, Th1 and Th17/Th1 cells among the FlaX, Fla2 and YidX-specific T cells. While the percentage of Th1 cells was similar, Th17 and Th1/Th17 frequency was higher in CD patients compared to healthy individuals. Microarray and real time-PCR gene analysis of sorted gut microbial-specific T cells showed a Th7-biased transcriptional profile in CD patients' microbial-specific T cells. Supernatants from CD activated FlaX, Fla2 and YidX-specific T cells induced higher expression of CXCL1 CXCL8, and CCL20 in healthy colonic crypts, which diminished upon IL-17 neutralization, demonstrating the pro-inflammatory potential of these cells in the intestinal mucosa. Collectively, our data indicate that T cells that react to gut microbial antigens have been differently imprinted towards a Th17 phenotype in CD. We hypothesize that these cells represent a circulating microbial-specific memory T cell pool that may contribute to sustain gut inflammation in CD; their identification opens new avenues for antigen-directed therapies.

T82. Type-III IFN Signaling Through PKR is Required for RipK3 Activation in Intestinal Epithelial Cells

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Interferons (IFNs) play a critical role in the antimicrobial host defense. Despite the fact that IFN-λ, a type-III IFN, has been shown to predominantly act on mucosal organs, *in vivo* studies have failed to elucidate a specific, non-redundant function. Here we investigated the influence of type III IFNs on intestinal homeostasis. IFN administration induced increased epithelial shedding which

was dependent on caspase-8 activation in wild type animals. Mice deficient for caspase-8 in intestinal epithelial cells (Caspase-8$^{\Delta IEC}$ mice) showed excessive cell death and villous atrophy associated with high mortality in response to IFN. Disruption of barrier function required RipK3, since RipK3-/-xCaspase-8$^{\Delta IEC}$ animals were protected from IFN induced epithelial cell death, indicating that this form of cell death is due to RipK3-mediated necroptosis. Interestingly IFN-λ induced necroptosis occurs independently of Tnf signaling and is instead mediated via the Stat1-PKR (RNA-responsive protein kinase) pathway. Moreover we could identify that under steady state conditions and after viral infection, IFN-λ is mainly expressed by intestinal epithelial cells and is transcriptionally regulated by the intestinal microbiota, since germ free animals have significantly lower IFN-λ mRNA levels. Taken together, our data reveal how IL28 mediates acute inflammation through Stat1/PKR mediated Rip dependent cell death.

T83. Effects of Narrow Spectrum Versus Selective Kinase Inhibitors on the Intestinal Pro-Inflammatory Immune Response in IBD

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Background & Aims. Intracellular kinase activation plays a pivotal role in intestinal inflammation. We profiled the effects of selective kinase inhibitors and the narrow spectrum kinase inhibitor (NSKI) TOP1106 on innate and adaptive immune mechanisms involved in IBD. Methods. Inhibition of P38a, Src and Syk kinase activity was assessed by recombinant ZLYTE^{TM}assays. Peripheral blood mononuclear cells (PBMCs) from healthy donors, HT-29 cells, or inflamed colonic IBD biopsies were cultured with TOP1106 or selective kinase inhibitors with pro-inflammatory cytokine release subsequently assessed by ELISA. Results. TOP1106 is a potent inhibitor of P38a, Src and Syk kinases. Compared to the selective kinase inhibitors, TOP1106 demonstrated broader activity across a range of innate and adaptive cellular response assays and in IBD organ culture experiments. Generally, the selective kinase inhibitors lacked efficacy and potency compared to TOP1106. Combination of the selective inhibitors led to an improved inhibitory profile in both cellular and IBD biopsy assays, with data indicating that TOP1106 could be achieving its broad anti-inflammatory activity through the synergistic effects of multi-kinase inhibition. Conclusions. TOP1106 inhibits key kinases involved in inflammatory signaling pathways. Simultaneous inhibition of these kinases with TOP1106 leads to an improved anti-inflammatory profile compared to selective kinase inhibitors, highlighting the potential of NSKIs as a treatment for IBD.

T84. Control of Intestinal Inflammation and Colon Cancer by Novel Genetic and Cytokine-Mediated Pathways

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Chronic intestinal inflammation is a colorectal cancer risk factor. Our group has characterized a model of chronic colitis-induced colon cancer that involves infection of 129/SvEv.Rag$^{-/-}$ mice with Helicobacter hepaticus (Hh). We previously described a congenic line, termed R17.Rag$^{-/-}$, that harbors a 1.7Mb locus of B6 origin on chromosome 3 and is resistant to Hh-induced colitis and colon cancer. Tumourigenesis in this model is dependent on interleukin (IL)-22. In addition to controlling chronic colitis, this locus regulates acute inflammation following Hh infection, involving pronounced recruitment of neutrophils and inflammatory monocytes within 2–5 days following infection. R17.Rag$^{-/-}$ mice are also protected from acute colitis, likely due to an enhanced ability to produce IL-10 in colon lamina propria macrophages and dendritic cells. Blockade of the IL-10 receptor during Hh colonization causes R17.Rag$^{-/-}$ mice to phenocopy the inflammatory pathology of 129/SvEv.Rag$^{-/-}$ animals. We are currently investigating mechanisms to account for the IL-10 deficiency observed in 129 mice. Genetic complementation studies have excluded several genes in the susceptibility locus as candidate disease-regulators, leaving several possibilities including Alpk1, which harbors several nonsynonymous polymorphisms between 129 and R17 mice, is highly expressed by disease-susceptible animals, and is associated with human inflammatory bowel disease and colon cancer.

T85. A Specific Expression Signature Suggests Intrinsic Defects in the Stem Cell Compartment of Colitic Epithelium

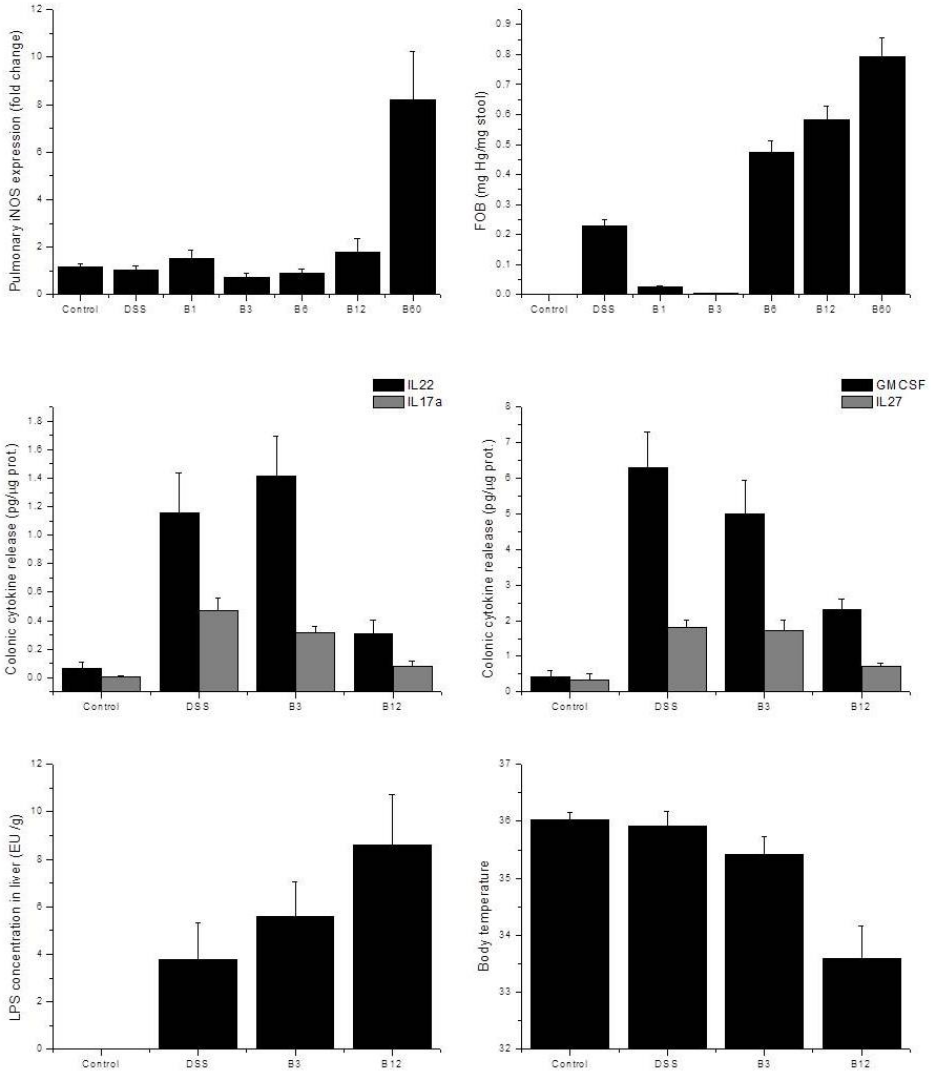
Isabella Dotti[1], Elena Ferrer[1], Núria Planell[2], Rut Mora-Buch[1], M. Carme Masamunt[3], Peter Jung[1], Eduard Batlle[1], Julián Panés[3] and Azucena Salas[1]. [1]Institut d'Investigacions Biomèdiques, Barcelona, Barcelona, Spain; [2]Networked Biomedical Research Center, Barcelona, Spain; [3]Hospital Clinic de Barcelona, Barcelona, Spain

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) that affects the superficial mucosal barrier. Scant data is available about the contribution of intestinal epithelial cell (EC) dysfunction to this disease. Using a recent human gastrointestinal epithelial stem cell culture system, we investigated whether primary defects in ECs could be present in UC. To this end, we

collected biopsies from the sigmoid colon of 11 non-IBD controls and 8 UC patients. Isolated crypt units were cultured in Matrigel, and stem ECs were expanded as 3D organoids. Total RNA from stem and differentiated organoids was extracted for transcriptional analysis. Our results show that control and UC epithelial stem organoids follow similar differentiation programs with comparable regulation of stem (i.e., Lgr5, AXIN2), proliferation (i.e., Myc, Ki67), and epithelial markers (i.e., MUC2, ANPEP). Microarray analysis, however, revealed a small panel of genes differentially expressed by UC compared to non-IBD organoids grown under the same conditions. Several of the UC up-regulated genes are characteristic of the upper regions of the intestine, while UC-derived organoids showed a marked down-regulation of genes normally expressed by the distal colon. Overall, we demonstrate that sigmoid ECs from colitic patients show a unique transcriptional signature including the acquisition of an upper intestinal tract phenotype. We hypothesize that this could ultimately drive permanent changes in colitic epithelium with implications on mucus composition and the nature of antimicrobial response.

T86. Intestinal TMIGD1 Expression in Crohn's Disease

Violeta Loren, Yamile Zabana, Arce Garcia-Jaraquemada, Elisabet Pedrosa, Miriam Mañosa, Eduard Cabré, Eugeni Domenech and Josep Manye. Germans Trias i Pujol Health Research Institute, Badalona, Spain



Introduction: A previous whole-genome microarray of affected and unaffected ileocecal resection specimens from Crohn's disease (CD) patients, showed a TMIGD1 differential expression (FC=-109 and -26; FDR=0 in unaffected and affected mucosa, respectively) compared to controls. TMIGD1 function is unknown in CD, and only existing data comes from colorectal cancer (Cattaneo, E et al.EMBO mol med.2011) Objectives: To define functional and molecular TMIGD1 expression changes between affected and unaffected CD ileocecal resections. Methods: TMIGD1 gene expression (Codelink microarrays) has corroborated by Taqman assay-PCR (Lifetechnologies), protein and glycosylation level has analyzed by Western-blot and GlycoProfile™-II (Sigma-Aldrich) test, respectively. By double immunolabeling (TMIGD1 vs NOD2 or Sucrase-isomaltase) has used to determinate their spatial localization in different mucosal compartments. Results: RT-qPCR correlates with TMIGD1 gene expression profiling results (Pearson correlation =-0.8 and r^2 0.66). Enzymatic assay and western-blot reveal that TMIGD1 is non-glycosylated protein, whereas it could be a truncated isoform (http://www.uniprot.org/). TMIGD1/Sucrase-isomaltase immunofluorescence shows continuous apical microvilli localization in healthy differentiated cells, but not in affected mucosa. Conclusions: Despite the lack of

knowledge about TMIGD1 function, our study demonstrates that presence of an apical truncated form in epithelial cells is associated with cell differentiation and inflammation degree.

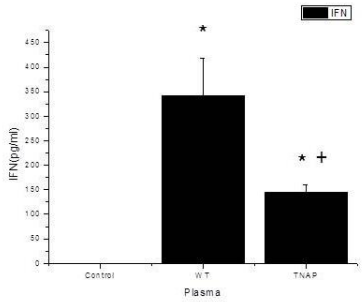
T88. Dual Role of Glucocorticoids on Intestinal Mucosa Integrity: Barrier Function Competence Versus Inflammation

Borja Ocon Moreno, Carlos J. Aranda, Maria Dolores Suarez Ortega, Olga Martinez Augustin and Fermin Sanchez De Medina López-Huertas. University of Granada, Granada, Spain

Glucocorticoids are widely used as anti-inflammatory drugs in inflammatory bowel disease. In the DSS induced model of colitis budesonide improves colonic inflammation, but it causes body weight loss and increased mortality. We hypothesized that glucocorticoids, and budesonide in particular, alter the mucosal barrier leading to these deleterious systemic effects. To test this hypothesis, C57BL6/J mice received budesonide (1-60 micrograms/day) by gavage and dextran sulfate sodium (DSS) in the drinking water for 7 days. A dose-dependent negative effect of budesonide, as shown by increased body weight loss, rectal bleeding and disease activity index, was observed. This effect correlated with higher bacterial translocation to mesenteric lymph nodes and liver, and higher concentration of LPS in the liver. Besides, animals receiving budesonide showed hallmark signs of sepsis: hypothermia, increased lung expression of iNOS and p-eNOS/eNOS ratio and myeloperoxidase activity, and higher levels of IL-18 and nitrates in plasma. Conversely, budesonide treated mice showed improved colonic inflammatory condition, studied by RT-PCR of colonic tissue. In line with this, budesonide inhibited the Th17-Th23 protective response axis within the mucosal compartment and additional data point to inhibition of epithelial cell proliferation (BrdU incorporation) and beta-catenin signaling. We conclude that budesonide exerts a dual effect in DSS-induced colitis, dampening intestinal inflammation but at the same time weakening mucosal barrier function.

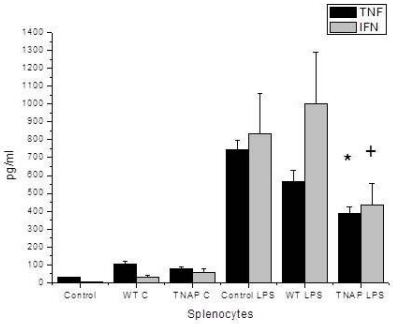
T89. T Lymphocytes from Tissue Non-Specific Alkaline Phosphatase Heterozygous Mice Induce a Milder Colitis in the T Cell Transfer Model of Colitis

Maria Gomez Belmonte[1], Cristina Hernandez Chirlaque[1], Patricia Martinez-Moya Bernal[1], Borja Ocon Moreno[1], Maria Dolores Suarez Ortega[1], Stefan Wirtz[2], Fermin Sanchez De Medina López-Huertas [1] and Olga Martinez Augustin[3]. [1]University of Granada, Granada, Spain; [2]Universitätsklinikum Erlangen, Erlangen, Germany



* p<0,05 vs Control
\+ p<0,05 vs WT

WT= TNAP +/+ C57BL/6J
TNAP= TNAP+/-



p<0,05 vs Control
* and + p<0,05 vs WT LPS

Intestinal and tissue non-specific alkaline phosphatase (IAP and TNAP) are coexpressed in mouse colon, and the latter predominates in inflammatory conditions. T cells from TNAP $^{+/-}$ mice (showing a dramatic drop in TNAP expression) produce less cytokines when stimulated than those from wild type animals *in vitro*. We tested the hypothesis that TNAP expressed in T lymphocytes is involved in the inflammatory response *in vivo*. RAG$^{-/-}$ mice were transferred with $CD4^+$ $CD62L^+$ cells, enriched in naïve lymphocytes, isolated by magnetic separation from spleen cells obtained from either TNAP$^{+/-}$ (TNAP $^{-/-}$ mice die early after birth) or the control TNAP $^{+/+}$ C57BL/6J mice. In this model the T cells transferred expand in the recipient mice and in the course of 4-8 weeks evoke a chronic inflammatory response in the large intestine. Our results indicate that colitis was attenuated in the animals transferred with TNAP $^{+/-}$ cells compared with those receiving wild type cells, based on macroscopic parameters (lower fibrosis), decreased plasma levels of IFNγ (57%) and dampened cytokine production by

splenocytes *ex vivo* (32-40% lower TNF and 57% lower IFNg). We conclude that TNAP of T lymphocytes modulates the inflammatory response in the mouse colon.

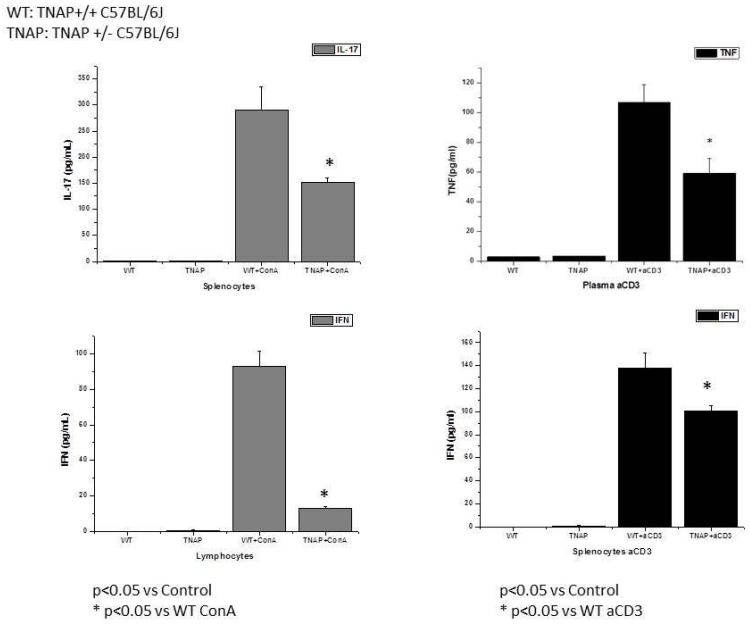
T90. Targeting Human CD2 by the Monoclonal Antibody CB.219 Reduces Intestinal Inflammation in a Humanized Transfer Colitis Model

Ulrike Erben[1], Nina Pawlowski[2], Katja Doerfel[3], Christoph Loddenkemper[4], Markus M. Heimesaat[1], Jörg Hoffmann[5], Britta Siegmund[1] and Anja Kühl[1]. [1]Charité Universitätsmedizin Berlin, Berlin, Germany; [2]Immatics Biotechnologies GmbH, Tübingen, Germany; [3]Cold Spring Harbor Laboratory, New York, NY; [4]Pathotres Joint Practice for Pathology, Berlin, Germany; [5]St. Marienkrankenhaus, Ludwigshafen, Germany

The cell adhesion molecule CD2 facilitates antigen-independent T cell activation. CD2 deficiency or blockade reduces intestinal inflammation in murine models. Transfer colitis induced by naúve CD_4-positive T cells expressing human CD2 (huCD2 tg) was treated with different monoclonal antibodies (mAb) specific for human CD2. Only one of the mAb, the clone CB.219 protected from severe colitis in a preventive treatment regimen. More importantly, therapeutic treatment ameliorated intestinal inflammation. Diminished intestinal tissue damage was paralleled by a profound suppression of the capacity of lamina propria lymphocytes to produce pro-inflammatory cytokines and tumor necrosis factor α as well as the neutrophil chemoattractant CXC-motif ligand 1 and the CC-chemokine ligand 3. Consequently, the infiltration with macrophages and T cells in the colonic mucosal tissue was low. Proposing that CB.219 prevents activation of engrafted T cells, we tested the antibody in huCD2tg mice orally infected with Toxoplasma gondii. While CB.219 did not affect the fulminant ileum inflammation, hence does not hamper the response to the pathogen, treated animals showed low levels of interferon g mRNA. Strongly suggesting a therapeutic potential for CB.219 in colitis treatment, this human CD2 mAb does not generally interferes with T cell-mediated inflammations but more specifically with the inflammation of the colonic mucosa.

T91. Tissue Non-Specific Alkaline Phosphatase Expression is Needed for the Full Stimulation of Lymphocytes

Cristina Hernandez Chirlaque, Maria Gomez Belmonte, Maria Dolores Suarez Ortega; Fermin Sanchez De Medina López-Huertas, Olga Martinez Augustin. University of Granada, Granada, Spain



Intestinal and tissue non-specific alkaline phosphatase (IAP and TNAP) are coexpressed in mouse colon, and the latter predominates in inflammatory conditions. It has been described that IAP dephosphorylates LPS and as a result prevents chemically induced colitis and metabolic syndrome. Because experimental colitis is differentially induced in $TNAP^{+/-}$ and wild type $TNAP^{+/+}$ mice ($TNAP^{-/-}$ mice die early after birth), we hypothesized that TNAP is involved in T cell activation. In order to explore this hypothesis, primary splenocytes and T lymphocytes isolated by negative magnetic separation were obtained from WT and $TNAP^{+/-}$ C57BL/6J mice and stimulated *in vitro* with concanavalin A. We also stimulated lymphocytes *in vivo* with anti-CD_3 antibody (50 µg/mouse i.p.). Cytokine production in primary splenocytes and cytokine plasma levels were studied. Cytokines were assessed by ELISA. Cells from $TNAP^{+/-}$ (with severely reduced TNAP expression) mice produced lower amounts of IFNg, IL-17 and IL-1β in response to concanavalin A. Stimulation of $TNAP^{+/-}$ with anti-CD_3 resulted in lower (45%) plasmatic TNFα levels and a decreased production of IFNg (28%) by splenocytes *ex vivo*. These results show that the lack of a TNAP allele hampers cytokine response and indicate a role for this enzyme in the immune response.

T92. Transfer of Alternatively Activated Macrophages Attenuates DNBS Colitis Independent of CCR2 or CX3CR1
Gabriella Leung, Arthur Wang and Derek McKay. University of Calgary, Calgary, AB, Canada

Alternatively activated macrophages (AAM) are considered anti-inflammatory cells. A transfer of AAMs has been shown to protect against experimental colitis. Our hypothesis is that transferred AAMs protect against colitis via CCR2-dependent migration to the gut. Methods. Bone marrow macrophages were stimulated with IL-4 for 48hr to induce alternative activation. *In vitro*, the polarization of WT, CCR2-KO, and CX3CR1-KO macrophages were compared phenotypically (PCR, flow cytometry) and functionally (arginase activity, nitric oxide production, cytokine secretion). *In vivo*, the ability of WT and KO AAMs were tested in their ability to block DNBS colitis. Cells were administered ip to WT mice 2d prior to the induction of colitis; mice were necropsied 3d later. Results. The lack of CCR2 or CX3CR1 did not impair the ability of macrophage to alternatively activate as observed by AAM mRNA expression of Arg1, Ym1, Retnla. AAMs were also similar in their functional parameters. In mice, both WT and KO transferred AAMs afforded protection against DNBS colitis as demonstrated by reduced colon shortening and reduced macroscopic/microscopic damage scores. Discussion. These data show that ip-transferred AAMs protect against DNBS colitis and do not require the expression of CCR2 or CX3CR1 to do so. As a treatment for inflammatory bowel disease, the results suggest that AAMs may be an effective therapy even in the absence of these chemokine receptors.

T93. The Immunological Function of Mesenteric Lymph Node in Diet Induced Obesity
Margarethe Smoczek[1], Isabelle Bekemeier[1], Stephan Schumacher[2], Wolfgang Bäumer[3], Melanie Bornemann[1], Ulrike Bode[1] and Manuela Buettner[1]. [1]Hannover Medical School, Hannover, Germany; [2]University of Veterinary Medicine, Hannover, Germany; [3]North Carolina State University College of Veterinary Medicine, Raleigh, NC

Obesity has emerged into a worldwide epidemic that is associated with cardiovascular disease, insulin resistance and probably intestinal inflammation. The mesenteric lymph nodes (mLN) are important for immune responses and acquisition of oral tolerance. However, the immunological status of mLN and their function in systemic inflammation after diet induced obesity is still unknown. Here we show that in a diet induced mouse model the cell subset composition in mLN change within 16-20 weeks. Analysis of the inflammatory response revealed that anti-inflammatory cytokines were reduced whereas pro-inflammatory cytokines such as IL-2 and IL-6 were increased. In addition, antigen specific immunoglobulines were induced. Although DNFB treatment as a model of allergic contact dermatitis showed no significant increased ear swelling in obese mice, mLN immune cells were strongly activated. This was characterized by increased homing on all lymphocyte populations. In summary, obesity resulted in mobilization of inflammatory cell subsets, an elevated level of pro-inflammatory cytokines and activated immune cells into mLN. These findings indicate a pivotal function of the mLN activating immune cells after diet induced obesity and provide new insights into the immunological mechanisms of obesity related systemic inflammation.

T94. T-Bet Expression by Th Cells Promotes Type 1 Inflammation but is Dispensable for the Clinical Manifestation of Murine Colitis
Jakob Zimmermann, Andreas Radbruch and Hyun-Dong Chang. German Rheumatism Research Center, Berlin, Germany

The transcription factor "T-box expressed in T cells" (T-bet) is highly expressed by Th cells isolated from the inflamed intestine of Crohn's disease patients. Previous analyses have suggested that T-bet is required for the induction of murine T cell-induced colitis. Here we demonstrate that this is not the case. T-bet-deficient Th cells efficiently induce colitis, with the clinical manifestation of weight loss, diarrhea and histopathological changes of the colonic mucosa. T-bet is regulating, however, the survival and positioning of the Th cells, their chemokine and chemokine receptor expression, their attraction of monocytes and macrophages into the inflamed colon, and the differentiation of monocytes and macrophages to the M1 type. T-bet-deficient Th cells show increased expression of IL-17, yet, remarkably, retain expression of IFN-γ. Th cell-derived IFN-γ, however, is not required to induce colitis, as we show using IFN-γ-deficient Th cells. Overall, we demonstrate that T-bet is not essential for the induction of murine colitis, but it is critical for the induction of type 1 inflammation.

T95. Neutralization of Pro-Inflammatory Monocytes by Targeting TLR2/6 Dimerization Ameliorates DSS-Induced Acute Colitis

Tegest Aychek[1], Liraz Shmuel-Galia[1], Avner Fink[2], Steffen Jung[1] and Yechiel Shai[1]. [1]Weizmann Institute of Science, Rehovot, Israel; [2]Harvard Medical School, Boston, MA

Rare Ly6Chi monocytes are present in the healthy intestinal lamina propria, where they give rise to resident CX_3CR1^+ macrophages that contribute to the maintenance of gut homeostasis. The same cells massively infiltrate the gut during challenge and become pro-inflammatory cells, recruited via CCR2 activated by TLR2 ligation. During DSS-induced colitis, these Ly6Chi monocyte-derived cells cause major damage to the tissue, including ulceration and sub-mucosal inflammation [1]. Here, we report a novel strategy to block TLR2 activation by inhibiting TLR2-TLR6 heterodimerization with the help of a TLR2 trans-membrane domain peptide (TLR2-p) [2]. Intra-peritoneal TLR2-p injection significantly ameliorated acute gut inflammation in DSS-treated mice. Reduction of colitis scores was accompanied by decrease of IL-23, IL-6, IL-12 and IFNg in the colon without affecting monocyte dynamics in the inflamed colon. Analysis of Ly6Chi monocytes isolated from gut of TLR2-p-treated mice revealed their decreased production of pro-inflammatory cytokines. Collectively, our data highlight the potential of TLR2-p treatment and blockade of TLR dimerization, as a novel therapeutic modality for inflammatory bowel disease (IBD). Ref.: (1) Zigmond, E. et al. Immunity 37, 1076 (2012); (2) Fink, A. et al. Jl 190, 6410 (2013).

T96. The Differential Role of Thymic Stromal Lymphopoietin (TSLP) Isoforms in Intestinal and Skin Pathologies

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Thymic stromal lymphopoietin is an IL-7 like cytokine produced by epithelial and stromal cells. TSLP has been shown to play a major role in homeostatic immune responses, mainly in the thymus and the gut. Conversely, overexpression of TSLP and TSLP-induced Th2 responses are also associated with allergic manifestations and with cancer progression. In humans, two isoforms of TSLP exist, the "long" and the "short" isoform. Even though the most well characterized form of TSLP is the long one, we found that the short isoform, always ignored, is readily expressed in various tissues. The short isoform is the only one expressed in healthy intestinal and skin tissue and it is downregulated in inflamed tissues. It also exerts anti-inflammatory activities by inhibiting the ability of DCs to respond to bacteria and to produce inflammatory cytokines. Consistently short TSLP administration in mice dampens inflammation after endoxin-shock and ameliorates experimental colitis. The long isoform is expressed only in disease conditions like ulcerative colitis and atopic dermatitis and is the isoform responsible for the induction of inflammatory Th2-like responses. In conclusion we showed that they have opposite expression patterns and elicit distinct immunological responses, probably explaining the dual role of the TSLP in the literature.

T97. TIMP1 Deficiency Aggravates the TNBS-Colitis Course in Mice and Promotes Post-Surgical Recurrence in Crohn's Disease

Arce Garcia-Jaraquemada, Anna Garcia-Hidalgo, Violeta Loren, Yamile Zabana, Farah Kamberovic, Eduard Cabré, Eugeni Domenech, Elisabet Pedrosa, Isabel Ojanguren and Josep Manye. Germans Trias i Pujol, Badalona, Spain

Introduction: The extracellular matrix (ECM) activity plays a key role in intestinal wound healing. Crohn's disease (CD) could disturb the intestinal balance between metalloproteinases (MMPs) and tissue inhibitor of MMPs (TIMP). Objective: 1) Study whether imbalances in ECM are related with early post-surgical recurrence (PSR) in the CD, and 2) its effect on TNBS-colitis. Methods:. ECM activity were measured by transcriptome analysis (RT-PCR validated) and protein microarrays from 20 CD ileo-cecal resections (5 of them with PSR<18 months post-surgery) and 10 controls. Paraffin embedded samples were stained with Masson'sTrichrome. Apart from that, TNBS-colitis course was assessed in wild-type and TIMP1-deficent mice by "*in vivo*" bioluminiscence imaging and tomography. Results: The transcriptome reflected a high activity of ECM in CD, while protein analysis showed increased levels of all TIMPs in ileo-cecal resections compared to control. In addition, TIMP1 levels significantly correlated with MMP1 [R^2=0.59] and MMP13 [R^2=0.58]. In contrast to non-early PSR, early PSR patients showed reduced levels of TIMP1 [4.2 (3.1-6.4) vs. 3.1 (2.0-8.1); mg/ml], TGFβ1 [7.7 (4.7-8.5) vs. 4.5 (1.7-5.5); 10^3 RU], and fibrosis [8 (5-10) vs. 4 (2-6)] Furthermore, TIMP1-deficient mice with TNBS-colitis showed higher bioluminiscence emission than wild-type mice (e.g. 6 days post-colitis: $6{,}93x10^4$ vs. $0{,}4x10^4$; $fot \cdot sr^{-1} \cdot cm^{-}$

[2]; p<0.05), and a greater transmural thickness (2.8 vs. 0.9 mm[2]) and stenosis [4 (2-5) vs. 0 (0-2)]. Conclusions: CD patients with early PSR show lower basal levels of intestinal TIMP1 and TGFβ1 than patients without early PSR. TIMP1 deficiency leads to more aggressive TNBS-colitis course.

T98. Glucocorticoids and Hypothalamic-Pituitary-Adrenal Interactions in the Modulation of Mucosal Immunity

Cristina Cardoso[1], Patrícia Reis de Souza[1], Helioswilton Sales-Campos[1], Paulo José Basso[1], Viviani Nardini[1], Vanessa Beatriz Freitas Alves[1] and Javier Emílio Lazo Chica[2]. [1]University of São Paulo, Ribeirão Preto, Brazil; [2]Federal University of Triangulo Mineiro, Uberaba, Brazil

Since immune responses in Inflammatory Bowel Disease (IBD) may be modulated by the hypothalamic-pituitary-adrenal axis (HPA), we aimed to understand the role of this neuroimmunendocrine interaction in experimental colitis. Therefore, C57BL/6 male mice were subjected to bilateral adrenalectomy and inflammation was induced by oral intake of water containing 3% dextran sulfate sodium (DSS). Colitis led to increased plasma corticosterone levels, which were lowered after adrenalectomy. The absence of endogenous glucocorticoids was associated to elevated disease clinical score and accumulation of CD_4 and CD8 T cells in lamina propria together with high local IFN-γ production, despite overall reduced infiltrate and accumulation of tolerogenic dendritic cells in gut mucosa. Furthermore, adrenalectomized mice exposed to DSS presented reduced accumulation of regulatory cells and increased susceptibility to colitis, as observed by the augmented mortality, which was not recued after exogenous glucocorticoid treatment. Finally, we concluded that HPA axis plays important role in the modulation of gut immunity, especially in experimental colitis.

T99. Aedes aegypti Salivary Gland Extract as Important Novel Therapeutic Option for Experimental Colitis

Helioswilton Campos[1,2], Paulo José Basso[1], Giuliano Bonfa[1,3], Viviani Nardini[1], Anderson Daniel Ramos[1], Anderson Sá-Nunes[1] and Cristina Cardoso[1]. [1]University of São Paulo, Ribeirao Preto, Brazil; [2]National Cancer Institute, Frederick, MD; [3]School of Medicine of Ribeirão Preto, Ribeirao Preto, Brazil;

Current therapies for inflammatory bowel disease (IBD) are not totally effective, resulting in continued disease burden for many patients. Mosquito saliva contains immunomodulatory molecules and therein could represent a novel therapy for IBD. To investigate the impact of Aedes aegypti salivary gland extract (SGE) on an experimental model of IBD, C57BL/6 male mice were exposed to 3% DSS in drinking water for 6 days and concurrently treated with SGE or PBS. On the 6th day, spleens and mesenteric lymph nodes (MLN) were harvested. Cytokine production and leukocytes profiles were assessed using flow cytometry. SGE treatment resulted in improved clinical disease outcome and postmortem scores. Within both the spleen and MLN, frequency of $CD_3^-CD49b^+$ cells was reduced. In the MLN, frequency of $CD_4^+CD25^+$ cells was increased. Cultured CD_4^+ cells from spleen and MLN of SGE-treated mice produced lower levels of IFN-γ, IL17, and IL4, when compared to CD_4^+ cells from PBS-treated mice (Fig. 3). These results indicate that SGE could be a source of immunomodulatory molecules with promising therapeutic activity for IBD.

T100. β7 Integrin Exacerbates Experimental Colitis in Mice by Directing Inflammatory Monocytes into the Colon

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Leukocyte recruitment is pivotal for the initiation and perpetuation of inflammatory bowel disease (IBD) and controlled by the specificity and interactions of chemokines and adhesion molecules. Interactions of the adhesion molecules α4β7 integrin and mucosal addressin cell-adhesion molecule-1 (MAdCAM-1) promote the accumulation of pathogenic T cell populations in the inflamed intestine. We aimed to elucidate the significance of β7 integrin expression on innate immune cells for the pathogenesis of IBD. We demonstrate that β7 integrin-deficiency protects recombination activating genes (RAG-2)-deficient mice from dextran sodium sulphate (DSS)-induced colitis and coincides with decreased numbers of colonic effector monocytes. We also show that β7 integrin is expressed on most $CD11b^+CD64^{low}Ly6C^+$ bone marrow progenitors and contributes to colonic recruitment of these pro-inflammatory monocytes. Importantly, adoptive transfer of $CD115^+$ WT monocytes partially restored the susceptibility of RAG-2/β7 integrin double-deficient mice to DSS-induced colitis, thereby demonstrating the functional importance of β7 integrin-expressing monocytes for the development of DSS colitis. We also reveal that genetic ablation of MAdCAM-1 ameliorates experimental colitis in RAG-2-deficient mice as well. In summary, we demonstrate a previously unknown role of α4β7 integrin/MAdCAM-1 interactions as drivers of colitis through directing of inflammatory monocytes into the colon.

T101. HDACs and Inflammatory Bowel Disease

Jacqueline Keye[1], Marie Sittig[1], Martin Wetzel[1], Elena Sonnenberg[1], Paolo Mascagni[2], Rainer Glauben[1] and Britta Siegmund[1]. [1]Charité Universitätsmedizin Berlin, Berlin, Germany; [2]Italfarmaco, S.p.A., Cinisello Balsamo, Italy

Inflammatory bowel disease is characterized by an overwhelming immune response causing an imbalance of the gut homeostasis. The etiology is still unknown and current therapies go along with severe side effects. While our group has already shown that pan-HDAC inhibitors ameliorate experimental colitis, we here further characterize this effect on a cellular level and analyze the role of specific HDAC in the inflammatory process. In the presence of the HDAC inhibitor ITF2357, the generation of $FoxP3^+$ cells from naïve T helper cells was enhanced, the polarization to the pro-inflammatory Th17 cells suppressed, which was paralleled by a profound down regulation of the IL6 receptor. In macrophages, ITF2357 treatment leads to a dose-dependent down regulation of pro-inflammatory cytokines. *In vitro* experiments revealed HDAC5 dependent changes in the inflammatory profile of T cells and macrophages and PCR analysis disclosed HDAC5 as differentially expressed in polarized T helper cells. Further experimental colitis models demonstrate a more severe disease pattern for HDAC5 knockout mice compared to wild type mice. This was shown by an increased histological disease score, higher weight loss and severe intestinal bleeding. Isolation and analysis of lamina propria mononuclear cells confirmed these results by demonstrating alterations in the T cell composition. This study demonstrates, that inhibition of HDAC results in an anti-inflammatory phenotype in both, cells from the adaptive as well as the innate immune system. Furthermore, our data suggest a critical role of HDAC5 in the pro-inflammatory immune response in general and in particular in the pathogenesis of Colitis.

T102. Wiskott–Aldrich Syndrome Protein (WASP) Regulates Anti-Inflammatory Macrophages in Mice and Humans and is Critical for Maintenance of Mucosal Homeostasis

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Patients with Wiskott-Aldrich syndrome develop recurrent infections, thrombocytopenia and eczema. Additionally, the majority of patients develop autoimmune sequalae and up to 10% of patients develop inflammatory bowel disease (IBD). WASP-deficient ($Was^{-/-}$) mice similar to human also develop spontaneous colitis. We found that WASP is critical for innate immune cell-mediated maintenance of intestinal homeostasis. Recently WASP was identified in an IBD causal sub-network and predicted to have potential role in regulating anti-inflammatory macrophage (Mφ) function (Jostins et al., Nature 2012). Therefore, we investigated the role of WASP in the generation/function of anti-inflammatory Mφ. Compared to wild-type mice, pre-colitic 5-week old $Was^{-/-}$ mice had an increased percentage of pro-inflammatory Mφ ($Ly6c^{hi}MHCII^{hi}$) and concomitant decrease in anti-inflammatory Mφ ($Ly6c^{-/low}MHCII^{hi}$) in the gut. Similarly, in mixed bone-marrow chimera experiment $Was^{-/-}$ compartment had more pro-inflammatory Mφ and less anti-inflammatory Mφ in the gut compared to wild-type compartment. In addition *in vitro* generation of M2 Mφ from bone marrow derived Mφ was also aberrant in $Was^{-/-}$ mice. $Was^{-/-}$ M2 Mφ produced more pro-inflammatory cytokines and induced higher T cell proliferation, and less Treg generation compared to WT M2 Mφ. We also observed that only WT M2 Mφ, but not $Was^{-/-}$ M2 Mφ, protected against colitis mediated by $CD4^+$ T cells in $Was^{-/-}Rag2^{-/-}$ mice. Similarly, the generation of M2 Mφ was also impaired in WAS patients producing elevated pro-inflammatory cytokines and were less efficient in promoting Treg generation. These data suggest that WASP plays a critical role in generation/function of anti-inflammatory Mφ in both mice and humans.

T103. Discovery and Characterization of Novel, Oral Peptide Antagonists of Human IL-23 Receptor that are Efficacious in a Rat Model of IBD

Xiaoli Cheng, Ashok Bhandari, Greg Bourne, Brian Frederick, Jenny Zhang, Jaimee McMahon, Sonya Scott, Herodion Celino, Genet Zemede, Natalie Spencer, Vinh Tran, Lu Bai, Thamil Annamalai, Mark Smythe, Larry Mattheakis and David Liu. Protagonist Therapeutics Inc., Milpitas, CA

Genome-wide association studies have demonstrated significant association of the IL-23 receptor (IL-23R) gene with inflammatory bowel disease (IBD) suggesting that perturbation of IL-23 signaling could be relevant to the pathogenesis of the disease. Here, we propose to modulate the IL-23 pathway through selective antagonism of IL-23R by oral treatment with peptides that are stable and restricted to the gastrointestinal (GI) tissue. Using a combination of medicinal chemistry and phage display, we have identified inhibitory peptides that are uniquely resistant to oxidative/reductive conditions and proteolytic degradation in a variety of assays that mimic the various compartments of the GI environment. Functionally, these peptides potently neutralize IL-23 mediated

signaling in a transformed human cell line and in human primary cells. The binding for IL-23R is selective since they do not antagonize the IL-12 signaling pathway. Furthermore, we have shown that these orally delivered peptides are efficacious in attenuating colitis in a DSS induced acute rat model of IBD as shown by a significant reduction in the disease activity index score, ratio of colon weight to length and colon macroscopic score. Overall, these data support the therapeutic potential of GI restricted IL-23R antagonists for the treatment of IBD.

T104. Probiotics in Crohn's Disease: Selecting Strains Able to Trigger Paneth Cells-Derived Antimicrobial Response and Down-Regulate Inflammatory Response

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Rationale: Paneth cells from Crohn's disease patients showed impaired secretion of antimicrobial peptides, which may contribute to an inappropriate mucosal immune response. Herein, we identified novel anti-inflammatory probiotic strains that are able to modulate the functionality of Paneth cells. Methods: The capacity of selected probiotic strains to induce defensin expression was tested *in vitro* using the murine epithelial cell line mICcl2. Induction of activation markers (CD40, CD80, CD86, MHCII) on bone-marrow dendritic cells (BMDC) after 24 hours of coculture with bacterial strains was followed by flow cytometry. The potential of probiotic strains to promote either Th17 or Tregs was evaluated by coculture of naive $CD4^{+}CD25^{-}$ cells with probiotic-primed dendritic cells. Mouse model of Citrobacter rodentium infection was used for testing the capacity of probiotic strains to decrease inflammation *in vivo*. Results: Strain and time dependent differences in the gene expression of defensins in mICcl2 were observed. Some strains E. coli, L. Reuteri or L. acidophillus were good inducers of defensin expression. L. acidophillus strains were the most potent inducers of activation markers on dendritic cells and the best inducer of IL-17 after six-day coculture of primed BMDC and naive CD4 cells, while L. reuteri and L. paracasei strains were the best inducers of Tregs. The aforementioned probiotic strains induced the colonic expression of several defensins *in vivo* and showed anti-inflammatory effects in response to C. rodentium. In conclusion, we have identified several promising probiotic strains for the treatment of Crohn's disease. This work was supported by the ANR project BIOpaneX.

T105. Role of SMAD7 in the Intestinal Epithelium for Gut Homeostasis and Tumor Development

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Besides the function as an inhibitor of the TGF-ß signaling pathway, polymorphisms of SMAD7 play a crucial role in increasing the risk for the development of colon cancer. In order to analyze the role of epithelial Smad7for colon cancer development we generated mice with a conditional knockout for Smad7 in intestinal epithelial cells ($Smad7^{\Delta IEC}$). $Smad7^{\Delta IEC}$ mice were born at mendelian ratio and did not show defects in the postnatal development of the gut. We next subjected $Smad7^{\Delta IEC}$ mice to an experimental model of colitis associated cancer (AOM-DSS model). Surprisingly, $Smad7^{\Delta IEC}$ mice developed fewer tumors in comparison to the control group ($Smad7^{fl.}$). To investigate whether the decreased number of colon tumors in $Smad7^{\Delta IEC}$ mice was dependent on colitis in the AOM-DSS model, we next used a sporadic tumor mouse model (APCmin). Comparison of $APCmin^{+/-}$ $Smad7^{\Delta IEC}$ and control mice ($APCmin^{+/-}$) revealed that $APCmin^{+/-}$ $Smad7^{\Delta IEC}$ mice not only showed a higher survival rate but also a reduced number of tumors. These findings strongly support our hypothesis that Smad7 plays a crucial role in the development of colon tumors independently from inflammation.

T106. Fine-Tuning the Balance of Mucosal Immunity by Protein Phosphatase 4

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The gut micro-environment is maintained by balancing inflammatory responses, which drive off the pathogens, and immune suppressions, which prevent tissue damages. This balance is kept through the complex interactions between innate, adaptive, and innate-like immune cells. Results from mouse models suggest that Treg cells play an important role in this process by negatively regulating the activities of many cell types. Our recent report showed that protein phosphatase 4 (PP4) is essential for Treg homeostasis, homing, and functions; moreover, T cell-specific ablation of PP4 resulted in the onset of spontaneous rectal prolapse and colitis. Here, we demonstrated that PP4 ablation also caused severe defects in T cell proliferation and adaptive immunity. These defects resulted from a partial G1-S block in activated PP4-deficient T cells, and were further associated with

altered ROS induction and AMPK activation. These data provide an additional perspective to suggest that, in addition to Treg defects, defective T cell responses may also contribute to the spontaneous colitis in mice with T cell-specific ablation of PP4.

T107. Differential Requirements for IL-17A and IL-22 in Cecal Versus Colonic Inflammation Induced by Helicobacter Hepaticus
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Th17-type cytokines have been implicated in the pathogenesis of inflammatory bowel disease (IBD), but no information is available regarding the contribution of these cytokines to pathology in different regions of the gut. To address this issue, we have used the Helicobacter hepaticus typhlocolitis model to investigate the role of IL-17A, IL-17F, and IL-22 in cecal versus colonic inflammation. We report that cecal, but not colonic pathology in C57BL/6 mice inoculated with H. hepaticus plus anti-IL-10R mAb is exacerbated by co-administration of anti-IL-17A mAb, suggesting a disease-protective role for IL-17A in the cecum. In contrast, anti-IL-17F had no effect on H. hepaticus-induced large intestinal pathology. Neutralization of IL-22 prevented the development of colonic, but not cecal inflammation in H. hepaticus-infected anti-IL-10R-treated mice, demonstrating a pathogenic role for IL-22 in the colon. By assessing transcript levels, we provide evidence that differential expression of IL-22R, IL-22BP and IL-23R between cecum and colon may help explain why these tissues respond differently following anti-IL-22 treatment. Analysis of microarray data from healthy human intestine further revealed significant differences in cytokine receptor transcript levels (including IL22RA1 and IL23R) in distinct parts of the human gut. Together, our findings demonstrate that individual Th17-type cytokines can have pro- or anti-inflammatory effects in different regions of the intestine, an observation that may have implications for interventions against human IBD.

T108. Irf5$^{-/-}$ Mice are Protected from Helicobacter hepaticus $^{+}$ Anti IL10 Receptor mAb-Mediated Intestinal Inflammation
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IRF5 was first described as a regulator of the innate antiviral response and cell cycle. More recently, IRF5 has been shown to polarize macrophages to a pro-inflammatory state which control Th1/Th17 responses, as well as act as a biomarker of inflammatory macrophages in the inflamed mouse knee. Gain of function IRF5 mutations predisposes patients to autoimmune conditions such as rheumatoid arthritis, systemic lupus erythematosus, and inflammatory bowel disease (IBD). IBD is a debilitating condition of the gastro-intestinal tract, where patients present with neutrophilia and Th1/Th17 responses. Here we show a fundamental role for IRF5 in the development of intestinal inflammation in an IL23-driven model of colitis. Irf5$^{-/-}$ mice infected with Helicobacter hepaticus in the absence of IL10 receptor signaling developed a strongly reduced colitis phenotype compared to wild-type controls, evidenced by lower histopathology and reduced infiltration of leukocytes. Therefore we conclude that IRF5 may present an attractive target for modulation of IBD.

T109. NEMO Maintains Intestinal Homeostasis by Preventing Epithelial Cell Apoptosis and Necroptosis
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The IKK/NF-κB signaling pathway regulates immune and inflammatory responses by inducing the expression of pro-inflammatory mediators, but also protects cells from cytokine-induced death. We showed previously that deletion of NEMO/IKKγ, the regulatory subunit of the IKK complex that is essential for activation of canonical NFkB signaling, in the intestinal epithelium of mice (NEMO^{IEC-KO} mice) caused the development of spontaneous chronic inflammatory colitis. Therefore, in contrast to the well-established pro-inflammatory function of the IKK/NF-κB pathway, inhibition of NEMO-dependent signaling in the intestinal epithelium triggered inflammation. Here we addressed the mechanisms by which NEMO knockout in IECs induces colitis. We show that colitis in NEMO^{IEC-KO} mice is dependent on the presence of the intestinal microbiota and is mediated by intestinal epithelial specific TNFR1 signaling. Furthermore we provide genetic evidence that IECs of NEMO^{IEC-KO} mice undergo cell death via both RIPK3-mediated necroptosis and FADD-dependent apoptosis. Inhibition of both apoptosis and necroptosis by combined knockout of FADD and RIPK3 protected NEMO^{IEC-KO} mice from intestinal epithelial cell death and inflammation. Therefore NEMO dependent signaling in the intestinal epithelium is essential to maintain intestinal homeostasis by preventing apoptosis and necroptosis.

T110. Dietary Porcine Plasma Protein Attenuates Inflammatory Severity in the mdr1a$^{-/-}$ Mouse Model of Colitis.
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We have previously shown that spray-dried plasma (SDP) can modulate the immune response in a rat model of acute intestinal inflammation. We now examine the effects of SDP in mdra1-/- (KO) mice lacking the multiple drug resistance gene, that develop spontaneous colitis Animals were supplemented with SDP (8% w/w) or milk proteins (Control) from weaning until day 56. The permeability of the colon mucosa was analyzed at functional and structural levels. The expression of different cytokines and the percentages of activated and regulatory T cells were also determined. Colonic permeability was increased in KO mice ($P<0.05$) in this was prevented by SDP supplementation ($P<0.05$). KO mice showed increased percentage of activated Th lymphocytes ($P<0.05$) that was attenuated by SDP ($P<0.05$). The expression of cytokines (TNFα, IFNγ, IL2, IL17), chemokines (MCP1, MIP1b) and iNOS in the colon was significantly increased but SDP could reduce them by 15-50% ($P<0.05$). Interestingly, SDP increased the expression of mucosal IL10 ($P<0.05$) as well as the percentage of regulatory Th lymphocytes in the lamina propria ($P<0.05$). We conclude that dietary supplementation with SDP is able to prevent in part the changes observed in the colon of mdr1 a KO mice during the development of colitis.

T111. Differential Regulation of IL-33 by NOD2 as a Potential Mechanism in the Pathogenesis of Chronic Intestinal Inflammation
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IL-33 is important in several chronic inflammatory disorders, including inflammatory bowel disease (IBD). NOD2 (nucleotide oligomerization domain protein 2) is a critical intracellular protein that senses bacteria and helps maintain normal gut homeostasis; it is also the major gene linked to Crohn's disease (CD). We determined whether NOD2 genetic variants differentially regulate IL-33 and whether genetic deletion of NOD2 may have an impact on an experimental model of CD-like ileitis. RAW264.7 mouse macrophages were stably transduced to express human WT, L469F, and R334Q NOD2 variants. IL-33 mRNA and protein levels were measured by qPCR and Western blots following stimulation with MDP, the bacterial ligand for NOD2. For *in vivo* studies, we evaluated the effects of NOD2 genetic deletion in SAMP1/YitFc (SAMP) mice, a spontaneous model of CD. Our results showed that although Il33 was increased after MDP stimulation in both WT ($P<0.005$) and L469F NOD2 macrophage variants ($P<0.0002$), no difference in MDP-induced Il33 was measured between these two groups. However, Il33 was significantly downregulated in R334Q NOD2 macrophages after MDP stimulation compared to both WT ($P<0.01$) and L469F NOD2 ($P<0.003$). In fact, in regard to IL-33, macrophages expressing the R334Q NOD2 variant did not respond at all to MDP. Analysis of IL-33 protein confirmed these trends. In addition, genetic deletion of NOD2 from SAMP mice resulted in a significant decrease in IL-33 ($P<0.001$), Th2 cytokines ($P<0.01$, and ileal inflammation ($P<0.01$). Our results show that IL-33 regulation by NOD2 may represent an important mechanism in the pathogenesis of chronic intestinal inflammation.

F127. The Role of Cdcs1 and Naive T Cell Subsets in the Onset of Colitis
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The interleukin-10-deficient mouse (Il10$^{-/-}$) is a suitable model for human inflammatory bowel disease research. It is known that the colitis severity in this model depends on the background strain. In a quantitative trait loci (QTL) analysis between B6.129P2/JZtm-Il10^{tm1Cgn} (B6-Il10$^{-/-}$) and C3Bir.129P2/JZtm-Il10^{tm1Cgn} (C3Bir-Il10$^{-/-}$) animals, 10 QTL, called cdcs1 (cytokine deficiency induced colitis susceptibility) to cdcs10 were identified. By breeding congenic and subcongenic strains, cdcs1 derived from C3Bir-Il10$^{-/-}$ was identified as the major modifier and the cause for an adaptive hyper-responsiveness. Here we show that naive CD4$^+$ T cell subsets are able to induce colitis depending on the cdcs1 region. In a transfer colitis of different naive T cell subsets (CD4$^+$CD62L$^+$, CD4$^+$CD45^{rbhigh}, CD4$^+$CD25$^-$), cells with parts of cdcs1 derived from C3Bir-Il10$^{-/-}$ were able to induce much more severe colitis compared to cells derived from B6-Il10$^{-/-}$. There was no difference between the used subsets and an influence of Il-10 producing regulatory T cells could be excluded. Furthermore, we showed that the delay until the onset of colitis depends on the number of transplanted cells. A microarray analysis of naive T cells revealed 47 differently expressed probes when comparing susceptible with resistant strains. The combination of a transfer colitis model and a microarray analysis is a promising approach to identify causative genes for colitis susceptibility within cdcs1.

INNATE LYMPHOID CELLS

OR.85. Crucial Role of Lymphotoxin Produced by ILC3 in the Development of Intestinal Adaptive Immune Responses and Autoimmunity

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Intestinal homeostasis depends on complex interactions between the microbiota, the intestinal epithelium and the host immune system. Induction of proper intestinal adaptive immune responses in the gut upon colonization of the gut with commensals ensures protection against invading pathogens, maintenance of epithelial barrier integrity and control of microbiota. However, mechanisms governing these responses still remain poorly defined. Here we report critical function of surface $LT\alpha_1\beta_2$ expressed by RORγt$^+$ innate lymphoid cells (ILC3) in controlling intestinal B and T cells in the absence of gut-associated lymphoid tissue (GALT). First of all, ILC3-derived $LT\alpha_1\beta_2$ is crucial for IL-6, IL-12 and IL-23 production by gut dendritic cells in naive state and during inflammation, resulting in defective production of IL-22 from T lymphocytes and ILC3s. Next, surface $LT\alpha_1\beta_2$ produced by RORγt$^+$ ILC controls T cell-independent IgA induction in the small intestine, resulting in the exclusive T cell-dependent IgA plasma cells induction in the gut. IgA produced by plasma cells from mice lacking $LT\alpha_1\beta_2$ expression on ILC3 exhibits enhanced reactivity to the commensal microbiota, leading to altered microbiota composition. Finally, LTα1β2 produced by ILC3 prevents development of autoantibodies via microbiota-dependent mechanism. Altogether, these data ascribe novel essential function for membrane-bound lymphotoxins produced by ILC3 in organizing adaptive immune responses in the gut, in the control of the commensal microbiota and subsequent development of autoimmunity.

OR.86. ICOS Signaling Regulates Expansion of Group 2 Innate Lymphoid Cells Under Homeostatic and Inflammatory Conditions

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Group 2 innate lymphoid cells are innate effectors playing an important role in the defense against helminthic infections and in the pathogenesis of allergic inflammation. Cytokines have been identified as the major stimuli driving ILC2 activation and expansion. Conversely, it is not clear whether costimulatory molecules contribute to regulation of ILC2 functions. ILC2 display high expression of Inducible T cell costimulator (ICOS), a costimulatory molecule belonging to the CD28 superfamily, which has been shown to control late effector T cell functions, and is of upmost importance for the humoral immune response. However, the biological function of ICOS expression on ILC2 is unknown. Here we show that ICOS but not CD28 signaling regulates ILC2 homeostasis independent of T cells and B cells, by promoting proliferation and accumulation of mature ILC2 as well as their activation and expansion under inflammatory conditions. Our data identify for the first time a role of ICOS in the innate immune system and indicate that not only cytokines but also costimulatory pathways can regulate the ILC2 pool. Thus, ICOS costimulation blockade which is currently under clinical evaluation for inhibiting the humoral immune response would also enable targeting of innate inflammatory circuits.

OR.87. Type 3 Innate Lymphoid Cells Maintain Intestinal Epithelial Stem Cells After Tissue Damage

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Disruption of the intestinal epithelial barrier allows bacterial translocation and predisposes to destructive inflammation. To ensure proper barrier composition, crypt-residing stem cells continuously proliferate and replenish all types of intestinal epithelial cells within days. As a consequence of the high mitotic activity of stem cells, the intestines are highly susceptible to chemotherapy-induced damage. The cellular mechanisms that control tissue protection and mucosal healing in response to intestinal damage are incompletely understood. Type 3 innate lymphoid cells (ILC3) are regulators of homeostasis and tissue responses to infection at mucosal surfaces. Therefore, we hypothesized that ILC3 could be involved in intestinal epithelial recovery after insult. We

demonstrate that ILC3 are required for epithelial STAT3 activation in response to small intestinal tissue damage inflicted by the chemotherapeutic agent methotrexate (MTX). Multiple cytokines are increased in distinct subsets of ILC3 early after MTX and absence of ILC3 leads to increased pathology and severe damage to the intestinal crypts. Using ILC3-deficient Lgr5 reporter mice we show that the maintenance of intestinal stem cells after damage is severely impaired in the absence of ILC3. These data unveil a novel function of ILC3 in limiting tissue damage by preserving tissue-specific stem cells.

OR.88. Coordinate Regulation of Inflammatory Responses by ILC3 and gp38$^+$ Colonic Stromal Cells During Chronic Intestinal Inflammation

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A dynamic interplay between innate lymphoid and tissue stromal cells governs the development and function of lymphoid organs. Although many of the mechanisms underlying these interactions are now well defined, little is known relating to such pathways in non-lymphoid tissues, particularly in the context of human inflammatory disease. Here we examined innate lymphoid cell (ILC) and intestinal stromal cell (iSC) interactions during inflammatory bowel disease (IBD) in humans and mice. We identified subsets of 'lymphoid like' iSCs in colonic tissue and found that stable phenotypic inflammatory alterations in a population of gp38$^+$ cells accompanied human IBD. Activated gp38$^+$ iSCs potentiated IL-12/23 production by myeloid cells in a 3D organotypic intestinal culture model and IL-23-responsive human ILC3s regulated colonic stromal cell activation *in vitro*, driving the development of gp38$^+$ICAM-1^{hi}VCAM-1^{hi} cells with enhanced expression of TNF and CSF2. Experimental therapeutic intervention using Remicade® (Infliximab-IFX) partially blocked human iSC activation mediated by ILC3s *in vitro*. We found an elevated frequency of TNFα-producing ILCs during human IBD, and colonic TNFα$^+$ ILC3s expanded in an innate murine colitis model, alongside highly proliferative ICAM-1^{hi}gp38$^+$ iSCs. Depletion of ILCs or therapeutic TNFα-blockade *in vivo* attenuated several parameters of colonic gp38$^+$stromal cell activation, including proliferation, ICAM-1 expression and GM-CSF production. Whole-tissue transcriptional analysis revealed that an ILC-iSC activation signature accompanied human IBD, correlating with both disease severity and patient IFX responsiveness. Thus, IL-23 responsive ILC3s drive TNFα-dependent gp38$^+$ inflammatory stromal cell activation during intestinal inflammation, with such pathways providing potential novel avenues for therapeutic development in human IBD.

T117. iCD8α Cell-Deficiency Increases Susceptibility to Intestinal Inflammation in the Anti-CD40 Antibody Colitis Model

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iCD8α cells constitute a population of innate immune cells present in close association with intestinal epithelial cells. Our previous work showed that iCD8α cells play a critical role during immune responses in the intestinal mucosa. For example, iCD8α cells are relevant for conferring protection against Citrobacter rodentium colonization of the colon. However, little is known about the possible role of iCD8α cells during inflammatory processes of the intestines. Using the anti-CD40 antibody model of colitis, we show that mice with reduced numbers of iCD8α cells (H2-T3$^{-/-}$Rag-2$^{-/-}$) lose more weight, have higher mortality and present more colon damage than mice with sufficient iCD8α cell numbers (Rag-2$^{-/-}$). Moreover, we show that iCD8α cell-deficient mice have higher levels of IFN-γ, IL-6 and osteopontin production in the colon. These results indicate that iCD8α cell-deficiency has a substantial effect on inflammatory processes of the intestines.

T118. Dedicated Immunosensing of Mouse Intestinal Epithelium Facilitated by Genetically Coupled Lectin-Like Receptors

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The integrity of the intestinal epithelium is constantly surveyed by innate-like, intraepithelial T lymphocytes (IELs). IELs are thought to act as "first-line" sentinels sensing the state of adjacent epithelial cells via both T cell receptors and auxiliary receptors. The latter include C-type lectin-like receptors (CTLR) encoded in the natural killer gene complex (NKC) such as NKG2D. We report fairly exclusive expression of the CTLR Nkrp1g by a subpopulation of mouse IELs enabling them to monitor the intestinal epithelium through ligation of the genetically coupled CTLR Clr-f, almost exclusively expressed on differentiated intestinal epithelial cells (IECs). As Clr-f expression strongly inhibits effector functions of Nkrp1g-expressing cells and is upregulated upon poly(I:C)

challenge, Clr-f molecules may quench reactivity of Nkrp1g$^+$ IELs towards the epithelial barrier. Of note, we also found that the orphan CTLR Clr-a, the closest Clr-f relative, is exclusively expressed by IECs. Using newly generated antibodies and in situ immunofluorescence, we localized Clr-a molecules in the small and large intestines showing that Clr-a and Clr-f are also highly related with regard to tissue expression. Altogether, we characterized genetically linked CTLR with an intestine-associated expression that apparently evolved to allow for a dedicated immunosurveillance of the mouse intestinal epithelium.

T119. Metabolites from Commensal Microbes Contribute to Establish the Immunity Comprising RORγt$^+$ ILC3-Treg Axis in Ileum Peyer's Patch

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Innate lymphoid cells (ILCs) are the most recently characterized cell populations by lymphoid cell morphology and do not express both somatically rearranged antigen receptors and cell lineage markers. The ILC family is categorized into three groups according to the characteristics of cytokine secretion and transcription factor expression. Among them, ILC3 population is heterogeneous and represents all RORγt$^+$ ILC subtypes. Although ILCs are small population in tissue, their strategic location at mucosal barriers enables them to regulate mucosal immune responses. Recent studies suggest the ILCs as crucial responder to metabolic stress although it was poorly understood. We have been interested in the association between Peyer' patch ILCs and butyrate, one of the metabolites from commensal microbiota, because it was reported as a modulator for Th17 and Treg cells in lamina propria. We monitored the level of butyrate and distribution of ILCs and Treg cells in ileum Peyer's patch and suggest that butyrate can contribute to maintain mucosal homeostasis through regulation of RORγt$^+$ ILC3-Treg axis in mucosal immunity. (S.-H. Kim, Y. N. Kim, H.-Y. Lee, and J. Park were supported by BK21 Plus program in the Department of Bioactive Material Sciences.

T120. Regulation of Group 2 Innate Lymphoid Cells

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Viral respiratory tract infections are the major causative agent of infection-induced asthma onset and asthma exacerbations. Despite their medical and economic impact, the mechanisms that regulate these conditions and associated pathologies remain elusive. Here we show that deficiency of type I interferon (IFN) receptor signaling in a mouse model of influenza infection results in infection-associated type 2 immunopathology and tissue fibrosis driven by deregulated priming of group 2 innate lymphoid cells (ILC2). Type I IFNs directly regulated ILC2 in an IFN-stimulated gene factor 3 (ISGF3)-dependent manner that led to reduced expression of GATA binding protein 3 (GATA3), altered cytokine production and cell proliferation, as well as increased cell death. Type I IFNs further induced IFNg *in vivo*, which directly altered ILC2 function in a signal transducer and activator of transcription 1 (STAT1)-dependent manner. Collectively, these results demonstrate that type I and type II IFNs synergistically restrict ILC2 and thereby limit type 2 immunopathology and fibrotic disease.

T121. Tissue-Resident NK Cells in Human Lung

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Natural Killer (NK) cells are the predominant innate lymphocyte population in human lung tissue. However, tissue-resident NK cells in the lung remain undescribed, and their role in physiological and pathological conditions is unknown. Here we characterize tissue-resident NK cell subsets in human lung tissue by multicolor flow cytometry. Tissue-resident NK cells can be identified by CD69, CD103, and CD49a. Whereas on bulk level terminally differentiated NK cells are accumulated in the lung as compared to matched peripheral blood, tissue-resident lung NK cells appear immature. In 10-15% of the donors we found a clonal-like expansion of tissue-resident NK cells, and accumulation of CD69$^+$ NK cells correlated strongly with decreased lung function. Finally, clonal-like expanded tissue-resident NK cells strongly expressed NKG2C, indicating a role for a preceding viral infection in shaping this NK cell population. Whereas lung NK cells are normally suppressed, activated clonal-like NK cell expansions might exert bystander cytotoxicity against healthy tissue and contribute to pathological conditions. We expect that our data will help in understanding the role of NK cells in the development and progression of lung diseases and improve the diagnosis and treatment strategies.

T122. Characterization of Intestinal Innate Lymphoid Cells
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Innate Lymphoid Cells (ILCs) derive from a lymphoid progenitor. They are divided into three groups based on the transcription factors and cytokines they express: ILC1, ILC2 and ILC3. We have recently shown that intestinal ILCs traffic to mesenteric lymph nodes (MLNs) where they populate the interfollicular area. To investigate the properties of migratory ILCs, we isolated cells from the small intestine (SI), colon and MLNs of C57BL/6 mice. ILC subsets were identified by staining for T-bet, GATA-3 and Roryt, and their proliferative capacity was assessed. ILC2s comprise the main population of ILCs in the colon (4,5% of CD45+ cells), whereas the SI contains mainly ILC2s and ILC3s. Strikingly, a population of Roryt and T-bet co-expressing ILCs is increased in the SI. However all three ILC populations are found at similar frequencies in the SI and colon-draining regions of the MLNs. Furthermore, the proliferative capacity of ILCs differs greatly between the ILC subsets and tissues; the ILC1 population in the MLNs is highly proliferative compared to ILC3s in the gut and MLNs. These data indicate that different mechanisms maintain steady state intestinal ILC populations. Future investigations will aim to uncover the functions of the migratory ILCs in the intestine.

T123. IL-15 and Notch Drive the Differentiation of a New Subset of a New Subset of Gut Innate Lymphoid Cells
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Recent work has highlighted how a spectrum of innate lymphoid cells (ILC) present in the intestinal lamina propria play a key role in the complex network of innate and adaptive immune mechanisms that control intestinal homeostasis and protect the gut against pathogens. Herein we intend to characterize a yet poorly defined population of ILC present in the gut epithelium and which may be the cellular origin of the rare and unusual lymphoma that complicates celiac disease (CD). They also represent approximately 50% of the intraepithelial lymphocytes at birth, pointing to a possible role in local defense when adaptive intestinal immunity is still weak. Our data indicate that those intraepithelial ILC (IE-ILC) form a distinctive subset of ILC both in human and mouse characterized by their expression of the CD103 integrin, the presence of intracellular $CD3$ chains ($iCD3^+$) which remains absent from the surface $CD3$ ($sCD3$-) and the presence of TCR rearrangements. This subset has overlapping features with NK cells and a strong dependence on interleukin-15 (IL-15) for their maintenance and their differentiation. In addition, we have deciphered the mechanism, by which IL-15 blocks the differentiation of lymphoid precursors into T cells and promotes their development into cytotoxic IE-ILC.

T124. Adaptation of Innate Lymphoid Cells to Nutrient Deprivation Promotes Type 2 Barrier Immunity
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Survival of the host relies on the establishment of a functional barrier immune defense that must be maintained in fluctuating states of food availability. Vitamin A deficiency is one of the most common nutrient deficiencies associated with defects in adaptive immune responses and profound immunosuppression. We found that vitamin A deficiency results in impaired immunity against bacterial infection accompanied by a fundamental decrease in IL-22 producing type 3 innate lymphoid cells (ILC3). Paradoxically deprivation of vitamin A results in a profound expansion of IL-13 producing ILC2 that confer enhanced type 2 immunity to helminth infections. Such changes in intestinal barrier immunity correlate with altered metabolic requirements of intestinal ILC2, suggesting a metabolic adaptation of ILC mediated barrier immunity. Thus the essential nutrient vitamin A regulates an inverse balance between intestinal ILC subsets promoting ILC3 while directly suppressing IL-13 producing ILC2. These results reveal ILC as primary sensors of the nutritional status and signify a powerful adaptation of the immune system during malnutrition to promote host survival in the face of limited nutritional availability.

T125. The Effect of the Diet on the Development and Homeostasis of Innate Lymphoid Cells (ILCs)
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ILCs are recently discovered heterogeneous populations of lymphocytes found mostly in the mucosal tissues that display several important functions, including regulation of intestinal homeostasis. ILCs appear to be primordial precursor of T cells, as ILCs lack an Ag-specific receptor, but closely resemble effector $CD4^+$ T cell subsets in terms of cytokine profiles and transcription factors. The precise role of commensal microbiota and dietary Ags in the intestinal immune homeostasis of ILCs is poorly understood. We found that only a minor fraction of Type 3 ILCs was partially depleted in germ-free (GF) mice. By contrast, Ag-free (AF) mice, which are devoid of both commensal bacteria and dietary Ags, were found to have a dramatically elevated number of Type2 ILCs, which produced considerably higher amounts of T_H2-associated effector cytokines than Type2 ILCs in conventional and GF mice. Moreover, Type3 ILCs in AF mice were severely depleted compared to that in conventional and GF mice. Accordingly, while the basal proliferation rate of Type 3 ILCs was similar in conventional and GF mice, it was minimal in AF mice. Various experiments confirmed the role of dietary antigens in modulating Type3 ILCs and Type2 ILCs. We are currently investigating whether the dietary Ags and/or various components of the diet directly or indirectly affect development, homeostasis and function of ILC subsets.

F1. Effects of Aryl Hydrocarbon Receptor Activation on Pulmonary Innate Lymphoid Cells and Dendritic Cells.
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Innate lymphoid cells (ILCs) are a unique group of innate immune cells present in small numbers in mucosal tissues. Increasing evidence suggests that ILC3s not only contribute to chronic inflammation, but may also mediate tissue repair in the lung. However, those mechanisms responsible for ILC regulation remain to be determined. Because differential production of pro- vs. anti-inflammatory cytokines (e.g. IL-17 vs. IL-22) is strongly dependent on signaling though the AhR, we hypothesize that activation of AhR signaling is a key mechanism regulating the function of pulmonary ILC3s. In this study, we investigated the effects of AhR activation on the phenotype and function of ILCs from C57Bl/6 and AhR^d mice. AhR activation results in the expansion and activation of ILCs, as measured by increased presence of ILCs in the interstitium of the lung, augmented fluorescent intensity of cell surface activation markers, and enhanced production of cytokines, as demonstrated in IL-22^{tomato} reporter mice. This activation was dependent on AhR expression, and more importantly dependent on AhR expression in $CD11c^+$dendritic cells (DCs). These novel data suggest that DCs may be potent regulators of ILC activation in the lung and that targeting DCs with select AhR ligands may be a viable therapeutic approach to inflammatory lung diseases such as asthma.

F2. IL-33 and TSLP Mediate Steroid-Resistant Airway Inflammation in Mice
Homayon Banie. Janssen-J&J, San Diego, CA

Steroid-insensitive asthma is most often described as a chronic inflammatory disease that presents airway constriction and recurrent exacerbations even when treated with steroid-based therapies. Thus far, pulmonary preclinical models have focused on steroid sensitive, Th_2driven, allergic inflammation. Here we describe a pulmonary mouse model that utilizes IL-33 and TSLP with steroid insensitive eosinophilic features. IL-33 plays a critical role in various inflammatory and immunological pathologies. Intra-nasal administration of IL-33 to wild-type and $Rag2^{-/-}$ mice creates an influx of inflammatory cells and cytokines to the airway. Dexamethasone treatment was effective at reducing the recruitment of eosinophils to the airway and suppressing IL-5 and IL-13 producing lung resident Innate Lymphoid cells type 2 (ILC2). Conversely, Dexamethasone was ineffective at modulating ILC2 and eosinophil recruitment into the airway once IL-33 plus TSLP administrated intra-nasally. In addition, multiple Th2 cytokines in the bronchoalveolar lavage fluid and lung homogenate were protected, potentially driving steroid "insensitivity." The IL33/TSLP mouse model has several steroid insensitive endpoints and could be a useful model for dissecting pathways and mechanism mediating steroid resistant eosinophilic lung inflammation.

MALT & ORGANOGENESIS

OR.62. The Gut-Associated Lymphoid Tissues in the Small Intestine, Not the Large Intestine, Play a Critical Role in Oral Prion Disease Pathogenesis

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Gut associated lymphoid tissue (GALT) is present throughout the small and large intestines (SI and LI), consisting of multi-follicular Peyer's patches (and equivalents in the caecum and colon) and solitary isolated lymphoid tissue. Determining the contribution of GALT in the SI and LI in diseases not confined to particular intestinal niches remains problematic as manipulations to alter GALT generally affect GALT in both the SI and LI. Oral infection with prion disease requires GALT, primarily for transport through M cells and replication within B cell follicles. Detection of prions within LI GALT has been suggested as a diagnostic in humans and animals, however whether this is a primary site of infection was unknown. Using a novel model in which mice lacked SI GALT, we have shown that an absence of SI GALT dramatically reduces susceptibility to oral prion infection. Additionally, co-infection with a LI restricted pathogen (Trichuris muris) did not alter prion disease susceptibility. Therefore, following oral exposure, the initial prion infection is restricted to SI GALT. Furthermore, these data suggest that whilst SI and LI GALT are similar, LI GALT is relatively deficient in the uptake of non-motile pathogens from the lumen.

F3. Absence of Endothelial MAdCAM-1 in Nkx2.3$^{-/-}$ Mice Leads to Accumulation of Solitary Intestinal Lymphoid Tissue with Immature Phenotype.

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We have recently reported that in the absence of endothelial MAdCAM-1 expression lymphocytes home to Peyer's patches of Nkx2.3$^{-/-}$mice via PNAd. However, the effect of this addressin switch on the components of solitary intestinal lymphoid tissues (SILT) has not been characterized yet. In this work we investigated the effect of Nkx2.3 absence on the distribution and DSS-induced colitis on the SILT spectrum. BALB/c and Nkx2.3$^{-/-}$mice received DSS in drinking water for 7 days (acute) or 3 cycles (chronic). SILT structure maturity was analyzed by immunofluorescence assessed by C-kit, B220, CD45, CR1.2, and peanut agglutinin. Vasculature was characterized by endothelial addressin labeling. In Nkx2.3$^{-/-}$ colons we observed structurally normal cryptopatches and isolated lymphoid follicles. However, the number of mature components was less compared to BALB/c mice. Isolated lymphoid follicles in mutant colons were characterized by a smaller degree of vascularization compared to wild type. Endothelial cells lacked MAdCAM-1 but expressed PNAd. DSS treatment increased the ratio of mature SILT structures harboring germinal centers in BALB/c mice but had only minor effects in Nkx2.3$^{-/-}$colons. Our results indicate the important role of MAdCAM-1 in the transformation of immature cryptopatches to mature isolated lymphoid follicles. In the absence of Nkx2.3 the appearance of PNAd does not provide complete functional compensation for the lack of MAdCAM-1.

F4. Dietary Plasma Protein Supplementation Ameliorates Intestinal Inflammation in a Mice Model of Double Challenge Inflammation

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Previous experiments in rodents have shown that dietary supplementation with spray-dried animal plasma (SDP) can attenuate intestinal inflammation induced by S. aureus enterotoxin B (SEB). We now wanted to assess whether supplementation with SDP is effective in animals exposed to a double challenge induced by the administration of SEB and LPS. Male C57BL/6 mice were fed diets supplemented with 8% SDP or milk proteins (Control group) from day 19 (weaning) until day 33. Mice were challenged with LPS from E. coli (12.5 µg; intranasal) at day 32 and 6h later they were given SEB (25 µg; intraperitoneal). Twenty-four h after LPS, leukocyte populations in mesenteric lymph nodes (MLN) and jejunum cytokine expression were analyzed. The challenge increased leukocyte recruitment into MLN, as well as the percentage of activated Th lymphocytes, monocytes and neutrophils (all $P<0.05$), which were attenuated by SDP supplementation (all $P<0.05$). There was also increased the expression of TNF-α, IFN-γ and IL-1β in jejunum mucosa (all $P<0.05$), which were reduced by SDP (all $P<0.05$). These effects were accompanied by increased expression of

IL-10, TGF-β as well as FoxP3 in jejunum (all P<0.05). Supplementation with animal plasma proteins attenuated intestinal inflammation by increasing the expression of anti-inflammatory cytokines.

MONOCYTES AND MACROPHAGES

OR.17. GPR35/CXCR8 is a New Chemokine Receptor for the Mucosal Chemokine CXCL17

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Chemokines are a superfamily of chemotactic cytokines that direct the movement of cells throughout the body under homeostatic and inflammatory conditions. Chemokines bind to G protein-coupled receptors expressed on target cells to trigger intracellular signaling cascades and induce chemotaxis. Although the receptors of most chemokines have been identified, the receptor for the mucosal chemokine CXCL17 was still undefined. Using a multifaceted approach we have identified GPR35 as the receptor for CXCL17, and have re-named it chemokine (C-X-C motif) receptor 8 (CXCR8). CXCR8 is expressed in CXCL17-responsive human monocytes, dendritic cells and monocytoid cell lines. Additionally, transfection of CXCR8 into Ba/F3 cells rendered them responsive to CXCL17 in calcium mobilization assays. CXCR8 is preferentially expressed in mucosal tissues that mirror CXCL17's expression. Importantly, a $Cxcl17^{-/-}$ mouse exhibits defective recruitment of mucosal macrophage populations, reduced expression of CXCR8 in mucosal tissues, and defective chemotactic responses to Cxcl17 *in vitro*. We conclude that CXCR8 is a novel chemokine receptor and that these observations strongly suggest that the CXCL17-CXCR8 axis represents a novel target for therapeutic intervention in inflammatory processes of the respiratory or digestive systems.

OR.18. Novel Tools Reveal Niche Availability and Inflammatory State Determines the Cellular Origin of Kupffer Cells.

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The study of Kupffer cells (KCs) remains in its infancy due to the lack of KC-specific tools. By comparing the KC gene expression with those of other mfs, we identified a number of KC-specific genes. Using Technitium99-labeled nanobodies, whole body imaging and analysis of liver homogenates we found one of these genes to be solely expressed in the liver by KCs and thus this gene was used to construct KC-DTR knock-in mice. Administration of a single dose of DT is sufficient to completely and specifically eliminate KCs, without inflammation. Despite their ablation, KC-like cells were found in the liver one-week later. By generating bone marrow chimeras in which the livers are not irradiated, we found the returning KCs derived from the bone marrow while adoptive transfer studies revealed $Ly6C^{hi}$ monocytes to be the progenitor. Importantly, these monocyte-derived KCs (mo-KCs) self-maintain for at least 4 months without any new input from circulating monocytes and acquire a functional KC phenotype. As monocytes do not contribute to KCs in adulthood in either parabiosis or bone marrow chimera studies, we sought to evaluate whether complete depletion of the KC niche is required to allow mo-KC generation. Thus, we administered a suboptimal dose of DT resulting in a partial depletion of KCs. Despite the presence of embryonically-derived KCs, mo-KCs were still identified. Interestingly, KC depletion as mediated by acetaminophen overdose did not result in the generation of mo-KCs. Taken together our results demonstrate that niche availability and inflammation state determines the cellular origin of KCs.

OR.19. CCR7 Expression of Intestinal Myeloid Cells and their Migratory Potential

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Intestinal myeloid cells play an indispensable role in antigen sampling from the lumen and its transport to the mesenteric lymph nodes (mLN) where they prime naïve lymphocytes. CD103 and CX3CR1 expression distinguishes non-overlapping populations whose migratory potential is yet to be elucidated. Our group demonstrated that $CD103^{+}$ DCs but not CX_3CR1^{hi} cells migrate to the mLN via lymph (Schulz et al, 2009). In contrast, Diehl et al suggested that in microbiota-depleted mice, CX_3CR1^{hi}cells become migratory and enter into lymphatics and mLN in a CCR7-dependent manner (Diehl et al, 2013). We therefore established a novel mouse model to monitor CCR7 expression and its regulation in-vivo. In this mouse model, insertion of GFP disrupts the function of

CCR7 gene. Upon stimulation, cells from CCR7$^{GFP/GFP}$ mice up-regulate CCR7/GFP but are not able to migrate because of the disrupted CCR7. In contrast, cells from CCR7$^{GFP/+}$ mice can up-regulate CCR7 and are capable of migrating to the draining LN. Mice were treated with antibiotics or left untreated and gavaged with R848 14 hours prior to analysis. Upon R848 stimulation, intestinal CD103^{+}and CD103^{-} DCs up-regulated CCR7/GFP, CD64^{+} macrophages didn't express CCR7/GFP in CCR7$^{GFP/GFP}$ mice even after dysbiosis induced by antibiotics. Moreover, in CX3CR1$^{GFP/+}$ mice, CX3CR1^{int}CD64^{-} but no CX3CR1^{hi}CD64^{+}cells were found in the mLN after R848 treatment in both antibiotics-treated and-untreated mice. Taken together, our data demonstrate that intestinal CD103^{+}CX3CR1^{-} and CD103^{-}CXCR1^{int} DCs but not CD64^{+}CX3CR1^{hi}resident macrophages up-regulate CCR7 and migrate to the mLN irrespective of the microbiota status of the mice.

OR.64. Specificity and Diversity of the Mouse Peyer's Patch Mononuclear Phagocyte System

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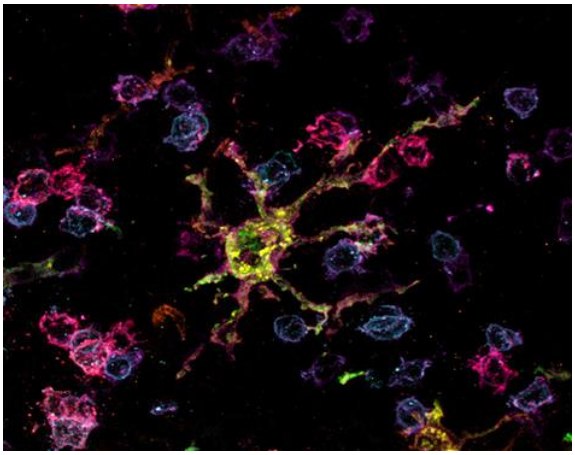

Peyer's patches (PP) are primary inductive sites of mucosal immunity. Defining PP mononuclear phagocyte system is crucial to understand the initiation of mucosal immune response. Although several mouse Peyer's patch dendritic cell (DC) subsets have been described in the literature, to our knowledge nothing is known about the macrophage diversity in PP. Here, we succeeded in distinguishing conventional DC, monocyte-derived DC and macrophage subsets of mouse PP. We defined their phenotype, distribution, origin, lifespan and transcriptional profiles. We show that monocytes differentiate locally into cells that display the characteristics of macrophages, i.e. long-lived cells with strong phagocytic activity but poor naïve T cell priming ability. However, these PP macrophages do not express classic intestinal macrophage markers which may explain their poor characterization so far. Interestingly, monocytes can also give rise in the same location to lysozyme-expressing DC (LysoDC) which, unlike macrophages, display a rapid renewal rate and strongly express genes of the MHCII presentation pathway.

OR.65. A20/TNFAIP3: Master Regulator of Intestinal Immune Homeostasis

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The transcription factor NF-κB is indispensable for intestinal immune homeostasis, but contributes to chronic inflammation and inflammatory bowel disease (IBD). A20, an inhibitor of both NF-κB and apoptotic signaling, was identified as a susceptibility gene for multiple inflammatory diseases, including IBD. Using tissue specific A20 knockout mice, we dissected the contribution of A20 to maintaining intestinal immune homeostasis. Despite absence of spontaneous intestinal inflammation in intestinal epithelial cell (IEC) specific A20 knockout mice, we found additional myeloid-specific A20 deletion to synergistically drive intestinal pathology through cell-specific mechanisms. A20 ensures intestinal barrier stability by preventing cytokine-induced IEC apoptosis, while A20 prevents excessive cytokine production in myeloid cells. Combining IEC and myeloid A20 deletion induces ileitis and severe colitis, characterized by IEC apoptosis, Paneth and goblet cell loss, epithelial hyperproliferation and intestinal microbiota dysbiosis. Continuous epithelial cell death and regeneration in an inflammatory environment sensitizes cells for neoplastic transformation and the development of colorectal tumors in aged mice. However, myeloid specific A20 knockout mice do not spontaneously develop intestinal pathology and are strongly protected in experimental colitis models. The pro-inflammatory environment in myeloid specific A20 knockout mice induces strong expansion of 'myeloid derived suppressor cells' (MDSCs) which suppress both innate and adaptive immunity in a tissue dependent manner. Although A20 defects are associated with multiple human inflammatory and auto-immune pathologies, myeloid-specific A20 deficiency results in protection from colitis. These studies underscore the delicate role of A20 in balancing intestinal immunity.

OR.66. Blood CD14⁺ Monocytes Constantly Replenish Mature Macrophages in the Human Small Intestine
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Tissue macrophages play a key role in preserving immune homeostasis in the gut mucosa by maintaining epithelial integrity, clearance of microorganisms and apoptotic material. As they also are key players in chronic inflammatory disorders, it is crucial to understand their origin and plasticity during steady state and inflammation. We distinguished three subpopulations of $CD45^+HLA\text{-}DR^+CD14^+$ mononuclear phagocytes in normal small intestinal mucosa. Two subsets were $calprotectin^+CCR2^+$ with monocyte morphology. HLA-DRintDC-SIGN- monocytes produced much higher levels of multiple cytokines including TNFα, IL-1ß and IL-6 compared with HLA-DRhiDC-SIGN⁺ monocytes (both with and without LPS). Monocytes showed a gradual change towards morphologically mature macrophages that were DC-SIGN⁺CD11c-calprotecin-CCR2- and produced no or very limited levels of cytokines even when challenged with LPS. However, they were highly phagocytic to soluble and particulate antigens. Examining transplanted human gut we found that monocytes were rapidly replaced, whereas mature macrophages showed delayed kinetics. However, one year post-transplantation all macrophages were also replaced. Our findings demonstrate that blood $CD14^+$ monocytes under homeostatic conditions constantly emigrate into the intestinal mucosa and differentiate into mature macrophages. Whereas recently recruited monocytes have strong pro-inflammatory properties, resident macrophages downregulate their capacity to produce cytokines but maintain their phagocytic capacity.

OR.67. H3K27me3 Contributes to the Establishment of Anergic Phenotype of Intestinal Macrophages
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Introduction: Intestinal macrophages (IMACs) are an integral part of first-line gut defense. A major feature of intestinal macrophages in the normal gut is inflammatory anergy, a state of tolerance essential for intestinal homeostasis, changes in which lead to inflammatory bowel disease (IBD). Aim: To explore the relationship between chromatin modification and the repression of inflammatory genes in intestinal macrophages. Methods: Lamina propria mononuclear cells (LPMCs) were obtained from gut tissue resections of healthy control (HC) and IBD patients. IMACs were purified using positive selection with anti-CD33 antibodies. Using the same method, blood monocytes were isolated from peripheral blood mononuclear cells (PBMCs) with anti-CD14 antibodies. Cells analyzed using chromatin immunoprecipitation (ChIP-qPCR) assay to determine associations between key histone modifications and tnfa gene expression. Results: Macrophages from normal control subjects displayed significantly ($P < 0.05$) higher binding of the silencing mark H3K27me3 to the tnfa transcription start site than CD macrophages. Moreover, the repressed state of tnfa promoter in HC macrophages was associated with increased binding of H3K9me3 and H3K9me1. Additional analysis of peripheral blood monocytes suggests that the acquisition of H2K27me3 is tissue type-specific. Conclusions: We have identified key epigenetic histone modifications at the tnfa gene, which may explain why the repressed state of the tnfa promoter in healthy macrophages is disturbed during gut inflammation, leading to an increased transcription and production of pro-inflammatory cytokines.

OR.68. The Expression of Interleukin-19 Distinguishes CX3CR1-positive Macrophages from Dendritic Cells
Anna Steinert, Jan Niess. University of Bern, Bern, Switzerland.

Introduction and Aims: Myeloid-derived cells in the intestine are rather an undefined cell population. We hypothesized that myeloid cells may be distinguished by the expression of the cytokine Interleukin (IL)-19, a member of the IL-20 cytokine family. Methods: Bone marrow derived macrophages and dendritic cells from $CX_3CR1^{GFP/+}$/$RAG\text{-}1^{-/-}$ animals and IL-19 expression was determined. To investigate the role of commensal microorganisms on IL-19 and on the type I IL-20 receptor (formed by the IL-20 receptor alpha (IL-20RA) and beta (IL-20RB) subunits) expression levels were determined in mice with different hygiene statuses. Results: CX3CR1-positive macrophages produced significantly more IL-19 than dendritic cells. IL-19 expression was further up-regulated after stimulation with Toll-like receptor ligands. Macrophages expressed IL-20RB but not IL-20RA. IL-19 did not induce an activation of macrophages in an autocrine fashion. In intestinal tissues of non-diseased mice, we did not detect IL-19 expression during steady state conditions. IL-19 expression was only found in mesenteric lymph nodes, but not in peripheral lymph nodes, skin, liver, spleen, stomach small and large intestine. In contrast intestinal inflammation lead to the expression of IL-19 by phagocytes isolated from

the large intestine of mice with colitis. IL-20RB was expressed in all investigated organs with the highest expression in the skin. Expression of IL-20RA was found in the epidermis, the stomach and proximal colon but not in the small intestine. Conclusions: CX_3CR1-positive macrophages are a cellular source of IL-19 acting on mucosal sites where the type I IL-20 receptor is expressed to maintain homeostasis on inner and outer body surfaces.

F5. Elevated Endoplasmic Reticulum Stress Reinforced Immunosuppression in the Tumor Microenvironment via Myeloid-Derived Suppressor Cells

Bo-Eun Kwon, Eun Hye Hong and Hyun-Jeong Ko. Kangwon National University, Chuncheon, South Korea

Although the role of endoplasmic reticulum (ER) stress in cancer has been studied in depth, ER stress is known to increase apoptosis of tumor cells and thus reduce tumor growth. In our study, persistent ER stress induced by multiple administrations of low-dose thapsigargin (Tg) accelerated tumor growth in mice. Tg-mediated ER stress increased the generation of $Ly6G^+CD11b^+$ myeloid cells, but did not alter anti-tumor effector T cells. 4-Phenylbutyric acid (PBA), a chemical chaperon which was widely used as ER stress reducer attenuated Tg-induced myeloid-derived suppressor cell (MDSC) expansion and tumor growth. Tg-mediated ER stress enhanced the immunosuppressive capacity of tumor-infiltrating MDSCs by increasing expression of ARG1, iNOS, and NOX_2, although splenic MDSCs were not affected. Consistent with these results, 4-PBA restored the anti-tumor immune response by regulating inflammatory cytokines such as TNF-α and CXCL1/KC, and activated tumor-infiltrating $CD8^+$ T cells that were inhibited by Tg-mediated ER stress. These results suggest that significant ER stress in a tumor-bearing host might induce tumor growth mediated by enhancement of MDSC-mediated suppression. Therefore, ER stress reducers such as 4-PBA could restore anti-tumor immunity by inhibiting suppressive MDSCs that are exacerbated by ER stress.

F6. Anti-Inflammatory Effect of the Recombinant Calreticulin from Taenia solium on Human Cells

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Chronic inflammatory diseases are highly prevalent worldwide. Currently prescribed treatments present a number of adverse side-effects, making necessary the quest for better options. Helminth eggs and excretion/secretion (ES) products induce Th_2-type regulatory responses; partly alleviating the symptoms in animal models and patients. However, details of the underlying mechanisms remain elusive, particularly for human cells. Our group identified calreticulin, a ubiquitous and multifunctional protein, among the ES products produced by Taenia solium. Additionally, we demonstrated that a recombinant version (rTsCRT) promotes the release of IL-4, -5 and -10 from hamster mesenteric lymph node and splenic cells. Therefore, the aim of this work is to address the consequences of the interaction between rTsCRT and human cells, specifically in the context of cytokine induction. Monocytes and monocyte-derived human macrophages (MDMs) were obtained from buffy coats. These cells will be immunophenotypified by flow cytometry, staining for the following surface markers: CD14, HLA-DR and CD68. Supernatants from rTsCRT-stimulated monocytes and MDMs will be collected, and Th_1 (TNF-α, IFN-γ and IL-12), Th_2 (IL-4,) and regulatory (IL-10 and TGF-β) cytokines ELISA measured. Cytokine profiling results will be presented at the congress. In agreement with previous publications, it is expected that rTsCRT will favor the production of Th_2-type/regulatory cytokines.

F7. Critical Role of MyD88 in Phagocytosis and Cell Signaling During *E. faecalis* Infection of Macrophages

Nathan Shankar and Jun Zou. University of Oklahoma, Oklahoma City, OK

Macrophages are the most abundant leukocytes in the lamina propria of the intestine and are involved in both induction of protective immunity as well as immune tolerance to endogenous bacterial flora. Enterococcus spp. are commensal bacteria residing in the gastrointestinal tract of mammals but can also cause serious antibiotic-resistant opportunistic infections. One of the mechanisms by which these commensals cause life-threatening infections is through bacterial translocation from the intestinal lumen to extraintestinal sites. Under such circumstances E. faecalis must interact with the host immune system including macrophages. Understanding the macrophage activation program during enterococcal infection, and the bacterial components that elicit it, will be useful to explore non-conventional therapies to combat enterococcal infections. In this study, using BMDM and RAW264.7 macrophages we show that enterococcal infection activates ERK, JNK and p38 MAPK as well as NF-κB, and drives polarization of macrophages towards the M1 phenotype. Inhibition of NF-κB activation significantly reduced the expression of TNF-α and IL-1β, as did the inhibition of ERK, JNK and p38 MAPK. Enterococci-induced activation of these pathways and subsequent cytokine expression was contact dependent, modest compared to activation by E. coli and, required the adaptor protein MyD88.

Phagocytosis of enterococci by macrophages was enhanced by preopsonization with E. faecalis antiserum and appeared to involve the ERK and JNK signaling pathways regulated by adaptor protein MyD88. These findings have important implications for understanding the interactions of E. faecalis at the gut mucosal interface.

F8. Identification of Factors Guiding Monocyte Differentiation Towards Distinct Resident Macrophage Fates in the Small and Large Intestine

Mor Gross, Biana Bernshtein, Eyal David and Steffen Jung. Weizmann Institute of Science, Rehovot, Israel

Gut health requires well-balanced tolerogenic responses to innocuous stimuli and efficient protective immunity towards harmful pathogens. Monocyte-derived macrophages are key players in maintaining this homeostatic balance. Intestinal macrophages engulf and neutralize pathogens, orchestrate ILC and DC functions, and contribute to wound healing.Recent studies from our laboratory have highlighted the heterogeneity of tissue macrophages, including significant differences in the gene expression patterns and enhancer landscapes of ileal and colonic macrophages. This might result from their differential exposure to microbiota or factors released by neighboring cells, such as epithelium. To define key factors that establish the distinct macrophage identities, we engraft mice, whose intestinal macrophages have been depleted, with monocytes that express a GFP reporter. This enables us to detect and isolate grafted cells by flow cytometry and ensures that the cells we retrieve from the intestine are synchronized with respect to their development. We have isolated and collected cells from the lamina propria of the colon and the ileum of mice at different days post-transfer and subjected them to RNAseq. We will report the comparison of their expression profiles, as well as the dynamics of transcription factors and their respective targets during monocyte differentiation.

F9. IL-10 Rapidly Inhibits Transcription of Secondary Response Genes in LPS-Stimulated Macrophages

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IL-10 is an anti-inflammatory cytokine that is essential for maintaining intestinal homeostasis. Here we examined the kinetics of IL-10-mediated suppression following LPS-stimulation of bone marrow-derived macrophages (BMDM). Following a delay, mRNA for the secondary response gene Il12b briefly increased after LPS stimulation, but then quickly leveled off at a steady state, concordant with the time that IL-10 was first detected in the culture medium. Treatment with actinomycin D in this steady-state phase demonstrated that the Il12b message was highly stable, indicating that IL-10 was not influencing mRNA stability. Using RT-PCR primers directed at pre-mRNA as a marker for active transcription, we found that production of Il12b pre-mRNA was transient, suggesting that IL-10 was inhibiting transcription of Il12b. Studies in $Il10^{-/-}$ BMDM confirmed that both Il12b mRNA and pre-mRNA were induced at significantly higher levels in the absence of IL-10. Remarkably, addition of IL-10 to LPS-stimulated $Il10^{-/-}$ BMDM that were actively transcribing Il12b led to the rapid inhibition of transcription, as well as loss of RNA Pol II association with the Il12b promoter. Interestingly, while at early time points following LPS stimulation IL-10 had little effect on transcription of the primary response gene Cxcl2, it did inhibit transcription and association of RNA Pol II with the Cxcl2 promoter at later time points. Thus, IL-10 rapidly inhibits the transcription of target genes during the secondary phase of the response to LPS. We hypothesize that IL-10 interferes with the function of an inducible transcriptional regulator involved in directing the secondary response to LPS.

F10. Loss of Myeloid Hypoxia Inducible Factor-1 Ameliorates Dextran Sodium Sulfate Induced Colitis in Mice

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Hypoxia is a hallmark of chronically inflamed tissue when compared to healthy tissue. Under oxygen deficiency, hypoxia, the transcription factor complex hypoxia inducible factor (HIF) regulates expression of genes potentially also involved in the pathogenesis of inflammatory bowel disease (IBD) which embraces Crohn's disease and ulcerative colitis. Among others HIF-1 is essential for myeloid cell life span and function during inflammation. The complex interplay between hypoxia and inflammation during IBD prompted us to investigate the role of HIF-1 in the pathogenesis of IBD and especially its role in myeloid cells during inflammation. Mice with a conditional knockout of HIF-1α in myeloid cells (LysMcre/HIF-$1\alpha^{+f/+f}$) were examined *in vivo* in a dextran sodium sulfate induced model of inflammatory bowel disease. The disease pattern was described macroscopic, histological and on RNA level. Furthermore, *in vitro* experiments with bone-marrow-derived macrophages lacking HIF-1α were performed to picture their general behavior. After cultivation under inflammatory and/or hypoxic conditions they were characterized on RNA and protein level. First experiments provide evidence that LysMcre/HIF-$1\alpha^{+f/+f}$ mice deficient in functional HIF-1 in myeloid cells show a milder

form of colitis with less weight loss and lower inflammatory parameters when compared to HIF-1α$^{+f/+f}$ control mice. This could indicate that HIF-1 could act as a pro-inflammatory regulator of myeloid cells during the course of inflammatory bowel disease.

F11. Differential Regulation of Intestinal Macrophage Function by Interleukin-10 and the Gut Microbiota in the Colon Versus the Small Bowel

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Background: Macrophages (MΦ) mediate intestinal immune tolerance. Interleukin (IL)-10 governs the regulatory functions of MΦ; production of IL-10 by immune cells is induced by the gut microbiota. How MΦ differ between the colon and small-bowel is poorly understood. Methods: Colonic and small-bowel MΦ from wildtype (WT), IL-10knockout (IL-10KO) and germ-free (GF) mice were analyzed by flow cytometry/qPCR. Results: Proportions of total MΦ were enhanced in the colon compared to the small-bowel. Mature (MHC Class II$^+$/Ly6C$^-$) MΦ expressed less ALDH1A2/ALDH1A3 in the colon, but colonic MΦ favored TGFβ/IL-1β expression. Colonic, but not small-bowel mature MΦ showed decreased expression of TGFβ and ALDH1A2 in IL-10KO mice. Furthermore, inflammatory intermediary (MHC Class II$^+$/Ly6C$^+$) MΦ accumulated in the colon, but not the small-bowel of IL-10KO mice. MΦ in GF mice were predominantly mature MΦ, with very few monocytes/intermediary MΦ. These mature MΦ in GF mice exhibited enhanced expression of cytokines IL-23/IL-6/TNFα and increased expression of the aryl-hydrocarbon receptor (for recognition of environmental antigens), alongside decreased expression of IL-10 and ALDH1A2. These differences were more striking in the small-bowel. MΦ numbers in the MLN were increased in IL-10KO and GF mice, suggesting that IL-10 as well the microbiota may act to limit MΦ migration from the intestine in the steady-state. Conclusions: These data provide an important role for IL-10 in MΦ-mediated immune tolerance in the colon in particular, and for the gut microbiota in MΦ-mediated immune tolerance in the small-bowel. We are currently identifying the role of the microbiota in synergizing with IL-10 to regulate MΦ homeostasis in the intestine.

F12. Chemokine Property of MIF Favors Immunosurveillance in Murine Colorectal Cancer

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Chronic inflammation has been related with cancer development by unbalancing immune response. The inflammatory cytokine macrophage migration inhibitory factor (MIF) is overexpressed in many carcinogenic tumors, stimulates angiogenesis and metastasis promoting proliferation and survival in malignant cells and binds to CD74 (ligand for CXCR2 and CXCR4). In ulcerative colitis MIF is early expressed and favors the inflammatory cytokine production like TNF-α and IL-β. As intestinal chronic inflammation may precede colorectal cancer, we are interested in determining the role of MIF on pathology of colorectal cancer. We used MIF$^{-/-}$ and WT mice to develop a colorectal cancer model of Dextran Sodium Sulfate and Azoxymethane. Strikingly, we found significantly more tumors and in higher development stage on MIF$^{-/-}$ (24.4±2) compared to WT (12.6±2). Higher number of activated macrophages were found in WT mice intestinal epithelium compared to MIF$^{-/-}$ mice. Therefore, although MIF has been recognized as a promoter of inflammation, which could favor cancer development; our results suggest that MIF is involved in important cells recruitment, like macrophages, to control colorectal cancer development, possibly by its chemokine property.

F13. Identification of Potential Therapeutic Macrophage-Related Targets for Inflammatory Bowel Disease (IBD)

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Background & Aims: Intestinal macrophages play an important role in IBD, however their gene expression profile is unknown. We therefore performed gene arrays on gut macrophages isolated from IBD patients and control subjects (HCs). Methods: 84 genes were analyzed in lamina propria CD33$^+$ macrophages isolated from the inflamed colon of 11 IBD patients and from the normal colon of 10 HCs. IL-24 was measured in the supernatants of IBD and control biopsies cultured *ex vivo*, and TNF-α concentration was determined in the supernatants of inflamed IBD biopsies cultured *ex vivo* with rhIL-24. Results: IL24, IL21, INHBA, TNFSF8, LTB and IL10 expression was significantly higher in IBD compared to HC intestinal macrophages, whereas MSTN, IFNA4, BMP5, BMP2 and PDGFA expression was significantly down-regulated in IBD macrophages. IL-24 was significantly higher in inflamed IBD (928.5 ± 171.3 pg./ml) than in HC (273.2 ± 37.8 pg./ml) biopsy supernatants. Compared to medium alone, *ex vivo* culture with rhIL-24

significantly increased TNF-α production by inflamed IBD biopsies (119.9 ± 41.0 and 436.9 ± 115.7 pg./ml, respectively). Conclusions: IBD gut macrophages show a dysregulated immune gene profile, which we are functionally evaluating in the search for macrophage-related therapeutic targets. Preliminary data show that IL-24 is up-regulated in IBD mucosa and promotes TNF-α release by IBD biopsies cultured *ex vivo*.

F14. Post-Transcriptional Control of Inflammasome Activation in Intestinal Macrophages
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Colonic macrophages (cMPs) are important for intestinal homeostasis as they efficiently kill microbes yet produce low levels of inflammatory cytokines. We found high constitutive mRNA expression but low protein levels for the inflammasome proteins NLRP3 and proIL-1beta as well as for certain pro-inflammatory cytokines, including TNF-α and IL-6 in cMPs compared to bone marrow-derived, and peritoneal macrophages. In contrast, high mRNA but low protein production was not found for ASC, procaspase-1 or IL-10. TLR activation enhanced mRNA for NLRP3 and proIL-1ß in cMPs, but resulted in minimal increase in protein expression, resulting in a correspondingly low production of mature IL-1ß following NLRP3 or NLRC4 activation. In contrast, during experimental colitis inflammatory monocytes and cMPs ($CD64^+F4/80^+$ cells) expressed high levels of both mRNA and protein for NLRP3, proIL-1ß and TNF-α, and produced high levels of IL-1ß following inflammasome activation indicating that microenvironmental signals during steady-state but not inflammatory conditions control the expression of inflammatory molecules, including the NLRP3 inflammasomes. Finally, blocking proteasome activity, as well as IL-10 signaling in cMPs ($CD64^+F4/80^+Ly6C^-$ cells) resulted in enhanced NLRP3 and proIL-1ß protein expression in cMPs from non-inflamed mice. These data indicate novel post-transcriptional mechanisms to control inflammasome activation in cMPs.

F15. HDAC5: A New Player in Macrophage Activation and Intestinal Homeostasis
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Histone deacetylases (HDACs) are key players in the process of gene regulation as they modulate chromatin structure and thereby gene transcription. Moreover they modify non-histone proteins as nuclear factor-κB (NF-κB). While inhibition of HDACs is well known for its anti-proliferative potency in various tumor models, recent studies revealed HDACs as essential mediators in chronic inflammation, especially in inflammatory bowel disease and colitis associated cancer. Here, we analyzed the immunological function and the expression profile of HDAC5 in macrophages. Furthermore, we investigate its relevance for gut homeostasis in mice with regard to macrophage-epithelial cell interaction, which has a direct impact on the integrity of the intestinal epithelial barrier. In the macrophage cell lines RAW 264.7 and U937, expression of HDAC5 mRNA is significantly down-regulated after toll-like receptor activation. HDAC5 overexpression in RAW 264.7 resulted in an increased LPS-dependent secretion of pro-inflammatory cytokines. Subsequently, knockdown of HDAC5 mRNA by specific siRNA significantly reduced the secretion of these cytokines. These effects were accompanied by a corresponding change in NF-κB activity, indicating a critical role of HDAC5 in the inflammatory response of macrophages. To address the impact of HDAC5 on colonic barrier function, colon samples of dextran sulfate sodium-treated knockout mice were analyzed by Ussing chamber experiments. Here, a decreased trans-epithelial resistance as well as a decreased flux of horseradish peroxidase was detected. These results indicate a regulatory function of HDAC5 in the pro-inflammatory response of macrophages and further point to a distinct role within intestinal homeostasis during experimental colitis.

F16. Precise Identification and Functional Characterization of Human Intestinal Macrophages in Health and Disease
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Intestinal macrophages have many critical functions, both in maintaining intestinal immunological homeostasis, and in orchestrating responses to pathogens. Not only do they contribute to regulating intestinal immune responses through production of IL-10 and PGE_2, but they also express TLRs and NLRs to sense bacteria, activate NFκB and secrete cytokines. We have previously shown that murine intestinal macrophages differentiate from monocytes, and that this process is altered under inflammatory conditions. Given their importance in intestinal immunity, precise identification and characterization of human intestinal

macrophages is key to understanding their role in inflammatory bowel disease (IBD). Our recent deep immunophenotyping of human colonic and small intestinal tissue revealed two macrophage populations, differentiated on CD14 expression, similar to those found in our murine studies. Defined as $CD64^{+}HLA\text{-}DR^{+}CD206^{+}CD14^{hi/lo}$, both macrophage subsets expressed higher levels of IL-10 and TNFα than either blood monocytes or intestinal dendritic cells. To unravel the roles played by macrophages in IBD we are assessing the abundance, proliferative capacity and functions of both these populations under steady state and inflammatory conditions. By performing this characterization and functional assessment of human macrophages we hope to elucidate specific roles of macrophages in human IBD, to aid development of future therapeutics.

F17. Innate and Adaptive Immune Functions of Peyer's Patch Monocyte-Derived Cells

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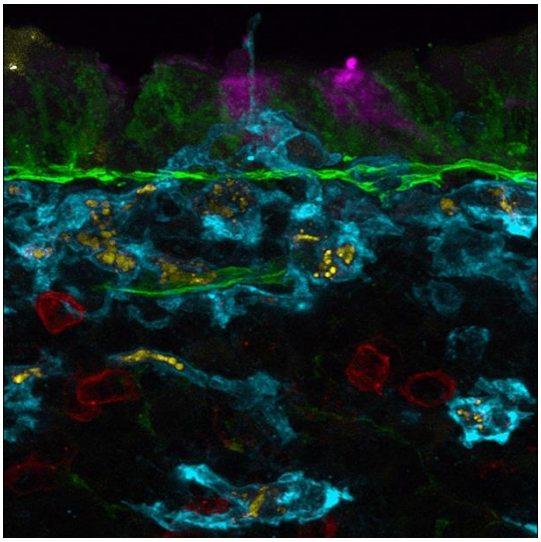

Peyer's patches of the small intestine are antigen sampling and inductive sites for the establishment of mucosal immunity. They comprise clustered domes formed by B cell follicles separated from each other by interfollicular regions enriched in T cells. The follicle-associated epithelium contains specialized epithelial cells, called M cells that bind and rapidly transport microorganisms from the lumen to the subepithelial dome (SED). The SED contains macrophages and dendritic cells (DC) among which we recently described a myeloid DC subset called Loud, which highly expresses the bactericidal agent lysozyme. Antigen uptake, degradation and presentation by these mononuclear phagocytes are crucial steps to induce the mucosal immune response. Here, we show that Loud and SED macrophages are both involved in particulate antigen uptake, display strong innate antiviral and antibacterial gene signatures and, upon TLR7 stimulation, secrete IL-6 and TNF but no IL-10 or IFNg. However, unlike macrophages, Loud display a rapid renewal rate, strongly express genes of the MHCII presentation pathway and efficiently prime naïve helper T cells for IFNg production.

F18. The Role of Free Fatty Acid on Modulating Regulatory Phenotype of MDSC

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Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous group of cells that expand during cancer and inflammation, have been reported to modulate cytokine production of macrophages, suppress T cell response, and promote tumor angiogenesis, tumor cell invasion and metastasis. An accumulation of MDSC in peripheral blood can be observed both in cancer and chronic inflammation, including Inflammatory Bowel Disease and Colon Cancer, while the mechanism of modulating MDSC inhibition is still unclear. Here we use the MDSCs cell line MSC-2 to describe a possible cellular mechanism for functional regulation of MDSCs. We found that not only IL-4, but also oleate, an unsaturated fatty acid, can induce a regulatory phenotype in these cells, paralleled by an increase in intracellular lipid droplet accumulation. Furthermore, this inhibitory effect can be reversed by treatment with TOFA, an inhibitor of droplet formation. We compared the ability of oleate (C18:1) and stearate (C18:0) to induce the regulatory phenotype of MDSCs. Our results demonstrate that both fatty acids can induce droplet formation while only oleate treated MSC-2 cells exhibit a suppressive ability. Analysis of nitric oxide (NO) production indicates that the oleate induced regulatory phenotype is mediated by NO and that the effect of oleate can be neutralized by L-NMMA, a non-selective inhibitor of NO synthetase. Thus we suggest a novel unsaturated fatty acid-dependent pathway to regulate formation of MDSCs, a mechanism which will inspire us a novel strategy for anti-tumor therapy.

F19. Interaction Between Enteric Glia and Immune Cells as New Players in Intestinal Homeostasis
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In the gastrointestinal tract balance between immune activation and tolerance is essential to maintain intestinal homoeostasis. Recently we have demonstrated that the enteric nervous system (ENS) has a potent anti-inflammatory effect in the gut via neuronal release of acetylcholine. Currently, we are investigating if also enteric glial cells (EGCs), the main cellular constituent of the ENS, may have immunomodulatory effects. Although EGCs are mainly described as supporting cells for enteric neurons, accumulating evidence suggest that EGCs may play a crucial role in modulating intestinal homeostasis influencing epithelial and immune cells. In line, we could show that EGCs are spread throughout the gut and are also present within the lamina propria where they are in close contact with immune cells. Moreover, we showed that glial-secreted molecules are capable to decrease expression of pro-inflammatory cytokines in myeloid cells after LPS stimulation. Our data further unravel the interaction between the enteric nervous system and the intestinal immune system, which might lead to the development of novel therapeutic strategies to treat intestinal immune-mediated diseases.

F20. Homeostatic Functions of Intestinal Macrophages
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Macrophages (mϕ) are highly abundant in the healthy intestine, where they reside close to the epithelial layer and are thought to be important in preventing invasion by commensal bacteria and other microbes. We have shown recently that resident intestinal mϕ are replenished continuously by circulating monocytes that differentiate locally into ant-inflammatory cells characterized by high levels of CX_3CR1, MHCII and IL10 production. Using microarray and phenotyping, we show here that maturing macrophages also acquire several scavenger receptors, tissue remodeling metalloproteases and receptors associated with clearance of apoptotic cells, including the $\alpha v\beta 5$ integrin known to be crucial for clearance of effete rods and cones in the retina. Functional studies confirm that resident mϕ are highly phagocytic for both bacteria and apoptotic thymocytes *in vitro*. Epithelial cell derived cytokeratin could also be detected with mϕ isolated from normal colon. Together with the fact that mϕ are still present in germ free intestine, these data suggest that the antimicrobial scavenger functions of these cells may be secondary to a vital physiological role in maintaining intestinal tissue homeostasis and we are currently exploring the role of $\alpha v\beta 5$ integrin in these processes.

F21. Potentiation of IL-4 and Vitamin D3 Receptor Signaling by Retinoic Acid in Intestinal Epithelial Cells and Macrophages: Mechanisms and Targets
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Previously we demonstrated all-trans retinoic acid (ATRA) increased mRNA expression of several IL-4 induced chemokines associated with alternative activation (M2a) in porcine macrophages. Herein, we describe several mechanisms whereby ATRA affects IL-4 signaling and extend these findings to epithelial cells. Treatment of primary explanted pig alveolar macrophages, blood-derived monocytes, and immortalized pig cell lines with ATRA alone induced expression of additional markers of M2a activation as shown by next generation sequencing (NGS) and real-time RT-PCR analysis. These markers included the adenosine A2b receptor ADORA2B, interleukin 1 receptor antagonist (IL1RN), Tissue inhibitor of metalloproteinase 1 (TIMP1) and the vitamin D3 (1,25- dihydroxyvitamin D3) receptor (VDR). Next generation sequencing also showed that an abundance of genes in the VDR activation pathway are also regulated by ATRA. Treatment with both ATRA and vitamin D_3 co-regulated the activities of each other and differentially affected the markers of M2a macrophages. In two intestinal epithelial cell lines, we observed that ATRA increased RNA and protein for the IL-4 receptor alpha chain and the VDR. ATRA increased IL-4 induced phosphorylation of signal transducer and activator of transcription 6 (STAT6) and mRNA expression of the chloride channel, calcium activated, family member 1 (CLCA1), important for mucus formation, and chemokine (C-C motif) ligand 26 (CCL26) a potent eosinophil chemoattractant. ATRA also increased vitamin D_3 induced expression of CYP24A1. Thus, in intestinal epithelial cells and macrophages, ATRA increased signaling in response to IL-4 and VD3. These findings have important implications for the regulation of inflammation at mucosal surfaces.

F22. The Nlrp3 Inflammasome Suppresses Colorectal Cancer Metastatic Growth in the Liver by Promoting Natural Killer Cell Tumoricidal Activity

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The crosstalk between inflammation and tumorigenesis is now clearly established. However, how inflammation is elicited in the metastatic environment and the corresponding contribution of innate immunity pathways in suppressing tumor growth at secondary sites are poorly understood. Here, we show that mice deficient in Nlrp3 inflammasome components had exacerbated colorectal cancer metastatic growth in the liver, which was mediated by impaired IL-18 signaling. Control of tumor growth was independent of differential cancer cell colonization or proliferation, intestinal microbiota effects or tumoricidal activity by the adaptive immune system. Instead, the inflammasome-IL-18 pathway impacted maturation of hepatic NK cells, surface expression of FasL and capacity to kill FasL-sensitive tumors. Our results define a regulatory signaling circuit within the innate immune system linking inflammasome activation to effective NK cell-mediated tumor attack that is required to suppress colorectal cancer growth in the liver.

MUCOSAL B CELLS

OR.9. B Cells and Dendritic Cells React to Intestinal Epithelial Stress with a Coordinated and Protective IgA Response.

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X-box binding protein-1 deletion in intestinal epithelium ($Xbp1^{\Delta IEC}$) induces endoplasmic reticulum (ER) stress resulting in microbiota-dependent enteritis. Compensatory mechanisms like autophagy have been shown to limit inflammation. Here, we show that plasma cell IgA production is another important compensatory mechanism dampening intestinal inflammation. IEC ER-stress increases lamina propria (LP) $CCR9^+IgA^+CD138^+$ plasma cells and IgA levels. $Xbp1^{\Delta IEC}$ mice with concomitant B cell deficiency (μMT) exhibit increased enteritis compared to $Xbp1^{\Delta IEC}$ mice. The IgA response was T cell dependent, and not independent, as observed by normal BAFF, APRIL and TSLP levels and increased Fas^+GL7^+ germinal center (GC) B cells and T follicular helper (Tfh) cells in Peyer's patches (PP) of $Xbp1^{\Delta IEC}$ mice. The microbiota was indispensable for the protective IgA response because germ free mice lacked increased GC and Tfh in PP and IgA^+ plasma cells in LP. In light of the T cell dependency, we investigated whether dendritic cell (DC) populations played a role in this IgA protective response. Interestingly, we found that $Xbp1^{\Delta IEC}$ mice had higher numbers of intraepithelial $CD11c^+CD11b^+CD103^+$ DCs. Although $Xbp1^{\Delta DC}$ mice lacked enteritis, loss of DC in $Xbp1^{\Delta IEC/DC}$ reversed the protective IgA response with increased enteritis like μMT- $Xbp1^{\Delta IEC}$. In conclusion, we show that microbially-induced, epithelial ER (dis)stress in a genetically at risk barrier is sensed by B cells and DC in PP resulting in a protective IgA response which is crucial to limiting enteritis. We hypothesize that epithelial ER (eu)stress under homeostatic conditions may be an important mechanism that controls small intestinal IgA responses.

OR.10. Microbial Influence in the Shaping of the Primary Immunoglobulin Repertoire

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The pre-immune Ig repertoire is generated in developing bone marrow B cells through V(D)J recombination, resulting in production of IgM—first expressed on the surface of immature B cells as the antigen binding B cell receptor (BCR). Antigen encounter then provides Ig repertoire-shaping influences on developing naïve B cells through BCR receptor editing, clonal deletion, anergy, and cell fate determination. While it is known that self-antigens play a role in influencing the naïve Ig repertoire, the role of environmental antigens is not understood. In light of our previous work showing that primary B cell development—including V(D)J recombination and BCR editing—occurs in the gut lamina propria (LP) of weanling mice, we hypothesized that gut luminal antigens influence naïve B cell Ig repertoires. Using an assay we developed to test IgM-dependent B cell binding to small intestinal luminal content (sILC), we find that B cells from germ-free mice do not demonstrate detectable IgM-dependent sILC reactivity. However, naïve B cells from littermates exposed to conventional microbiota results in the appearance of IgM-dependent sILC reactivity into the naïve B cell

repertoire. In contrast, B cells from both groups show no IgM-dependent reactivity to germ-free sILC. In addition, intravital microscopy reveals model luminal antigen in contact with naïve LP lymphocytes in the gut mucosa of weanling mice. These results suggest that non-pathogenic commensal/mutualistic microbes may benefit their hosts by providing antigenic substrates to naïve mucosal B cells that serve as selection forces resulting in the injection anti-microbial specificities into the primary Ig repertoire.

OR.11. Asymmetric Recirculation of $\alpha_4\beta_7$-Expressing Memory B Cells Throughout the Gut-Associated Lymphoid Tissues
Davide Agnello, Damien Denimal, Cécile Pitoiset, Amandine Lavaux and Pierre Pothier. Centre Hospitalier Universitaire de Dijon, Dijon, France

Memory B cells (MBCs) are important for secondary humoral responses, but their homing properties are largely unknown. We previously showed that immunization by the intra-rectal route induces IgA antibody-secreting cells (ASCs) which express high levels of the integrin $\alpha_4\beta_7$ and migrate not only to the colon but also to the small intestine. Here we investigate the distribution of MBCs induced by intra-rectal immunization with a particulate antigen in comparison with other immunization routes. We identified MBCs in secondary lymphoid organs of immunized mice as isotype-switched B cells ($B220^+$, $sIgD^-$, $sIgG_1/sIgA^+$) that lack germinal center-specific markers ($GL7^-$, $CD38^+$) and bind to fluorescent antigen. Whereas only ~40% of antigen-specific MBCs induced by subcutaneous or intra-nasal immunization express $\alpha_4\beta_7$, virtually all MBCs induced by intra-rectal immunization are $\alpha_4\beta_7^+$. Therefore, antigen-specific MBCs induced by intra-rectal immunization preferentially recirculate among the gut-associated lymphoid tissues (GALTs), such as Peyer's patches (PPs) and mesenteric lymph nodes (MLNs), which express MAdCAM-1, but are instead largely excluded from extra-intestinal LNs. When mice deficient for β_7-integrin were immunized by the intra-rectal route, MBCs were reduced in MLNs and increased in peripheral LNs. Likewise, adoptive transfer of antigen-specific MBCs from β_7-deficient mice immunized by the intra-rectal route generated fewer antigen-specific ASCs in PPs of recipient mice upon rechallenge, but higher number of ASCs in spleen, in comparison with MBCs from normal mice. In contrast, after intra-nasal immunization, antigen-specific cells were instead significantly increased in MLNs of β_7-deficient mice, suggesting that competition among lymphocytes is important for MBC homing towards the GALTs.

OR.12. Clonal Distribution of B Cells Between Distant Foci of Gut-Associated Lymphoid Tissue in Humans.
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Gut-associated lymphoid tissue (GALT) in humans is not easily visible to the naked eye, even from the luminal surface of the gut. However, lymphoid follicles can be observed through the colonoscope and can be selectively biopsied. So far, only B cells isolated from the relatively prominent follicles of the Peyer's patches sampled at endoscopy have been studied in cell suspensions. Here we compare B cells from three sites in the gut by flow cytometry and we compare the clonal distribution of B cells between these sites by next generation sequencing. The three sites compared were the Peyer's patches of the terminal ileum, the prominent follicles located around the mouth of the appendix and the follicles in the rectum. We have observed that the cellular composition of GALT in the three sites is similar. All sites contain germinal centre, naïve and marginal zone B cells and the T2 but not T1 subsets of transitional B cells and the proportions of each are similar in each site. We observed IL-10 production by B cells at all sites that was higher than production by B cells in the blood and that was highest by the T2 subset of transitional B cells in GALT. B cells clones identified by next generation sequencing were common to all three sites in the gut in 4 tissue donors studied in this way. The features of these clones and their distribution across B cell subsets will be discussed.

OR.90. In Utero Lymphotoxin Signaling Is Required for Mucosal B Cell Function in Adults
Conglei Li, Lesley Ward, Tian Sun, Alberto Martin and Jennifer Gommerman. University of Toronto, Toronto, ON, Canada

Lymphotoxin-beta Receptor (LTβR) signaling is required for development of secondary lymphoid organs, including Peyer's patches. Serum IgA levels are also severely reduced in LT-deficient mice. While it has been shown that B cell migration to the lamina propria is impaired in LT-deficient mice, it is unknown whether LTβR signaling affects mucosal B cell function. Here we show that expression of LTαβ ligand on hemapoietic cells was dispensable for induction and maintenance of gut B cell response against a mucosal rotavirus infection in mice where LTβR signaling was intact in utero. However, expression of LTαβ ligand on hemapoietic cells was required for optimal mucosal B cell responses in mice where LTβR signaling was absent in utero. Specifically, compared to

WT→WT chimeras, AID induction in mucosal B cells was impaired in WT→LTβ$^{-/-}$ chimeric mice concomitant with reduced global fecal IgA levels as well as weaker, slower and non-persistent fecal anti-rotavirus IgA responses. Furthermore, mice treated in utero with LTβR inhibitors also exhibited attenuated mucosal anti-rotavirus responses and alterations in gut resident stromal cells in adulthood. Thus, in utero LTβR signaling shapes gut stromal cell populations and has an impact on mucosal B cell function in adult mice.

T128. Characterisation of T follicular helper cells (Tfh) in human nasopharynx-associated lymphoid tissue and effect of CpG-DNA on TFH-mediated antibody production

Abdullah Aljurayyan[1], Nav Upile[2], Casey Vaughan[2], Carol Xie[2], Ravi Sharma[2], Priya Achar[3], Maxwell McCormick[3], Stephen Gordon[1] and Qibo Zhang[1]. [1]University of Liverpool, Liverpool, United Kingdom; [2]Alder Hey Children's Hospital, Liverpool, United Kingdom; [3]Royal Liverpool and Broadgreen University Hospital, Liverpool, United Kingdom

Considering the importance of T follicular helper cells (T_{FH}) for B cell antibody response, novel adjuvants to boost T_{FH} function may be an attractive vaccination strategy. Adenotonsillar tissues are major component of nasopharynx-associated lymphoid tissue (NALT) and important in mediating immunity to respiratory pathogens. We studied T_{FH} in human NALT and effect of CpG-DNA on T_{FH}-mediated B cell immunity. Adenotonsillar MNC were stained for T_{FH} markers including $CD4^+CXCR5^{high}$ $ICOS^{high}$ by and intracellular cytokine staining. Purified T_{FH} and non-T_{FH} cells were co-cultured with B cells in the presence of influenza antigen and CpG-DNA. Purified pDC were added to T_{FH}-B cell co-culture to study their importance in T_{FH} cell-mediated response. Haemagglutinin (HA)-specific antibody production was analysed. We have found a prominent number of T_{FH} in human NALT considerably higher than in PBMC. There was an age-associated decrease in T_{FH} frequency in NALT. T_{FH} in NALT were shown to express IL-4, IL-10 and IL-21. A good correlation between GC B cell and T_{FH} cell was seen. Co-culture of purified T_{FH} but not non-T_{FH} with B cells promoted antibody production. Stimulation by CpG-DNA increased T_{FH} and that was correlated with HA-specific antibody production following influenza antigen stimulation. Co-incubation T_{FH}-B cell with pDC enhanced the antibody production. Blocking IL-21R reduced T_{FH} that was correlated with reduction of HA-specific antibody production. Enhancing vaccine immunogenicity through modulation of T_{FH} numbers or function in human NALT using modern adjuvants such as CpG-DNA may be an effective vaccination strategy against respiratory pathogens.

F23. Oral Challenge with Wild-Type Salmonella Typhi Induces Distinct Changes in B Cell Subsets in Individuals who Develop Typhoid Disease

Franklin Toapanta[1], Paula Bernal[1], Stephanie Fresnay[1], Thomas Darton[2], Claire Jones[2], Claire Waddington[2], Christoph Blohmke[2], Gordon Dougan[3], Brian Angus[2], Myron Levine[1], Andrew Pollard[2] and Marcelo Sztein[1]. [1]University of Maryland, Baltimore, Baltimore, MD; [2]University of Oxford, Oxford, United Kingdom; [4]Wellcome Trust Sanger Institute, Hinxton, United Kingdom

A new human oral challenge model with wild-type Salmonella Typhi (S. Typhi) was recently developed. In this model, 10^4 CFU resulted in 65% of participants developing typhoid fever (TD) 5-10 days post-challenge. TD was diagnosed in participants meeting clinical (≥38°C for ≥12h) and/or microbiological (bacteremia) endpoints. Changes in B cells subpopulations following S. Typhi challenge remain to be explored. To address this, a subset of volunteers (6 TD and 4 who did not develop TD -NoTD-) was evaluated. The most notable changes included an increase in plasmablasts frequency during disease days (TD-oh to TD-96h). Plasmablasts also showed enhanced binding to S. Typhi, decreased expression of IgA and increased expression of CD21. Additionally, the percentages of B memory subsets (IgD/CD27 classification) were decreased during disease days, most notably in switched (Sm) and unswitched (Um) memory cells. Of note, IgA expression was upregulated in Sm cells. Moreover, the percentages of Sm $CD27^+$ cells phosphorylating Erk1/2, p38MAPK and Btk were increased during disease days. These changes were absent in NoTD volunteers. This is the first study to describe differences in B cell subsets related directly to clinical outcome following oral challenge with wild-type S. Typhi.

F24. Characterization of T Follicular Helper Cells (Tfh) in Human Nasopharynx-Associated Lymphoid Tissue and Effect of CpG-DNA on T_{FH}-Mediated Antibody Production

Abdullah Aljurayyan[1], Nav Upile[2], Casey Vaughan[2], Carol Xie[2], Ravi Sharma[2], Priya Achar[3], Maxwell McCormick[5], Stephen Gordon[1] and Qibo Zhang[1]. [1]University of Liverpool, Liverpool, United Kingdom; [2]AlderHey Children's Hospital, Liverpool, United Kingdom; [3]Royal Liverpool and Broadgreen University Hospital, Liverpool, United Kingdom

Considering the importance of T follicular helper cells (T_{FH}) for B cell antibody response, novel adjuvants to boost T_{FH} function may be an attractive vaccination strategy. Adenotonsillar tissues are major component of nasopharynx-associated lymphoid tissue (NALT) and important in mediating immunity to respiratory pathogens. We studied T_{FH} in human NALT and effect of CpG-DNA on T_{FH}-mediated B cell immunity. Adenotonsillar MNC were stained for T_{FH} markers including $CD4^+$ $CXCR5^{high}$ $ICOS^{high}$ by and intracellular cytokine staining. Purified T_{FH} and non-T_{FH} cells were co-cultured with B cells in the presence of influenza antigen and CpG-DNA. Purified pDC were added to T_{FH}-B cell co-culture to study their importance in T_{FH} cell-mediated response. Haemagglutinin (HA)-specific antibody production was analyzed. We have found a prominent number of T_{FH} in human NALT considerably higher than in PBMC. There was an age-associated decrease in T_{FH} frequency in NALT. T_{FH} in NALT were shown to express IL-4, IL-10 and IL-21. A good correlation between GC B cell and T_{FH} cell was seen. Co-culture of purified T_{FH} but not non-T_{FH} with B cells promoted antibody production. Stimulation by CpG-DNA increased T_{FH} and that was correlated with HA-specific antibody production following influenza antigen stimulation. Co-incubation T_{FH}-B cell with pDC enhanced the antibody production. Blocking IL-21R reduced T_{FH} that was correlated with reduction of HA-specific antibody production. Enhancing vaccine immunogenicity through modulation of T_{FH} numbers or function in human NALT using modern adjuvants such as CpG-DNA may be an effective vaccination strategy against respiratory pathogens.

F25. Early Treatment of HIV-Infected Patients Favors the Preservation and the Development of HIV-Specific B Cells as well as TFH Cells in the Gastrointestinal Mucosa

Cyril Planchais[1], Laurent Hocqueloux[1], Ayrin Kök[3], Nicolas Ruffin[4], Thierry Prazuck[2], Laurent Lefrou[2], Nabila Seddiki[6], Jean-Daniel Lelièvre[5], Yves Lévy[5], Hugo Mouquet[3] and Sophie Hüe[5]. [1]Institut Cochin, Paris, France; [2]Centre Hospitalier Régional d'Orléans, Orléans, France; [3]Institut Pasteur, Paris, France; [4]Institut Curie, Paris, France; [5]Université Paris Est Créteil, Créteil, France

HIV-specific broadly neutralizing antibodies (nAbs) are highly-mutated immunoglobulins rarely found in HIV-infected patients. They result from the interaction between follicular helper T cells (Tfh) and B cells in germinal centers. We, recently, demonstrated the beneficial effects of early treatment on the preservation of intestinal lymphoid structure (ILS). Here, we analyzed the impact of treatment initiation either at the early phase of the primary infection (e-ART) or later during the chronic phase (l-ART) on blood and gastrointestinal mucosal (GM) human GP140-specific B cells (B140) and on GM Tfh cells. e-ART patients displayed a higher frequency of GM B140 than l-ART patients whereas no difference was observed in the blood. B140 were significantly more represented in e-ART patients among resting memory B cells (73.60±2.16% versus 50.03±9.77%, p=0.01), and less represented among naive (12.46 ± 4.65% versus 37.87 ± 10.1%, p=0.04) and tissue-like memory B cells (0.58±0.23% vs. 3.69±0.95%, p=0.006) than l-ART patients. The frequency of Tfh cells was higher within e-ART than l-ART patients (11.43±1.96% vs. 3.30±0.71%, p=0.01), which strongly correlates with the frequency of B140 in GM (r=0.77, p=0.002). Our results demonstrate that early initiation of antiretroviral therapy preserves the Tfh cells present in ILS that maintain the HIV-specific B cells and could help to the development of nAbs.

F26. IgD Activates an Ancestral Galectin-9-CD44-Dependent Protective Pathway that Uncouples Th2 Immunity From Th2 Inflammation

Meimei Shan[1], Cooper Walland[2], Jorge Carrillo[3], John Yeiser[2], Kang Chen[4] and Andrea Cerutti[2,5,6]. [1]Mount Sinai School of Medicine, New York, NY; [2]Icahn School of Medicine at Mount Sinai, New York, NY; [3]IrsiCaixa Institute for AIDS Research, Badalona, Barcelona, Spain; [4]Wayne State University, Detroit, MI; [5]Institut Hospital del Mar d'Investigacions Mèdiques, Barcelona, Spain; [6]Catalan Institute for Research and Advanced Studies, Barcelona, Spain

Immunoglobulin D (IgD) is an ancestral antibody class that emerged in lower vertebrates before the inception of specific Fc receptors. Besides serving as a B cell antigen receptor, IgD mediates enigmatic fluid-phase functions after its release by plasma cells positioned at mucosal and systemic sites of antigen entry. We found that some plasma cells released IgD during both pre-immune and post-immune antibody responses. Basophils captured secreted IgD through a mechanism involving interaction of IgD

with the galactose-binding lectin galectin-9 and CD44, a galectin-9 receptor with immunoregulatory function. Cross-linking of soluble IgD by cognate antigen triggered basophil secretion of interleukin-4 (IL-4), IL-5 and IL-13 as well as expansion of IL-4-expressing T follicular helper cells. The resulting T helper type-2 (Th2) response enhanced B cell production of high-affinity IgG1 antibodies through a basophil-regulated germinal center pathway that required IgD, CD44 and galectin-9 but not FceRI, a degranulation-inducing IgE receptor involved in allergy. Despite enhancing IgE production, IgD mitigated IgE-mediated basophil degranulation and allergy induction through a mechanism possibly involving galectin-9 and CD44, two known FceRI signaling inhibitors. Thus, higher vertebrates may retain an ancestral IgD secretory pathway to maximize antigen clearance via induction of non-inflammatory Th2 responses.

F27. Marginal Zone B Cells in Human Gut-Associated Lymphoid Tissue

Jo Spencer[1], Yuan Zhao[1], Mohamed Uduman[2], Yu-Chang Wu[1], Wayel Jassem[1], Konstantia-Maria Chavele[1], Andrew Wotherspoon[3], Jeremy Sanderson[1], Mats Bemark[4] and Steven Kleinstein[2]. [1]King's College London, London, United Kingdom; [2]Yale School of Medicine, New Haven, CT; [3]Royal Marsden Hospital, London, United Kingdom; [4]University of Gothenburg, Gothenburg, Sweden, Sweden

Marginal zone B cells in gut-associated lymphoid tissue (GALT) in humans are those that surround the mantle zone of naïve B cells and that infiltrate into the follicle associated epithelium. As such they are the B cells located on the boundary between the host and the microbiota and other constituents of the intestinal lumen. They are considered to be the benign analogues of marginal zone B cell lymphomas of mucosal-associated lymphoid tissue (MALT lymphomas). The only other lymphoid tissue in the body with a marginal zone, in health, is the spleen. The splenic marginal zone contains some memory B cells and also B cells responsible for immune responses to TI-2 antigens. Circulating analogues of marginal zone B cells express CD27 and IgM, and includes subsets that are IgD^+ and IgD-. We have compared marginal zone B cells in human Peyer's patches to those in spleen and to putative circulating analogues in blood, by immunohistochemistry, flow cytometry and by next generation sequencing of immunoglobulin heavy chain variable region genes. We have observed that GALT and splenic marginal zone B cell populations differ markedly in the proportion of B cells that express IgD. GALT marginal zone B cells are mostly IgD- whilst splenic marginal zone B cells are IgD^+. The alignment of each of these subsets to blood circulating analogues identified by next generation sequencing and to class switch variants with memory B cell phenotypes will be discussed.

MUCOSAL IMMUNOLOGY IN THE NEONATE

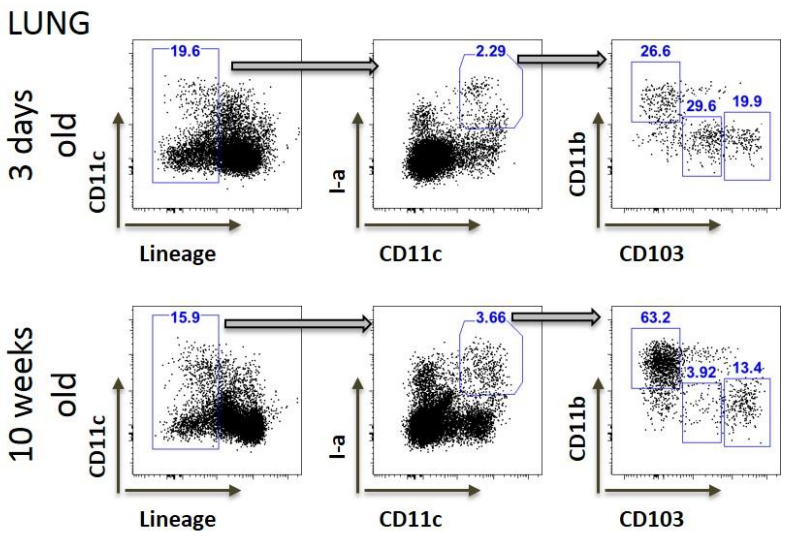
OR.93. Maternal Microbiota-Derived Metabolites Shaping the Neonatal Immune System are Transferred to the Offspring Ante- and Postnatally

Stephanie Ganal[1], Mercedes Gomez de Agüero[1], Anna Steinert[1], Tobias Fuhrer[2], Uwe Sauer[2], Kathy D. McCoy[1] and Andrew J. Macpherson[1]. [1]University of Bern, Switzerland, Bern, Switzerland; [2]ETH Zurich, Zürich, Switzerland

Metabolic capacity of the host and immune system development are dependent on colonization with commensal microbiota. There is evidence that signals originating from commensals during early life or from maternal microbiota before birth are required to shape the neonatal immune system. Our system of reversible colonization of germ-free mice with the auxotrophic E. coli strain, HA107, allows us to expose pregnant mice to microbiota without subsequently colonizing their offspring. We use this system to detect maternal microbiota-derived metabolites that are transferred to the offspring and to assess their influence on neonatal immunity. After colonization of pregnant germ-free mice with ^{14}C-labeled HA107, microbiota-derived products were present in placenta and fetus as well as in the maternal milk as detected by liquid scintillation. Likewise, using ^{13}C-labeled HA107 and mass spectrometry, we identified ^{13}C-labeled products in the maternal milk and the offspring, indicating that maternal microbiota-derived products can reach the offspring. Exposing pregnant mice to HA107 increased the number of intestinal $NKp46^+$ type 3 innate lymphoid cells (ILC3s) and $F4/80^+$ $CD11c^+$mononuclear cells (MNCs) in the offspring and altered the offspring's sensitivity to LPS challenge. Cross-fostering between reversibly colonized and germ-free mothers revealed that both ante- and postnatal transfer of maternal microbiota-derived metabolites are required to fully shape the neonatal immune system.

OR.94. CD103^{int} CCR7^{+} cDCs in Neonatal Lungs have Poor Antigen Presenting Capacity that Limits T Cell Responses Under Non-Inflammatory Conditions

Aaron Silva Sanchez, Selene Meza-Perez, Scott Simpler, Andre Ballesteros-Tato and Troy Randall. University of Alabama at Birmingham, Birmingham, AL



The neonatal immune system must rapidly distinguish newly encountered environmental antigens and commensal organisms from pathogens without compromising the function of still-developing organs, such as the lung. Here we compared neonatal (2-7 days) and adult (>6 weeks) conventional dendritic cells (cDCs) in the lung. We found that neonates had a greater frequency of pulmonary CD103^{+} cDCs than adults. The CD103^{+} cDCs in neonates were comprised of two populations, a CCR7^{+}CD103^{int} population that was not observed in adults and a CCR7-CD103hi population that was similar to the population in adults. Alterations in the composition of pulmonary DCs in the neonate were not due to microbial exposure, as a similar composition was observed in neonatal and adult germ-free mice. The composition of DCs in the mediastinal lymph node (LN) reflected that in the lung, with a higher frequency CD103^{+} migratory DCs in neonatal LNs than in adult LNs. In neonates, the uptake, processing, and presentation of antigen was performed mostly by CD103^{hi}, followed by CD11b, and lastly CD103^{int} DCs. As a result, the T cell-priming capacity of neonatal DCs was limited and activated T cells produced more TNF-a than IL-4 or IFN-g. In summary, lung CD103^{int} CCR7^{+} cDCs are characteristic of the neonatal period and their low APC-activity contributes to poor T cell responses in the neonate.

OR.95. Maternal Microbiota Educates the Immune System of the Offspring Through Natural Antibodies

Mercedes Gomez de Aguero Tamargo, Stephanie Ganal, Sandra Rupp, Anna Steinert, Kathy D. McCoy and Andrew J. Macpherson. University of Bern, Bern, Switzerland

The impact of maternal microbiota during fetal life on the overall immune system development and health of the offspring is not clearly understood. We have found that reversible colonization of pregnant germ free mice impacts on the development of innate immunity in the pups. The offspring from mothers exposed to intestinal microbes only during the pregnancy showed an increase in intestinal NKp46^{+}Rorγt^{+} innate lymphoid cells and F4/80^{+}CD11c^{+} mononuclear cells (iMNC). Local F4/80^{+}CD11c^{+} iMNC differentiation was controlled by maternal microbiota. Moreover, the expression of genes involved in microbial adaptation was imprinted by maternal microbiota. The offspring from microbial treated mothers were protected against microbiota challenge preventing bacterial translocation to mesenteric lymph nodes and modulating gene expression. In the absence of natural antibodies and B cells, maternal microbiota failed to shape the innate immune system in the offspring resulting in increased bacterial translocation following challenge. Furthermore, maternal antibodies required loading with maternal bacterial products to control the development and the efficiency of the innate immune system of the offspring. Our results reveal the tremendous role played by maternal microbiota and natural antibodies in setting the baseline of the innate immune system in the offspring.

OR.96. Neonatal Intestinal Macrophage Subsets: Characterization and Recruitment During an Intestinal Infection

Laurent Potiron, Sonia Lacroix-Lamande, Mathilde Marquis and Fabrice Laurent. French National Institute for Agricultural Research, Nouzilly, France

Recent publications have shown that origin of tissue-resident macrophages varies with age (1). In the intestine, the first macrophages present at birth derived from embryonic precursors originate from yolk sac and fetal liver and express high levels of F4/80. These initial macrophages are progressively replaced around weaning by a CCR2–dependent influx of Ly6Chi blood

monocytes by a process driven largely by the microbiota (2). In the present work we investigated the nature of the macrophage subsets present in the neonatal intestine at homeostasis and during a Cryptosporidium parvum infection in presence of conventional or reduced microbiota. C. parvum is a zoonotic protozoan that infects and completes its parasitic life cycle in the intestinal epithelial cells of the small intestine and affect primarily neonates. We previously demonstrated the importance of intestinal dendritic cells in the control of the infection by innate immune mechanisms (3) and decided to further explore changes in the macrophage subset composition during the infection with conventional and transgenic animals. One striking observation was the presence of a limited number of $CD11c^{+}CX_3CR1^{int}$ monocytes/macrophages in the neonatal intestine. However, these cells were strongly recruited (13-fold) during the infection while $CD11c^{+}CX_3CR1^{hi}$ cells were only modestly affected by the infection. The influence of the infection and of the bacterial flora on these neonatal macrophage subsets will be discussed. (1) Davies et al., Nature Immunology 14, 986–995 (2013); (2) Bain et al., Nature Immunology 15, 929–937 (2014); (3)Lantier et al. PLoS Pathog. 2013 Dec;9(12):e1003801.

F28. Development of the Respiratory Polymeric Immunoglobulin Secretory Immune System (PISIS) in a Porcine Model

Marco Vega-Lopez, Edgar Guzman-Bautista, Carmen Ramirez-Estudillo and Oscar Rojas. Instituto Politécnico Nacional, Mexico City, Mexico

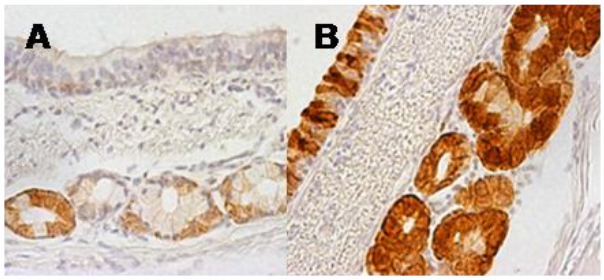


Figure. Immunohistochemical staining of secretory component (pIgR) in porcine trachea. A. Sample taken at birth. B. Sample of a 6-month-old pig. 200 X.

The mucosal secretion of polymeric immunoglobulins (Igs) is mediated by the polymeric immunoglobulin secretory immune system (PISIS), composed by J-chain and antibody producing cells (pAPC), the expression of the polymeric immunoglobulin receptor (pIgR) by epithelial cells and the efficient release of the pIg-pIgR complex to the mucosal lumen. In spite its importance, few detailed studies about their development have been described in humans. Since the porcine model has been reported as an option for translational medicine to humans, we studied the development of the PISIS in trachea and bronchi of healthy, non-vaccinated SPF, miniature Vietnamese pigs, from birth to adulthood using immunohistochemistry and ELISA. Our results showed that colostrum is a source of IgM, SIgA, total IgA and IgG in respiratory secretions (nasal secretion, saliva, trachea and bronchoalveolar lavages) in neonates. Moreover, the pIgR was present at birth, increasing expression with age (Figure). In contrast, pAPCs were low in neonatal pigs, steadily increasing in post-weaned, young and adult pigs. Considerable increases in secretory and total Igs were found in trachea and bronchi with age, correlating with the tissue density of APCs. These data suggest a compensatory role of maternal Igs in the absence of a structured PISIS before weaning. Passive transfer of Igs in elderly people may also compensate PISIS impairment. Besides, it was evident that monomeric Igs may also play an important role in respiratory protection and deserves a more thorough study.

F29. Dysbiosis-Associated Mortality During Dextran Sulfate Sodium (DSS) Induced Murine Colitis is Reduced by 2′-Fucosyllactose, a Human Milk Oligosaccharide

YingYing He[1], David Hill[2], Paul McCabe[1] and David Newburg[1]. [1]Boston College, Malden, MA; [2]University of Michigan Medical Center, Ann Arbor, MI

The mouse DSS model of IBD exhibits many similarities with human ulcerative colitis. Our model uses antibiotic-induced dysbiosis to increase sensitivity to DSS damage. Human milk oligosaccharides (HMOSs) and a principal component, 2′-fucosyllactose (2'-FL), protect the vulnerable neonatal gut through inhibition of pathogen binding, prebiotic activity, and direct inhibition of inflammation. The ability of 2′-FL to ameliorate DSS damage was tested in dysbiotic mice. 12-week old male C57BL/6 mice, following a 14 day course of antibiotics (kanamycin, gentamicin, colistin, metronidazole, and vancomycin), were treated for 7 days with 3.5% DSS with or without 2'-FL. After 3 days recovery, animals were sacrificed. The antibiotic treatment caused dysbiosis of gut microbiota. The most dysbiotic (lowest colony number) were most sensitive to DSS, exhibiting exacerbated body weight loss, bleeding, pro-inflammatory cytokines, and invasive pathobionts in their liver, kidney and lung. In these mice, 2′-FL decreased body weight loss, disease activity index scores, and increased survival. This protection by 2′-FL is associated with decreased pathobiont colonization in colon and invasion to organs, and reduced IL-17 levels. Amelioration of colitis by HMOS is consistent with lower risk of inflammatory diseases in breastfed infants, and suggests utility for 2′-FL in treating clinical inflammatory diseases.

F30. Modulation of CD14 Expression in Human Enterocytes by the Human Milk Oligosaccharide 2'-Fucosyllactose Attenuates Type 1 Pili LPS-Mediated Inflammation

YingYing He[1], Shubai Liu[2], David Kling[1], Serena Leone[3], Nathan Lawlor[1], Yi Huang[1], Samuel Feinberg[1], David Hill[4] and David Newburg[1]. [1]Boston College, Boston, MA; [2]Brigham and Women's Hospital, Boston, MA; [3]University of Naples, Napoli, Italy; [4]University of Michigan Medical Center, Ann Arbor, MI

Gram-negative pathogenic bacteria activate mucosal inflammation through lipopolysaccharide (LPS) binding to intestinal toll-like receptor 4 (TLR4). This study tested whether human milk oligosaccharides (HMOSs) influence pathogenic Escherichia coli-induced interleukin (IL)-8 release by intestinal epithelial cells (IECs), identified specific proinflammatory signaling molecules modulated by HMOSs, and specified the active HMOS and determined its mechanism of action. Inflammation models were IECs invaded by type 1 pili enterotoxigenic E. coli (ETEC) *in vitro*, with T84 modeling mature, and H4 modeling immature, IECs. HMOSs attenuated LPS-dependent induction of IL-8 caused by ETEC, UPEC, and AIEC infection, and suppressed CD14 transcription and translation. CD14 knockdown recapitulated HMOS-induced attenuation. Overexpression of CD14 increased the inflammatory response to ETEC and sensitivity to inhibition by HMOSs. 2'-fucosyllactose (2'-FL) activity was equivalent to total HMOSs *in vitro* and *in vivo*. HMOSs and 2'-FL directly inhibit LPS mediated inflammation during ETEC invasion of T84 and H4 IECs through attenuation of CD14 induction. CD14 expression mediates LPS-TLR4 stimulation of the 'macrophage migration inhibitory factors' inflammatory pathway via SOCS 2/STAT 3/NF-κB. HMOS and 2'-FL inhibition of inflammation supports their functioning as components of an innate immune system whereby the mother's milk quenches hyper-inflammation of the neonatal gut, and suggests their clinical utility.

F31. Active Suppression of Intestinal $CD4^+TCR\alpha\beta^+$ T Lymphocyte Maturation During the Postnatal Period

Natalia Torow[1], Kai Yu[2], Jenny Freitag[3], Kasra Hassani[2], Matthias Lochner[3], Andé Bleich[2], Tim Sparwasser[3], Reinhold Förster[2], Oliver Pabst[1] and Mathias Hornef[1]. [1]RWTH Aachen, Aachen, Germany; [2]Hannover Medical School, Hannover, Germany; [3]Twincore, Hannover, Germany

Priming of the mucosal immune system during the early postnatal period substantially influences the host-microbial interaction and susceptibility to immune-mediated diseases in adult life. The underlying mechanisms, however, are ill defined. Here, we investigated the migratory routes, immune function, and maturation kinetic of intestinal mucosal $CD4$ T lymphocytes during the postnatal period. Shortly after birth, $CD4$ T cells populate preformed lymphoid structures and quickly acquire a distinct transcriptional profile. T cell recruitment is independent of microbial colonization, innate immune receptor or T cell receptor (TCR)-mediated stimulation but requires β7 integrin expression. Surprisingly, neonatal $CD4$ T cells remain immature throughout the postnatal period, despite exposure to the rapidly evolving enteric microbiota. Yet, they are able to readily undergo maturation and gain effector function upon barrier disruption by invasive infection with rotavirus or Salmonella enterica as well as upon adoptive transfer into adult recipients. Maternal SIgA and signals intrinsic to Peyer's patches act to prevent immune stimulation and maintain the immature phenotype of $CD4$ T cells in the postnatal intestine. Our results identify mechanisms that actively suppress $CD4$ cell maturation during the postnatal period, and that might significantly contribute to prevent auto-reactivity, sustain a broad TCR repertoire and establish life-long immune homeostasis.

F32. Dendritic Cell Development in the Neonatal Intestine

Tamsin Zangerle Murray[1], Calum Bain[2] and Allan Mowat[1]. [1]University of Glasgow, Glasgow, United Kingdom; [2]University of Edinburgh, Edinburgh, United Kingdom

Dendritic cells (DCs) are crucial for the ability of the intestinal immune system to decide between inducing tolerance or active immunity. We and others have identified four populations of intestinal DCs based on CD11b and CD103 expression, with $CD103^+CD11b^+$ DCs being unique to the intestine. As this suggests the presence of specific differentiation factors in the local microenvironment, here we have explored when this population appears during early life. All four DC subsets are present in the lamina propria (LP) from birth, but $CD103^+CD11b^+$ DCs are scarce at this time and do not reach adult levels until around weaning. This is not simply due to delayed expression of CD103, as there is parallel acquisition of TREM-1 expression by this subset of DCs. A similar pattern of subset maturation is seen amongst migratory DCs in the draining mesenteric lymph node (MLN), although this is delayed compared with LP, consistent with these cells arriving from the mucosa. Thus the profound changes that occur in the intestinal environment after birth appear to be responsible for the selective differentiation of $CD103^+CD11b^+$ DCs and identification

of the factors involved will aid understanding of how DCs assist induction of tolerance against commensal bacteria and food proteins.

F33. Maturation of the Avian IgA System Critically Depends on Microbial Colonization
Bernd Kaspers[1], Sarah Lettmann[1], Susanne Röll[1], Philippe Velge[2] and Catherine Schouler[2]. [1]University of Munich, Munich, Germany; [2]French National Institute for Agricultural Research, Nouzilly, France

The avian immune system shows striking differences to its mammalian counterpart such as the complete lack of lymph nodes and the development of B cells in a unique gut associated lymphoid organ the bursa of Fabricius. At present, little is known about relevance of microbial colonization for the development of the mucosal immune system in birds. In order to address this question, we raised germ free chickens and reconstituted them with the E.coli strain Nissle alone or in combination with an Enterococcus, Lactobacillus and Clostridium strain. Analysis of GALT structures and mucosal immune cell populations revealed no impact of the microbiota on bursal development but a significant role in the development of the mucosal secondary immune organs. Most strikingly, germ free birds completely lacked IgA plasma cells in the lamina propria, systemic and mucosal IgA production and germinal center formation in the caecal tonsils, the most prominent GALT structure in birds. Mono-reconstitution with E.coli Nissle only partially reverted this phenotype but induced a significant IgA response to this bacterium. Tetra-reconstitution further improved but did not fully mature the mucosal immune system as compared with the status in SPF birds. The striking IgA deficiency in germ free birds was paralleled by significantly reduced expression levels of J-chain, AID, BAFF, BAFF-R and poly-IgR mRNAs. We conclude that maturation signals seem to be identical in mammals and birds further supporting the importance of the IgA system in tissue homeostasis which has been conserved for more than 300 million years since birds and mammals segregated.

F34. Mice with Neural Crest Conditional Deletion of EdnrB Model Neonatal Hirschsprung's Associated Enterocolitis
Ankush Gosain, Amanda Barlow-Anacker, Ken Kudsk and Miles Epstein. University of Wisconsin, Madison, WI

Hirschsprung's disease (HSCR) is a common cause of intestinal obstruction in the newborn and is characterized by absence of the enteric nervous system in the distal hindgut. Up to 40% of infants develop potentially life-threatening Hirschsprung's-Associated Enterocolitis (HAEC) prior to definitive surgery. Clinical series have suggested that abnormal goblet cell function, decreased secretory IgA secretion, leukocyte dysfunction, altered microbiota, and bowel obstruction may all contribute to the pathogenesis of HAEC. We have characterized mice with a neural crest conditional deletion of endothelin receptor B (EdnrB), which display distal colonic aganglionosis and develop HAEC in the fourth post-natal week. Our investigations reveal decreased luminal SIgA and impaired SIgA transport (polymeric immunoglobulin receptor), alterations in goblet cell numbers and morphology, alterations in splenic and Peyer's Patch lymphocyte populations, and persistence of primarily anaerobic species (Bacteroides, Tanerella, Clostridium, Paludibacter) in the colonic microbiota during HAEC. These findings mimic the human condition and provide targets for understanding the multifactorial pathogenesis of HAEC.

F35. Prophylactic Interleukin-2 Treatment Prevents Fetal Gut Inflammation and Injury in an Ovine Model of Chorioamnionitis
Maria Nikiforou, Joris Vanderlocht, Boris Kramer and Tim Wolfs. Maastricht University Medical Center, Maastricht, Netherlands

Chorioamnionitis, which results from an infection of the fetal membranes, is associated with adverse fetal intestinal outcomes. Using a translational ovine model, we showed that intra-amniotic (IA) exposure to inflammatory stimuli decreased the regulatory/effector T (Treg/Teff) cell balance in the gut, which was accompanied by intestinal inflammation and mucosal injury. We aimed to augment the Treg/Teff cell ratio in the fetal gut by systemic prophylactic IL-2 treatment, to prevent chorioamnionitis-induced intestinal inflammation and subsequent injury. Fetal sheep were intra-amniotically exposed to LPS for 2 or 7 days, with or without prophylactic IL-2 treatment. Infiltration of inflammatory cells and cytokine gene expression in the fetal ileum were analyzed and correlated with gut wall integrity. IL-2 administration increased intestinal Treg cells and thus the Treg/Teff cell ratio. Prophylactic IL-2 treatment reduced the LPS-induced influx of neutrophils and $CD3^+$ cells and decreased the mRNA levels of pro-inflammatory cytokines including IL-6 and IL-17 in the fetal ileum. Importantly, prophylactic IL-2 treatment prevented mucosal damage without inducing fetal adverse treatment outcomes. Our data show that prophylactic IL-2 treatment prevents fetal intestinal inflammation and mucosal injury in the context of experimental chorioamnionitis. Modulation of the Treg/Teff cell balance may contribute to these IL-2-induced protective effects.

MUCOSAL INFECTIONS

OR.22. Unravelling a Novel Mechanism by Which Cytokines Induce ER Stress to Halt Viral Protein Synthesis in Mucosal Epithelial Cells and its Implications for Sterile Inflammatory Diseases

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Whilst it is accepted that endoplasmic reticulum (ER) stress initiates inflammatory signaling via the unfolded protein response, the influence of local inflammatory factors on ER stress remains unclear. We recently demonstrated in mucosal epithelial cells and pancreatic β-cells that specific inflammatory cytokines such as IL-17A, IL-23, IL-24 and IL-33 are potent initiators of oxidative stress, which induces protein misfolding and ER stress. Conversely, IL-22, and to a lesser extent, IL-10, suppress ER stress and facilitate protein folding[1, 2]. Using a murine pneumovirus infection model, we now report that cytokine-induced ER stress prevents viral protein synthesis by mucosal epithelial cells. Neutralization of IL-24 or administration of IL-22 through the early stage of infection increased pneumovirus replication, epithelial apoptosis and lung injury. In mouse models of non-infectious mucosal inflammation cytokine-induced ER stress is initiated inappropriately and exacerbates disease. For example, this mechanism explains the characteristic goblet cell depletion seen in colitis which leads to loss of mucus, exposing the epithelium to microbes and further exacerbating inflammation. In murine colitis neutralizing stressor cytokines or replenishing suppressor cytokines restored goblet cell mucin production. While manipulation of cytokine-induced ER stress provides a therapeutic opportunity for inflammatory disease, consequences for viral infection need to be considered. 1. Hasnain SZ, et al. Glycemic control in diabetes is restored by therapeutic manipulation of cytokines that regulate beta cell stress. Nat Med 2014;20:1417-26. 2. Hasnain SZ, et al. IL-10 promotes production of intestinal mucus by suppressing protein misfolding and endoplasmic reticulum stress in goblet cells. Gastroenterology 2013;144:357-368 e9.

OR.23. Oral-Resident Natural Th17 Cells and γδ T Cells Control Opportunistic *Candida albicans* Infections

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Oropharyngeal candidiasis (OPC) is an opportunistic mucosal infection caused by the commensal Candida albicans. To date, there is a paucity of research focusing on oral mucosal immune responses and fungal immunity. Individuals with rare genetic defects have shown the protective role of Th17-associated cytokines IL-23 and IL-17 to OPC. OPC is frequent in HIV/AIDS, implicating adaptive immunity. It has long been assumed that classic iTh17 cells are the primary source of IL-17 in OPC. However, multiple innate cell populations can produce IL-17, though their role in oral immunity is largely unknown. Since rodents are naïve to Candida albicans, we used a mouse OPC model to focus on innate antifungal immunity. T cell receptor rearrangement is required, as $Rag1^{-/-}$, SCID, $IL\text{-}7R\alpha^{-/-}$ mice are susceptible to OPC. However, classic iTh17, NK and NKT cells were dispensable. Using fate-tracking IL-17 reporter mice, we found that IL-17 is produced within 1-2 d by tongue-resident populations of γδ T cells and $CD3^{+}CD4^{+}CD44^{hi}TCR\beta^{+}CCR6^{+}$ natural Th17 (nTh17) cells, but not by innate lymphoid cells (ILCs) or NK cells. The role of γδ T cells during dermal candidiasis is well understood, and this cell type is known to mediate host defense at mucosal surfaces through the production of IL-17. To date the function of nTh17 cells is poorly understood, and little is known about the role of nTh17 cells in infections. This is the first demonstration of a host-protective activity for nTh17 cells, and suggests that this population serves as a mucosal sentinel that controls oral pathogens.

OR.24. Resistance to a Helminthic Infection is Dependent on Mast Cell Activation Mediated by ATP in Spi-B-deficient Mice

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We have evaluated protective roles for mast cells against infection with an intestinal nematode, Heligmosomoides polygyrus (Hp) utilizing a mutant mouse strain that genetically lacks the Spi-B transcriptional factor (Spi-BKO). These mice exhibiting the increase in mast cells very quickly expelled the worms in association with induction of group 2 innate lymphoid cells (ILC2) producing IL-13 and goblet cell hyperplasia in the intestinal epithelium. Mast cells were rapidly activated in response to ATP, released from apoptotic intestinal epithelial cells immediately after Hp infection, followed by production of IL-33 and IL-25. *In vivo* inhibition of

ATP with P_2X_7 receptor on mast cells using a selective inhibitor abolished mast cell activation and induction of IL-13-producing ILC2, which rendered Spi-BKO mice susceptible to Hp. These results clearly demonstrate that activation of mast cells by ATP orchestrates the development of protective type 2 responses by producing IL-33 and IL-25 both of which are crucial for ILC2 activation.

OR.28. A Crohn's Disease Susceptibility Gene, ATG16L1 Confers Protection From Urinary Tract Infections Caused By Uropathogenic *Escherichia Coli* in Mice And Humans

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A polymorphism (T300A) in the ATG16L1 gene is associated with increased susceptibility to Crohn's disease (CD). We identified a protective role of ATG16L1 deficiency in urinary tract infection (UTIs), very common recurrent infections caused predominantly by uropathogenic E. coli (UPEC). We posit that the protection to infectious disease might provide selective pressure on this CD risk allele. We demonstrate that ATG16L1 plays epithelial-intrinsic and macrophage-specific roles in UTI pathogenesis. Loss of ATG16L1 in epithelial cells results in reduction of persistent niches for UPEC and renders them unable to cause a recurrent UTI. Loss of ATG16L1 in macrophages enhances IL-1β release in response to UPEC, in a NOD2-independent but caspase-1/NLRP3 inflammasome-dependent manner. Inhibition of IL-1β signaling abrogates ATG16L1-dependent protection from UTIs. These phenotypes are replicated in mice carrying the T300A point mutation. Our work suggests that aberrant pro-inflammatory responses to commensals in the gut mucosa leading to CD pathogenesis may occur as a trade-off for productive responses to pathogens in the urinary tract mucosa. To extend our findings from mice to humans, we are conducting genomic studies to investigate associations between the T300A mutation and incidence of single and multiple UTIs and have found that presence of the T300A allele is associated with reduced incidence of UTIs in humans. Together, our findings have implications for elucidating how UPEC is able to evade host innate defenses to cause a UTI and suggest that ATG16L1 polymorphisms may be maintained in the population because of protective effects from this common infection.

OR.60. Host Directed Therapy for Chronic Tuberculosis via Intrapulmonary Aerosol of siRNA Targeting the STAT3 Pathway

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In this study is shown that host directed therapy [HDT] targeting IL-10 and STAT3 during chronic pulmonary infection with Mycobactrium tuberculosis has bactericidal effect against drug tolerant bacilli. When chronically Mtb infected C57BL/6 mice received two weeks of standard TB chemotherapy consisting of isoniazid and rifampin (HR) as expected the pulmonary bacterial load decreased significantly and a population of drug tolerant bacilli remained after therapy. When compared to infected animal controls not receiving chemotherapy, the lungs of mice receiving HR had higher concentrations of IL-10 and STAT3 whereas the concentration of antimicrobial end effector products were decreased. On the other hand if chronically Mtb infected mice received combined therapy regimens (HR-HDT) of HR chemotherapy followed by HDT consisting of intrapulmonary aerosols of siRNAs targeting il-10 and stat3 the pulmonary drug tolerant bacterial load was significantly reduced by more than 90% when compared to control mice receiving HR but not HDT. Furthermore, mice receiving HR-HDT have increased pulmonary antimicrobial capacity as evidenced by higher expression of antimicrobial end effector molecules and decreased arginase activity. Moreover, important checkpoints regulating cell apoptosis-autophagy were also affected by HR-HDT therapy. As a proof of concept, here, it is shown that a successful targeting of the host IL-10-STAT3 pathway via aerosol delivery of siRNAs can modulate the lung immunity to enhance its own antimicrobial capacity and can reduce the pulmonary drug tolerant bacterial burden.

W127. Relationship between mucosal Th17 and Treg in Nasopharynx-Associated Lymphoid Tissue and Their Association with Pneumococcal Carriage in Children and Adults

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Streptococcus pneumoniae is a leading cause of respiratory tract infection in humans. It colonizes human nasopharyngeal mucosa and pneumococcal carriage, it is most common in young children which may account for the high incidence of pneumococcal

disease in this age group. Pneumococcal carriage rate in humans decreases with age. However, the immunological factors that mediate the clearance or persistence of carriage in human nasopharynx remain unclear. Recent studies in mice suggest Th17 is important in host clearance of *S. pneumoniae*. We have studied the relationship between frequencies of mucosal Th17 and T regulatory cells (Foxp3+ Treg) in human nasopharynx-associated lymphoid tissue (NALT) and age, comparing their association with pneumococcal carriage in children and adults. The frequencies of Th17 and Treg in adenotonsillar tissue were significantly higher than in peripheral blood ($p<0.01$) in both children and adults. There was an inverse correlation between frequencies of Th17 and Treg in adenotonsillar tissue ($r=-0.52$, $p<0.01$). Tonsillar Th17 frequency was shown to increase with patient's age ($r=0.62$, $p<0.01$), whereas the Treg frequency inversely correlated with age ($r=-0.45$, $p<0.01$). Furthermore, there was a positive correlation between the ratio of Th17/Treg in NALT and age of patients. Also, the Th17/Treg ratio was significantly higher in pneumococcal carriage negative than positive children ($p<0.05$). Our findings suggest that the balance/ratio of mucosal Th17 and Treg in nasopharynx is a critical determinant of pneumococcal clearance/carriage, and support efforts to promote mucosal Th17 in vaccination strategy against pneumococcal infection in humans.

F36. The Immune Response Against Chlamydia Suis Genital Tract Infection Partially Protects Against Re-Infection

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Background: Chlamydia suis is widespread in commercial pig production and causes important economic losses. Currently, C. suis infections are mainly associated with conjunctivitis and reproductive disorders in sows and boars. Objectives: The aim was to reveal the characteristics of genital C. suis infection and re-infection in female pigs by studying the immune response, pathology, replication of chlamydia in the genital tract and bacterial excretion. Methods: Pigs were intravaginally infected and re-infected with the C. suis reference strain S45. Results: S45 is pathogenic for the urogenital tract. Chlamydia replication occurred throughout the urogenital tract, causing inflammation and pathology. The infection elicited both cellular and humoral immune responses. Compared to the primo-infection of pigs with C. suis, re-infection was characterized by less severe macroscopic lesions and less chlamydial elementary bodies and inclusions in the urogenital tract. This indicates the development of a certain level of protection following the initial infection. Protective immunity against re-infection coincided with higher chlamydia-specific IgG and IgA antibody titers in sera and vaginal secretions, higher proliferative responses of peripheral blood mononuclear cells (PBMC), higher percentages of B lymphocytes, monocytes and $CD8^+$ T cells and upregulated production of IFN-γ and IL-10 by PBMC. Data on immunohistochemistry will be presented. Conclusions: Although C. suis is often still considered as an insignificant pathogen of pigs, it was demonstrated to be a primary pathogen of the urogenital tract. Furthermore, we established an experimental challenge model, suitable for further pathological and immunological investigations and will probably also be useful for studying vaccine development.

F37. Elucidating Pathways of Toxoplasma gondii Infection in the Gastrointestinal Tract: Involvement of the Tight Junction Protein Occludin

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Toxoplasma gondii (T. gondii) is an obligate intracellular parasite, infecting one third of the world's population through the consumption of contaminated undercooked meat, water and soil. The parasite's primary route of infection is via the small intestine, although the routes of entry remain unclear. Using epithelial cells derived from the small intestine we set out to determine if the parasite transmigrates epithelial cells via the paracellular route by interacting with and/or disrupting tight junction complexes. *In vitro* invasion assays revealed that T. gondii infects and transmigrates through polarized epithelial cell monolayers without altering barrier integrity. However, during invasion Toxoplasma co-localized with occludin, which was subsequently redistributed from tight junction complexes to intracellular sites. Reduction of occludin expression reduced the ability of Toxoplasma to penetrate epithelial cells, consistent with the involvement of occludin in parasite transmigration. Furthermore, *in vitro* binding assays using recombinant fragments of occludin confirmed the ability of the parasite to interact with occludin and in particular with the extracellular loops. Following immunoprecipitation of occludin from infected cell lysates, we have identified potential candidate occludin-binding partners from T. gondii, by mass spectrometry. Together, we provide evidence that T. gondii can interact with occludin to help facilitate its infection of epithelial cells.

F38. Mechanism for Persistent Salmonella Enterica Serovar Enteritidis Infections in Chickens: Induction of Anti-Inflammatory Host Cell Signaling and the Migration and Activation of Regulatory T Cells

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Non-typhoidal Salmonella enterica induce an early pro-inflammatory response in chickens that is asymptomatic of disease, resulting in a persistent colonization of the ceca. The underlying mechanisms that control this persistent infection of chickens by Salmonella are unknown. We hypothesize that a tolerogenic response is induced by alterations of host signaling pathways that mediate the influx and functional activation of $CD4^+CD25^+$ T regulatory (Treg) cells. Here, we evaluated the development of immunological tolerance in chickens infected with S.Enteritidis in a persistent infection model (4-14 days post infection). For the first time, we outline the induction of a tolerogenic response in the cecum of chickens infected with S. Enteritidis beginning around 4 days post- infection. The response is induced by a series of phosphorylation-mediated changes in the cecal tissue of chickens during the development of a persistent Salmonella infection. The tolerance is characterized by alterations in T cell signaling (dephosphorylation of phospholipase c-γ1) and mTOR signaling pathways (increased phosphorylation of AMP-activated protein kinase) and blockage of IFN-γ protection through the disruption of the JAK-STAT signaling pathway (dephosphorylation of JAK2, JAK3, and STAT4). Further, we found a reduction in pro-inflammatory cytokine mRNA expression and an increase in anti-inflammatory cytokine mRNA expression. Lastly, we found an expansion of the Treg population and subsequent increased *in vitro* immunosuppressive functions of the $CD4^+CD25^+$ cells isolated from the ceca of the Salmonella-infected chickens. These studies define a mechanism by which Salmonella infection influences the host responsiveness that establishes a persistent colonization of the avian cecum.

F39. Regulation of Type 2 Diabetes by Helminth-Induced Th2 Immune Responses

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Helminth-induced Th2 cytokines increase the number of regulatory T cells and M2 macrophages, resulting in the modulation of the host immune system. Studying parasite-induced immune regulatory mechanisms might contribute to the development of novel therapies for the treatment of inflammatory diseases, including Type 2 diabetes mellitus (T2DM). Previous studies have suggested the progression of obesity-associated metabolic abnormalities is under the pathophysiological control of $CD4^+$ T cells. Furthermore, glucose absorption through intestinal epithelium is decreased following infection in a STAT-6-dependent manner. In this study, we investigated whether infection with the gastrointestinal nematode parasite, Heligmosomoides polygyrus, could modulate disease severity in a mouse model of T2DM (KK-Ay/Tajcl). KK-Ay mice were inoculated with infective, third-stage H. polygyrus larvae. Studies were conducted 8 days after infection. Uninfected KK-Ay mice had more elevated serum glucose levels 120 minutes after i.p. administration of glucose than mice in the infected group. HOMA-IR, fat accumulation and hepatic FAS gene expression were significantly decreased by H. polygyrus infection. Gene expression of GLUT2 was significantly decreased in infected mice compared with that in uninfected diabetic mice, which is possibly involved in decreased intestinal glucose absorption by parasite infection. In conclusion, helminth-induced Th2 cytokines may reduce T2DM disease severity.

F40. Prior Cryptosporidium Infection Leads to Protective Th-1 Type Mucosal Immunity Despite Protein Malnutrition

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Cryptosporidium is a major cause of childhood diarrhea and stunting in resource-limited countries. Malnutrition increases risk of cryptosporidiosis and could impair mucosal vaccine uptake. The immunologic consequences of malnutrition on mucosal immune responses, and specifically related to Cryptosporidium infection, are poorly understood. Malnourished children with cryptosporidiosis have increased fecal IL-13, which has been interpreted to indicate a Th-2-biased response. Using a murine model of protein malnutrition (PM), we show that despite decreased baseline IFN-γ, and severe enteropathy, Th-1 type immune responses in cryptosporidiosis are enhanced. Primary infection with 10^6 C. parvum challenge conferred resistance to parasitism and weight loss after secondary challenge with 10^7 C. parvum 21 days later. C. parvum 10^6 challenge resulted in minimal inflammation at three days post-infection, but through 23 days post-infection, CCL5 progressively increased (68.83 vs 38.43 pg/ml, $P<0.05$) and $CD4^+$ and $CD8^+$ cells, including those with a $CD8^+CD103^+$ phenotype, expanded within the lamina propria. At re-challenge, previously exposed mice had further increases in CCL5 (122.38 vs 34.61, $P<0.001$), as well as greater IFN-γ (21.38 vs 11.47 pg/ml, $P=0.056$) and IL12p40

(26.17 vs 1.90 pg/ml, P<0.05) compared with primary infection. Re-challenge associated with significantly lower IL-13 (113.30 vs 0 pg/ml; P<0.05) than primary infection. IL-4 and IL-5 were not elevated, suggesting a non-Th2 source of IL-13. This is the first model to demonstrate protective mucosal immunity in cryptosporidiosis despite PM. Future investigations will confirm whether the $CD8^+CD103^+$ cells have a tissue-resident memory phenotype and are protective, and whether IL-13 promotes barrier dysfunction in cryptosporidiosis.

F41. Secretory IgA Deficiency in Small Airways Results in COPD-Like Lung Inflammation and Remodeling
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To investigate the role of secretory IgA (SIgA) deficiency in chronic obstructive pulmonary disease (COPD), we analyzed 1,104 small conducting airways from 50 former smokers with COPD and 39 healthy controls. Based on detection of IgA-immunospecific fluorescent signal on the mucosal surface, we found an increased proportion of SIgA-deficient airways in COPD patients (<5% airways in healthy controls vs. 47% airways in patients with mild-to-moderate COPD and 71% airways in patients with severe COPD). Independent of disease state, SIgA-deficient airways had thickened walls, accumulation of neutrophils and macrophages, increased susceptibility to bacterial colonization, and NF-κB pathway activation. In contrast, airways with intact SIgA showed none of these findings. Polyimmunoglobulin receptor (pIgR)-deficient mice, which have airway SIgA deficiency, showed bacterial invasion into the airway epithelial border, NF-κB activation, and progressive COPD-like inflammation and remodeling by 6-12 months of age. Reconstitution of airway SIgA in $pIgR^{-/-}$ mice reduced acute inflammation following bacterial challenge. In addition, treatment of $pIgR^{-/-}$ mice with the long-acting phosphodiesterase-4 inhibitor roflumilast blocked progression of the COPD-like phenotype. Together, our findings support the concept that loss of the mucosal immune barrier in small airways and persistent activation of innate immune response contribute to COPD progression.

F42. Helminth-Based Therapy of Colitis: Influence on Tumor Growth and Progression in Colitis-Associated Colon Cancer
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Ulcerative colitis (UC) is a chronic inflammatory disorder of the gastrointestinal tract, associated with an increased risk of colorectal cancer development. Since the etiology of UC is still unknown, current therapies are able to alleviate the acute inflammation but fail to cure the patients. The IBD patients' exposure to helminths appears to be a novel promising approach as helminths provoke an immunosuppressive state in the host by releasing immunomodulatory molecules and inducing regulatory T cells (Tregs). However, whether this increase in regulatory T cell numbers interferes with the development of colitis-associated colon cancer (CAC) is not yet known. In the present study we demonstrate that the treatment of mice with Heligmosomoides (H.) polygyrus at the onset of tumor progression in a mouse model of CAC does not alter tumor growth and distribution. In contrast, H. polygyrus infection in the early inflammatory phase of CAC seems to strengthen the inflammatory response and to boost tumor development accompanied by a reduced frequency of colonic $CD8^+$ effector T cells. Together, our results demonstrate that the therapeutic application of helminths during CAC might have tumor-promoting effects. Therefore the specific time point of helminth infection may dictate the therapeutic outcome.

F43. Disease in the Absence of Virulence Factors: "Avirulent" (TTSS-1/2-Deficient) Salmonella Mutants Induce Colitis in NADPH Oxidase KO-Mice
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Infections permanently challenge the intestinal immune system. The production of reactive oxygen species (ROS) by NADPH oxidase is thought to be a key element of defense. This becomes evident in patients lacking one of the subunits of the NADPH oxidase, as these are hyper-susceptible to infections, e.g. have a 10x higher likelihood to suffer from Salmonellosis. We used NADPH oxidase deficient ($Cybb^{-/-}$) mice and Salmonella Typhimurium (S. Typhimurium) infections to assess the microbe handling by the large intestinal mucosa. Wild type S. Typhimurium is known to employ two key virulence factors to cause enteropathy. Thus, a S. Typhimurium mutant lacking these virulence genes ($S.Tm^{avirulent}$; invGsseD), cannot cause enteropathy in wild type mice. Strikingly, in $Cybb^{-/-}$ mice the same mutant strain can trigger pronounced colitis. These mice showed enteropathy dependent on Myd88 and $CD11c^+CX_3CR1^+$ monocytic phagocytes by day 4 post infection. Surprisingly, a partial reconstitution of

Cybb-proficiency in the bone marrow derived compartment was sufficient to reduce disease severity significantly. Thus, we concluded that NADPH oxidase expression is restricting the growth of S.Tm^{avirulent} in the mucosal lamina propria. The disease is the result of a complex interplay between the pathogen, its virulence factors and the innate defenses of the host's mucosa.

F44. Constitutive, Intestinal IgA Secretion is Regulated by NLRP3-Derived IL-1 and Confers Protection from Enterotoxin-Induced Fluid Aaccumulation

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A role for interleukin (IL)-17 in the regulation of polymeric immunoglobulin receptor (pIgR) expression at mucosal surfaces has recently been described (Jaffar et al, Cao et al). In this study we investigated whether constitutive IL-17 production in the gastrointestinal tract (GIT) was dependent on NLRP3 inflammasome-derived IL-1β and analyzed the consequences of deficiency in components of this pathway on homeostatic secretory antibody responses. We found that both NLRP3$^{-/-}$ and IL-1R$^{-/-}$ mice had significant impairments in their ability to produce and secrete IgA in the GIT and lungs under steady-state conditions. Moreover, intestinal exposure to cholera toxin (CT) triggered rapid secretion of IgA into the lumen in an IL-17, IL-1 and NLRP3-dependent manner. We hypothesized that this secretory IgA might be protective in an enteric insult setting. Indeed we observed an increased susceptibility to CT-induced enterotoxicity in IL-17R$^{-/-}$, IL-1R$^{-/-}$ and NLRP3$^{-/-}$ mice correlating with reduced faecal IgA levels in these strains. These findings suggest a key role for the inflammasome-IL-1-IL-17 axis in facilitating optimal humoral immunity at the mucosae and identify a protective role for non-specific, secretory IgA in an enterotoxin challenge model.

F45. IL-18: A Key Modulator of Mucosal Inflammation During Salmonella Infection

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Pathogen control at mucosal surfaces relies on an efficient innate immune response. However, the mucosal defense mechanisms are not completely understood. We have analyzed the initial phases of mucosal infection by Salmonella typhimurium (S.Tm) using the streptomycin mouse model. Epithelium-intrinsic recognition of S.Tm was recently shown to rely on the NLRC4/Caspase-1 inflammasome and the shedding of infected enterocytes. However, it had remained unclear if/how inflammasome-activation may activate additional mucosal defenses. In the present study, we show that IL-18, a downstream target of Caspase-1, is required for rapid and efficient stimulation of mucosal inflammation, while IL-1α/β appears dispensable. Knockout mice, cytokine inhibition and cytokine injection revealed that IL-18 accelerates the inflammatory response. IL-18 mediated inflammation was independent of a functional IFNγ response. However, IL-18 facilitated an effective local NK cell response, which was important for a rapid mucosal inflammation. Collectively, these data establish that epithelium-intrinsic NLRC4/Caspase-1 activation orchestrates the shedding of infected enterocytes with mucosal inflammation and implicate IL-18 in the initiation of mucosal inflammation in response to bacterial infection via the induction of an efficient NK cell response.

F46. Elmo-1 Regulates the Induction of Autophagy and Bacterial Clearance During Salmonella Infection

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Macrophages are specialized phagocytic cells involved in clearing invading pathogens by phagolysosomal and autophagic degradation. Earlier, we reported that Brain Angiogenesis Inhibitor 1 (BAI1) recognizes bacterial lipopolysaccharide (LPS). BAI1 binds to Engulfment and Cell Motility 1 (ELMO1) that mediates the engulfment of Salmonella and regulates inflammatory responses. Here, we hypothesize that BAI1-ELMO1 pathway plays a crucial role in bacterial clearance by modulating host cell immune responses. Salmonella infection in J774 increases accumulation of an autophagic marker LC3B in an ELMO1-dependent manner. Silencing ATG5 in ELMO1-knockdown cells confirms that ATG5 is essential for ELMO1-mediated LC3B regulation. This result indicates that ELMO1 regulates conventional autophagy. Subcellular fractionation shows that like BAI1, ELMO1 is present in phagosomes. Furthermore, we show that the lysosomal environment of ELMO1-knockdown cells is more acidic and proteolytic in nature as compared to the empty vector-transfected cells. In addition, we confirm that faster recruitment of the Early Endosomal Antigen 1 (EEA1) and the Lysosomal marker LAMP1 occurs in ELMO1-shRNA cells. These results suggest there is an interrelationship between autophagic regulation and the clearance of pathogens through ELMO1-mediated events. Taken

together, we conclude that ELMO1 is an important modulator for the clearance of enteric pathogens by controlling cellular autophagy.

F47. Interleukin-1 Signaling in Intestinal Stromal Cells Prevents Bacteremia upon Citrobacter rodentium Infection via Migration of IL-22 Secreting Neutrophils at Early Stages of Infection

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Attaching and effacing pathogens, including enterohemorrhagic Escherichia coli in humans and Citrobacter rodentium in mice, raise serious public health concerns. Here we demonstrate that interleukin-1 receptor (IL-1R) signaling is indispensable for protection against C. rodentium infection in mice. Oral challenge with C. rodentium led to severe loss of body weight and high mortality in IL-1R$^{-/-}$ mice at 7–14 days after infection whereas wild-type mice recovered from mild symptoms within 3 weeks. At day 10 after infection, mRNA and protein levels of KC/CXCL1 were significantly reduced in colon homogenates of infected IL-1R$^{-/-}$ mice as compared with wild-type one. Of note, infiltration of IL-22-secreting CD11b^{+}Ly6C^{+}Ly6G^{+} cells was significantly defective in the colons of IL-1R$^{-/-}$ mice at day 4 after infection. Of most interest, colonic stromal cells isolated from IL-1R$^{-/-}$ mice secreted lower levels of KC/CXCL1 than stromal cells from wild-type mice during C. rodentium infection. Similar effects were found when mouse colonic stromal cells and human nasal polyp stromal cells were treated with IL-1R antagonists (i.e., anakinra) *in vitro*. These results suggest that IL-1 signaling plays a pivotal role in activating mucosal stromal cells to secrete chemokines, which are essential for infiltration of innate immune cells upon bacterial infection.

F48. Toll-Like Receptor 5 Agonist Improves the Therapeutic Efficacy of Antibiotic Treatments of Primary and Post-Influenza Pneumococcal Infections

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Antibiotics are widely recognized as an effective medical intervention against bacterial infections. However, antibiotic resistance increases while development of new antibiotics dries out. Previous reports showed that stimulation of innate immunity through intranasal administration of flagellin, the agonist for the Toll-like receptor 5 (TLR5), promote the clearance of pathogenic bacteria in pneumonia models. We hypothesized that a TLR5-mediated stimulation of lung immunity could improve the therapeutic index of antibiotics to cure Streptococcus pneumoniae respiratory infection in mice. Treatments were performed by combining intranasal administrations of flagellin with either oral regimen of amoxicillin or systemic injection of co-trimoxazole. Compared to standalone treatments, the combination of antibiotic and flagellin increased the survival rate and reduced the bacterial load in lung and spleen. As observed in antibiotic therapy, combinatory treatments improved lung architecture of infected animals but did not induce any inflammation. Moreover, combinatory treatment was also more effective than standalone antibiotic treatment in post-influenza pneumococcal infection. Finally, TLR5 signaling was shown mandatory for the effectiveness of therapy. Therefore, this study represents the first evidence that treatment combining antibiotic and a TLR agonist can improve the course of respiratory infections, thereby representing a new antibacterial strategy.

F49. IL-22 Plays a Key Role in Nontypeable Haemophilus influenzae Clearance in Chronic Obstructive Pulmonary Disease

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Infection by Nontypeable Haemophilus influenzae (NTHi) often exacerbates chronic obstructive pulmonary disease (COPD). Th17 cytokines are essential in the anti-bacterial defense, however their role remains unknown in the protection against NTHi. Mice were chronically exposed to cigarette smoke to induce COPD symptoms or to ambient air (Air mice) and then challenged with NTHi. Infection with NTHi strongly increased the levels of IL-17 and IL-22 in the lungs of Air mice. Whereas IL-17 was increased in COPD mice after NTHi, IL-22 production was not increased due to a defective innate lymphocyte activation. To determine the implication of IL-22, IL-22$^{-/-}$ mice were infected with NTHi. IL-22$^{-/-}$ mice had higher bacterial load in the lung compared with WT mice. This was associated to an increased inflammatory reaction (TNF-α, IL-6 in the BAL and IFN-γ in the lung) and the recruitment of neutrophils

and NK cells. A severe acute pneumonia with congestion of alveolar spaces was only observed in infected IL-22$^{-/-}$ and COPD mice. Moreover, supplementation with recombinant IL-22 before NTHi challenge of COPD mice improved bacterial clearance and decreased inflammation. In conclusion, the defect in IL-22 related to COPD could explain the susceptibility to NTHi infection and the associated deleterious inflammation.

F50. Characterization of Mast Cell in Inflamed Gingiva in Mouse Model

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Mast cells (MCs) are a key cell type of the hematopoietic lineage that the evolutionarily conserved functions in pathogen surveillance. At the earliest stages of infection, mast cells are important for communicating the presence of a pathogen to many cell types located nearby in the site of infection and distally in draining lymph nodes. We already reported that our established murine periodontal disease model shows significant inductions of IL-17 expressing $CD4^+$ T cells in inflamed gingiva with dramatically inductions of inflammatory cytokines were detected of mice orally infected with Porphyromonas gingivalis (Pg.). In this study, we elucidate the mechanism of interactions between MCs and effector T cell in inflamed gingiva. A murine periodontal disease model was dissect and isolated mononuclear cells from inflamed gingiva for determined the frequencies of MCs ($FceRIa^+$/c-Kit^+) by flow cytometer. Three days after the initial P.g-infection, significant induction of MCs was detected in inflamed gingiva. Those expressions were maintained during the infection period. Further, these MCs significantly express CD63, as a maker of MCs activation, at same P.g-infection period. Taken together, these results indicated that MCs in inflamed gingiva might key role for initiate inflammation for periodontal disease in early stage.

F51. Mucosal Associated Invariant T Cells (MAIT) in the Human Gastric Mucosa and Blood: Role in Helicobacter pylori Infection

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Mucosal-associated invariant T (MAIT) cells represent a class of antimicrobial innate-like T cells that have been characterized in human blood, liver, lungs and intestine. Here, we investigated, for the first time, the presence of MAIT cells in the stomach of children, adults and the elderly undergoing routine endoscopy and assessed their reactivity to Helicobacter pylori (H. pylori-Hp), a major gastric pathogen. We observed that MAIT cells are present in the lamina propria compartment of the stomach and display a similar memory phenotype to blood MAIT cells. We then demonstrated that gastric and blood MAIT cells are able to recognize and respond to H. pylori-infected macrophage stimulation by producing cytokines (IFNg, TNFa, and IL-17A) and exhibiting cytotoxic activity. Interestingly, we observed that blood MAIT cells frequency in Hp^{+ve} individuals was significantly lower than in Hp^{-ve} individuals. However, gastric MAIT cells frequency was not significantly different between Hp^{+ve} and Hp^{-ve} individuals, demonstrating a dichotomy between blood and gastric tissues. These results contribute important new information to the understanding of MAIT cell function on peripheral and mucosal tissues and demonstrate that MAIT cells might be important in the host response to H. pylori.

F52. Intestinal Barrier Dysfunction During Whipple's Disease

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Background: Classical Whipple's disease (CWD) is caused by chronic infection with Tropheryma whipplei. Most of the patients suffer from malabsorption and chronic diarrhea accompanied by articular, neuronal, and multisystemic affections. Immunologically, the disease is characterized by a dichotomy of mucosal immune suppression and systemic immune activation. Today, especially mechanisms of diarrhea are largely unknown. To understand underlying pathomechanisms we characterized the mucosal barrier function and possible links to chronic systemic immune activation in CWD-patients. Methods: Data of 73 CWD patients were compared to the results of 101 control subjects. Small intestinal biopsies or biopsy supernatants, respectively, were studied for villous length, epithelial cell turnover and inflammatory response. In sera markers of microbial translocation such as LPS and activation-associated mediators and cytokines were determined. Results: In duodenal specimens of CWD patients compared to healthy subjects villus atrophy, increased apoptosis of epithelial cells, and goblet cell hyperplasia indicated barrier dysfunction. In sera of CWD patient's intestinal barrier dysfunction becomes evident as enhanced values of LPS in the sera as a marker of microbial

translocation indicate microbial translocation. Enhanced values of soluble CD14 in duodenal biopsy supernatants and elevated serum concentrations of soluble CD14 and LPS binding protein hint a gut microbiota driven immune response. Conclusion: CWD patients exhibit intestinal barrier dysfunction that might provoke diarrhea with subsequent microbial translocation and systemic immune activation at time of diagnosis.

F53. The Mucin Muc1 Suppresses Helicobacter pylori-Induced Gastritis by Regulating the NLRP3 Inflammasome

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Background: Expression of the mucin MUC1 by epithelial cells is a critical component of the barrier lining the mucosal surface of the gastrointestinal tract. Polymorphisms in MUC1 are linked to increased susceptibility to gastric cancer caused by infection with the mucosal pathogen, Helicobacter pylori. Previously, we have shown, using MUC1 ($Muc1^{-/-}$) deficient mice, that this mucin plays an important role in suppressing H. pylori-induced gastritis, though the mechanism by which this occurs was unknown. RESULTS: Using bone marrow chimeras, we surprisingly found regulation of H. pylori-induced gastritis is not mediated by MUC1-expressing epithelial cells, but rather depends on mucin expression by immune cells. Severe gastritis in $Muc1^{-/-}$ mice was associated with significant mucosal IL-1ß, and bacteria-stimulated $Muc1^{-/-}$ cells secreted increased IL-1ß and IL-18. Hypothesizing that MUC1 regulates an inflammasome, we used specific ligands to demonstrate that MUC1 specifically suppresses activation of the NLRP3 inflammasome, but not other complexes. Further analyses revealed MUC1 controls the expression of NLRP3 itself, via an interaction with the NF-kB pathway at the level of IRAK4 or above. Infection of $Muc1^{-/-}$xCaspase-$1^{-/-}$ and $Muc1^{-/-}$x$Nlrp3^{-/-}$ double knock-out mice showed that the severe H. pylori-induced gastritis observed in $Muc1^{-/-}$ mice was both caspase-1 and NLRP3 dependent. Flow cytometric analysis revealed neutrophils and dendritic cells as 1) the main cell-types infiltrating the infected $Muc1^{-/-}$ gastric mucosa, 2) the predominant cells expressing active caspase-Conclusion: This study demonstrates the important and previously unrecognized role that MUC1-expression by mucosal immune cells has in regulating bacterial-driven inflammation, via regulation of the NLRP3 inflammasome.

F54. Mapping the Cellular Source and Role of IL-22 in Murine Lung Infections

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Interleukin-22 (IL-22) belongs to the IL-10 cytokine family and is expressed in the lung during infection with Chlamydia muridarum (Cmu) and Pneumonia Virus of Mice (PVM). IL-22 can have both pro-inflammatory and tissue protective roles depending on the inflammatory context, tissue tropisms, and local cytokine milieu. Several cell types can produce IL-22, including innate lymphoid cells, neutrophils, γδ T cells, and $CD4^+$ Th cells. While IL-22 has previously been associated with Th17 cells, recently a novel lineage of $CD4^+$ T helper cells (known as Th22 cells) have been identified that predominantly produce IL-22 in the absence of IL-17. Current knowledge of the cellular source of IL-22 in inflammatory lung infections is unclear and our understanding of a role for IL-22 in the pathogenesis of these diseases remains limited. We have mapped, for the first time, the cellular source of IL-22 in both Cmu and PVM lung infections using novel transgenic dual reporter mice. We show that IL-22 expression only partially overlaps with IL-17a expression and identify Th22 cells as the major source of IL-22 in the lung. Using IL-22 deficient mice, we show a functional role for IL-22 in Cmu infection, but fail to identify a role in PVM infection. Our study provides novel insight into the cellular source of IL-22 in the context of lung infections and identifies a role for IL-22 in Cmu lung infection.

F55. Atrophy of Jejunal Peyer´s Patches (JPP) as a Consequence of Experimental Infection of Goats with Mycobacterium avium subsp. paratuberculosis (MAP)

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Introduction: MAP may cause granulomatous enteritis predominantly in ruminants. Initial lesions are detectable during the clinically non-apparent phase of infection in organized gut-associated lymphoid tissues (oGALT). The objective of this study was to characterize re-organization of JPP as part of oGALT during the first year after infection of goats with MAP. Material and methods: Goat kids were orally inoculated with a total dose of about 10^9 CFU of MAP. Six goats were necropsied at 3 mpi and ten at 12 mpi and JPP were collected. Lesions were assessed in H&E stained paraffin sections. Lymphocyte subsets and macrophages were

labeled in consecutive frozen sections. Results: At 3 mpi, JPP (especially in the proximal jejunum) were thickened and sometimes ulcerated. Severity of lesions varied along the intestine and between individuals. Extensive granulomatous infiltrates of $CD68^+$ epitheloid cells, $CD4^+$ and gd T cells had replaced the oGALT. At 12 mpi, respective JPPs were thin, inconspicuous and often recognized by a circumscribed serositis only. oGALT was segmentally to diffusely atrophic and replaced by a mild to moderate infiltrate of T cell subsets, plasma cells and macrophages. Conclusions: The mycobacterial-host interaction caused marked tissue lesions even during the clinically non-apparent phase of infection. This resulted in a partial loss of oGALT.

F56. Relationship Between Mucosal Th17 and Treg in Nasopharynx-Associated Lymphoid Tissue and their Association with Pneumococcal Carriage in Children and Adults

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Streptococcus pneumoniae is a leading cause of respiratory tract infection in humans. It colonizes human nasopharyngeal mucosa, and pneumococcal carriage is common in young children that may account for the high incidence of pneumococcal disease in this age group. Pneumococcal carriage rate in humans decreases with age. However, the immunological factors that mediate the clearance or persistence of carriage in human nasopharynx remain unclear. Recent studies in mice suggest Th17 is important in host clearance of S. pneumoniae. We have studied the relationship between frequencies of mucosal Th17 and T regulatory cells (Foxp3+ Treg) in human nasopharynx-associated lymphoid tissue (NALT) and age and their association with pneumococcal carriage in children and adults. The frequencies of Th17 and Treg in adenotonsillar tissue were significantly higher than in peripheral blood ($p<0.01$) in both children and adults. There was an inverse correlation between frequencies of Th17 and Treg in adenotonsillar tissue ($r=-0.52$, $p<0.01$). Tonsillar Th17 frequency was shown to increase with patient's age ($r=0.62$, $p<0.01$), whereas the Treg frequency inversely correlated with age ($r=-0.45$, $p<0.01$). Furthermore, there was a positive correlation between the ratio of Th17/Treg in NALT and age of patients. Also, the Th17/Treg ratio was significantly higher in pneumococcal carriage negative than positive children ($p<0.05$). Our findings suggest that the balance/ratio of mucosal Th17 and Treg in nasopharynx is a critical determinant of pneumococcal clearance/carriage, and support efforts to promote mucosal Th17 in vaccination strategy against pneumococcal infection in humans.

F57. Fungus-Reactive T Cells as Sensitive and Specific Sensors to Diagnose Fungal Infections in Cystic Fibrosis Patients

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Cystic fibrosis (CF) patients frequently suffer from chronic infections of the lung, resulting in substantial disease exacerbation. Environmental fungi play a major role in this process as infecting pathogens. The clinical relevance of fungal pathogens ranges from colonization of the lung to sensitization of the immune system to even invasive fungal infections. The major bottleneck for timely and specific treatment of CF patients is the lack of sensitive and specific diagnostic tools allowing differentiating between fungal species and stages of fungal diseases. T lymphocytes are specific sensors for invading pathogens. By using ARTE (antigen-reactive T cell enrichment) for the characterization of antigen-reactive T cells directly from human peripheral blood, we show that fungus-specific $CD4^+$ T cells in CF patients strongly differ in their phenotype and function as compared to healthy donors. Patients with CF can further be sub-classified according to fungus-specific T cell phenotype which can be correlated with clinical parameters. In particular, we show that increased frequencies of fungus-reactive T cells can be used as a specific diagnostic parameter for invasive fungal infections in CF patients. These data imply that the characterization of fungus-specific $CD4^+$ T cells is a valuable tool to resolve the contribution of various specific fungal pathogens to the disease state in individual patients with CF. This approach will help to determine the role of specific fungal pathogens to CF disease exacerbations and will improve diagnosis and prognosis of fungal infections.

F58. Reduced Intestinal Production of CCL20 during Cryptosporidiosis in Neonatal Mice: A Mechanism by Which Cryptosporidiosis Parvum Escapes Antimicrobial Activity of CCL20?
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Cryptosporidium parvum is an intestinal parasite that completes its life cycle in epithelial cells. This protozoan is frequently associated to diarrheal disease outbreaks and is now recognized as the second cause of diarrhoea in infants in Africa and Asia. In a neonatal mouse model, we previously showed that C. parvum infection of epithelial cells induces secretion of a broad range of chemokines allowing the recruitment of inflammatory cells to the site of infection. In this work, we observed that surprisingly, CCL20 production in the intestine of infected neonatal mice was significantly reduced by a mechanism independent of the enteric flora and IFNγ, a key cytokine for the resolution of this infection. MiR21 that functionally targets CCL20 is upregulated during the infection, and might participate to the downregulation of the chemokine. The tertiary structures of CCL20 and the anti-microbial peptide b-defensin are very similar, this confers to this chemokine a direct antimicrobial activity against Escherichia coli, Staphylococcus aureus and Candida albican. We observed that oral administration of recombinant CCL20 to neonatal mice reduced significantly intestinal parasite load by a mechanism independent of immune cell recruitment but rather by a direct cytolytic activity on free stages of the parasite as confirmed by *in vitro* experiments. Our findings demonstrate for the first time the direct antiparasitic activity of CCL20 against an enteric protozoan and its down-regulation during C. parvum infection, that might reflect a mechanism developed by the parasite to escape the protective innate immune response.

F59. Murine Norovirus Exacerbates Intestinal Inflammation in IL10-Deficient Mice Colonized with Defined Flora
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Noroviruses are highly prevalent RNA viruses which infect gastrointestinal tract of different mammalian species. Murine noroviruses (MNV) are highly prevalent in mouse colonies worldwide. Recently was shown that MNV is capable of inducing histopathological changes in conventionally housed mice. However, the underlying mechanism of the inflammation development is not well understood yet. Altered Schaedler Flora (ASF) is a defined microbiota community composed from eight bacterial species. In this study we analyzed the influence of MNV on immunological and histological characteristic of intestinal inflammation in germ-free (GF) and ASF colonized IL10-deficient ($Il10^{-/-}$) mice.
In the present study GF and ASF colonized B6-$Il10^{-/-}$ mice were monitored for structural and functional intestinal barrier alterations by histology, TUNEL staining, qRT-PCR and ELISA. Intestinal inflammation was not observed in GF and MNV infected $Il10^{-/-}$ mice, but the histological score increased after ASF colonization and was even higher after MNV infection. Gene expression of tight junction proteins was not changed after MNV infection, but it was changed within 4 weeks in MNV infected mice colonized with ASF. Mice colonized with ASF and infected with MNV showed 4 weeks post infection increased production of IFNg and IgA. MNV can induce mucosal inflammation and changes in intestinal epithelial barrier in the susceptible host in the presence of defined intestinal flora.

F60. Resident Memory γδ T Cells Orchestrate Response to Secondary Oral Lm Infection
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Resident T cell memory provides protection to mucosal tissues by sensing infection and recruiting both innate and adaptive arms of the immune system. Using a model of oral infection with Listeria monocytogenes (Lm), we tested the hypothesis that memory Vγ4 γδ T cells (Lm-elicited) are resident and participate in driving the accelerated immune response to secondary infection in the mesenteric lymph node (mLN). We found that CXCR3+ Lm-elicited γδ T cells reside in the mLN and are the main producers of IL-17A, critical to control burden and induce clearance of bacterial, 1 day after secondary oral Lm infection. Most strikingly, we observed that Lm-elicited γδ T cells formed clusters with neutrophils surrounding Lm aggregates in the mLN that were disrupted by IL-17A blockade, neutrophil depletion, TCR γδ downregulation or CXCR3 blockade. Moreover, CXCL1 and CXCL9 were found in the cluster area. Together, these observations demonstrate that during secondary oral Lm infection IL-17A secreted by resident Lm-elicited γδ T cells is critical to recruit monocytes and neutrophils into clusters that contain Lm and may also attract memory αβ T cells. These findings support an exciting role for memory γδ T cells to orchestrate a hierarchy of immune upon infection to contain intestinal pathogens.

F61. Early Events of Rectal Transmission of Simian Immunodeficiency Virus
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To better understand the early events of HIV rectal transmission, we studied the timing, location and the cell types of virus-infected cells in rhesus macaque model of HIV rectal transmission. 49 adult male Indian rhesus macaques were intrarectally inoculated with SIVmac251 (3.4 x10^4 $TCID_{50}$) and euthanized at various days post inoculation (0, 1, 2, 3, 4, 6 10, 12 day, 3 and 6 months PI). The tissues from over a dozen different anatomic sites were analyzed for the presence of SIV vRNA using qRT-PCR and in situ hybridization (ISH). At 3 and 4 dpi, SIV was only detected in the rectum and draining lymph nodes (LNs), but not in the distal sites. All monkeys at 6 and 10 dpi were systemically infected. SIV vRNA+ cells in rectum and LNs were primarily $CD4^+$ T cells revealed using combined ISH/IHC. Host transcriptome analyses revealed a dramatic alteration in host gene expression in the rectum and draining LNs. Our results indicate that SIV rectal transmission follows a stage dissemination mode and virus infected-cells are primarily $CD4^+$ T cells. The implications of this study for developing of vaccine and other preventive measures against HIV rectal transmission will be discussed.

F62. The Effects of *Salmonella typhimurium* on Intestinal Dendritic Cells
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Dendritic cells (DCs) trigger the adaptive immune response. In the intestinal lamina propria (LP) this process begins with antigen uptake and subsequent migration of DCs to the mesenteric lymph nodes (MLN). DCs from the LP and MLN are defined as $CD64^-$ $B220^-$ $CD11c^+$ MHC II^{hi} cells and can be further subdivided into 4 functionally-different DC subsets, based on their expression of CD11b and CD103. We aimed to characterize the mechanisms enabling Salmonella typhimurium (STM) to reach the MLN, and to discover whether DCs are responsible for transporting bacterial antigens during STM infection. To allow efficient STM colonization we pre-treated animals with streptomycin (ABX). Our results show that CX_3CR1^- and CX_3CR1^{int} DCs but not CX_3CR1^{hi} cells migrate to the MLN before and after STM infection. Preliminary data indicate that all DCs subsets continue to migrate effectively to the MLN after STM infection, albeit mice that received ABX + STM showed higher proportion of total MLN DCs, with a lower frequency of $CD11b^+$ $CD103^-$ DCs and more $CD103^+$ $CD11b^-$ DCs, compared to mice receiving STM only. Our next aim is to establish, by collecting lymph from the thoracic duct of STM-infected mice, which migrating cells are responsible for transporting STM to the MLN.

F63. Oral Enteropathogenic E. Coli (EPEC) Infection of Newborn Mice
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Enteropathogenic E. coli (EPEC) represent a major causative agent of infant diarrhea associated with significant morbidity and mortality in developing countries. Although studied extensively *in vitro*, the investigation of the host-pathogen interaction *in vivo* has been hampered by the lack of a suitable small animal model. Here, we orally infected newborn mice with EPEC and observed a spontaneous intestinal colonization restricted to the postnatal period. Intimate attachment of the bacteria to the epithelial plasma membrane and microcolony formation were observed by immunofluorescence and occurred in a type III secretion system (TTSS) dependent manner. Similarly, EPEC-induced innate immune stimulation required an intact TTSS and led to the upregulation of a restricted set of enterocyte response genes. Together, we present the first suitable small animal model to study the pathogenesis of EPEC.

F64. Identification and Characterization of the "Gut Vascular Barrier" that is Exploited by *Salmonella typhimurium* for its Systemic Dissemination

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Orally administered antigens can reach systemic sites inducing a systemic state of tolerance, while the microbiota is contained in the mucosal compartment and is systemically ignored. How this discrimination is achieved is unknown. We describe the existence of a gut vascular barrier (GVB) both in human and mouse that plays an important role in controlling the translocation of bacteria to systemic sites. We characterized the intestinal endothelial cells (ECs) in terms of expression of tight and adherent junction proteins. In addition, we observed that the ECs were associated with enteric glial cells and pericytes forming together the "gut vascular unit". GVB integrity could be modified by Salmonella typhimurium. Indeed, upon infection the vascular ECs up-regulated the expression of PV1, marker of damaged vascular barriers, which correlated with higher endothelial permeability. S. typhimurium could modify barrier properties of the ECs through the negative regulation of the Wnt/β-catenin signaling pathway. Indeed, we found that β-catenin activation was reduced upon infection *in vitro*. Consistently, Salmonella was incapable to modify ECs permeability and to spread systemically in mice where β-catenin was constitutively activated by genetic means in vascular ECs. Furthermore, we demonstrated that Salmonella pathogenicity island (spi)-2 was involved in the regulation of Wnt/β-catenin signaling pathway.

F65. Herpes Simplex Virus (HSV) Suppressive Therapy in HSV-2/HIV-1 Co-Infected Women is Associated with Reduced Markers of Inflammation in the Systemic but not the Mucosal Compartment

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Herpes simplex virus 2 (HSV-2) infection has been associated with higher genital chemokine levels and increased numbers of human immunodeficiency virus type 1 (HIV-1) target cells in the genital mucosa, suggesting local immunologic alterations may increase HIV-1 replication. Archived specimens from an 18 week randomized, placebo-controlled, cross-over trial of daily HSV-2 suppressive therapy (valacyclovir) in HIV-1 and HSV-2 dually infected women conducted in Peru were analyzed to determine the effect of HSV-2 treatment on the systemic and mucosal inflammatory environment. Levels of 31 cytokines in plasma and 14 cytokines in endocervical swabs were assessed by multiplex bead array at time points collected weekly throughout the trial. Valacyclovir treatment was found to significantly reduce plasma levels of CXCL10, but did not significantly alter concentrations of the other factors measured in either compartment, suggesting that new HSV-2 therapies must aim to reduce local inflammation of the mucosae to reduce viral transmission.

F66. Innate Lymphoid Cells Control Early Colonization Resistance Against Intestinal Pathogens through Id2–Dependent Regulation of Microbiota

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Bacterial infections remain highly prevalent diseases in developing countries. Infections, especially those that are caused by antibiotic-resistant bacteria, cause high morbidity and mortality in hospitalized patients. Although most studies focus on innate and adaptive immune responses, recent publications suggest that pathogen colonization resistance could be dependent on direct inhibition by microbiota before innate and adaptive immune responses take place. However, it is unknown whether and how the host shapes the microbiota to mediate colonization resistance to pathogens. Innate lymphoid cells (ILCs) are newly defined immune cells that protect the host from various infections. Id2, an essential transcriptional regulator for the development of innate lymphoid cell (ILC) progenitors, remains highly expressed in differentiated ILCs with unknown function. Using conditionally deficient mice that delete Id2 in differentiated ILC3s, we observe that these mutant mice exhibit greatly impaired gut colonization resistance against Citrobacter rodentium. Utilizing gnotobiotic hosts, we show that the Id2-dependent early colonization resistance was through IL-22 mediated regulation of microbiota. We also demonstrated that, in addition to controlling the development and maintenance of ILC3s, Id2 regulated IL-22 production through an Ahr and IL23R pathway. We conclude that ILC3s can mediate

immune surveillance that constantly maintains proper microbiota to mediate early colonization resistance through an Id2–dependent regulation of IL-22. Considering the difficulty to modify the microbiota in adult patients, our study suggests that a combination of immune molecule supplementation and microbiota transplantation may be a better approach to introduce a stable healthy microbial community.

F67. Mucosal Associated Invariant T (MAIT) Cells are Highly Activated in Duodenal Tissue of Humans with *Vibrio cholerae* Infection

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Cholera is a diarrheal disease caused by infection by V. cholerae O1/O139. The mechanisms of protection against cholera are poorly understood, particularly with regard to immune responses in the intestinal mucosa. We have previously shown that circulating MAIT cells are activated during cholera and associated with V. cholerae LPS-specific antibody responses. In this study, we enrolled 6 adults with confirmed V. cholerae O1 infection, and obtained blood, stool, and duodenal biopsy specimens by endoscopic procedure, on days 2 and 30 after onset of disease. We found that the frequency (~2%) of MAIT cells as % of $CD3^+$ cells are similar in the periphery and the lamina propria (LP) and do not change between acute and convalescent phases. We found that a greater percentage of MAIT cells are activated ($CD38^+$) in the LP at day 2 compared to day 30. At all time points, MAIT cell activation was higher in the LP than in the periphery. Stool alpha-1-antitrypsin, a marker of intestinal permeability, was correlated with decrease in % of activated MAITs between days 2 and 30. Enrollment for this study is ongoing, as well as analysis of corresponding antibody responses, and updated results will be available for presentation and discussion.

F68. Dysbiosis Associated with Intestinal Pathology in Mice Infected with Malaria Parasites

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Malaria caused by infection with protozoan parasites, genus Plasmodium, produces 200 million patients and 600 thousand deaths every year. In addition to malarial trias (fever, anemia, and splenomegaly) and fatal complications (cerebral malaria, renal failure), it is well known that gastrointestinal symptoms such as abdominal pain and diarrhea are frequently observed in malaria patients. However, the interactions between malaria pathology and intestinal microbiota have never been investigated. Here we investigated the intestinal pathology and microbiota in mice infected with a rodent malaria parasites P. berghei ANKA (PbA). We found that severe inflammatory changes occurred in the small intestines of C57BL/6 mice susceptible to experimental cerebral malaria (ECM) during PbA infection. Notably, we also found remarkable changes in composition of the intestinal microbiota, dysbiosis; decrease in symbiont bacteria and increase in pathobiont bacteria. The degree of intestinal pathology and dysbiosis was much milder in BALB/c mice resistant to ECM. Furthermore, the amount of some bacterial genera clearly correlated with disease severity of malaria, suggesting the relevance of intestinal microbiota to malaria pathogenicity. These results provide novel insights into host-parasite relationship in the intestines during malaria. We are now further investigating immune responses in gut-associated lymphoid tissues in these mice, and microbiota in patients with malaria.

F69. Male IgG Seroconversion to Urogenital Chlamydial Infection Exacerbates Immunopathology in Females

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Urogenital Chlamydia trachomatis infects over 100 million people per annum and can cause inflammation, scarring, pelvic inflammatory disease and infertility in women. However, infections are often asymptomatic with an unknown underlying trigger for immunopathology. Male IgG serostatus to C. trachomatis is also a correlate of the disease outcome of female partners in fertility clinics. Using IgG purified from chronically C. muridarum-infected male mice, the role of IgG and Chlamydia in transcytosis, cellular uptake, antigen presentation and immune responses was determined in female mice. Opsonization of chlamydia with IgG enhanced uterine epithelial transcytosis of infectious Chlamydia to the lamina propria, which increased phagocytosis, antigen

processing/presentation and draining lymph node migration of DCs. Primed DCs in the lymph node then expanded more $CD4^+$ and $CD8^+$ T cells. The expansion of T cells did not assist in host clearance of chlamydial shedding, but surprisingly enhanced fallopian tube scarring and blockage, dependent of chlamydial opsonization with IgG. When $FcRn^{-/-}$ mice were infected with opsonized Chlamydia the enhancement of pathology was abrogated and the infection was resolved 50% quicker. When female mice were depleted of $CD8^+$ T cells prior to infection, $FcRn^{-/-}$ mice (but not $FcRn^{+/+}$ mice) had a 61% reduction in the incidence of infertility. Taken together, these data demonstrate that opsonization of Chlamydia with male IgG exacerbates epithelial transcytosis, dendritic cell priming, production of immunopathogenic $CD8^+$ T cells, and infertility in mice. These results in some part may help to explain the asymptomatic nature of Chlamydia-induced pathology and infertility in human females.

F70. The Human Cytomegalovirus Glycoprotein UL16 Selectively Mediates Intracellular Sequestration of the MHC Class I-Related Neonatal Fc Receptor (FcRn) for IgG

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FcRn transports IgG across polarized epithelial cells, protects IgG from degradation and improves presentation of immune complexed antigens to T cells. FcRn and MHC class I molecules consist of a heavy chain with beta2m. Several pathogens including human cytomegalovirus (HCMV), escape $CD8^+$ T or natural killer cell cytotoxicity by destroying MHC class I or its related molecules to prevent antigen presentation. Whether pathogens have similar mechanisms for affecting FcRn is not known. By screening HCMV immune evasion proteins, we found that UL16 binds to FcRn and is a candidate component of immune evasion. Binding was confirmed by immunoprecipitation with lysates from HeLa cells expressing FcRn and UL16. A GST-UL16 protein could bind FcRn from Caco-2 intestinal epithelial cells. UL16-FcRn were co-localized in Caco-2 cells infected with wild-type, but not UL16-deleted HCMV. By using beta2m-null FO-1 cells transfected with FcRn heavy chain alone, we showed that UL16 binding occurred within the ER prior to recruiting beta2m. Confocal microscopy and endoglycosidase digestion revealed that UL16 selectively retained FcRn in the ER and reduced FcRn accumulation in endosomes, and HCMV infection decreased IgG transcytosis across Caco-2 monolayers. Because FcRn is expressed in epithelial, endothelial, macrophage and dendritic cells, the same cells capable of supporting HCMV replication, our results suggest that UL16 sequesters FcRn occurs during virus infection. By inhibiting the capacity of FcRn to bind IgG, the UL16 protein inhibits IgG transcytosis, enhances IgG catabolism in infected cells or tissues, and blocks other FcRn functions that are important for protective immunity against HCMV.

F71. Clearance of PTX3 Pre-Opsonized Conidia of A. Fumigatus from the Lung of Rats Immunosuppressed by Cortisone Acetate

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PTX3 is multimeric glycoprotein that binds to A. fumigatus and promotes phagocytosis and killing of conidia by innate immunity cells, a process defined as opsonization. In order to provide proof of concept that direct administration of PTX3 to the respiratory mucosa would preserve protein mediated clearance of A. fumigatus from the lung of rats immunosuppressed by cortisone acetate, PTX3 pre-opsonized conidia were intra-tracheal administered in these animals. Three PTX3 concentrations were selected for the *in vivo* study corresponding to saturation, intermediate and low binding of PTX3 to conidia. Twenty four hours after intra-tracheal administration with $5x10^7$pre-opsonized conidia, rats were sacrificed and fungal burden evaluated in lung and blood by CFU and galactomannan (GMI) respectively. The rats challenged with PTX3-saturated conidia, showed similar CFU and GMI compared to rats challenged with PTX3-free conidia. A reduction of lung CFU was observed in rats infected with intermediate and low PTX3 concentrations suggesting a pro-zone effect in the mechanisms of opsonization. Present results are consistent with PTX3 opsonic activity and suggest that local administration of PTX3 on the respiratory mucosa, for instance by aerosol, could be useful but dose selection should be carefully identified.

F72. Mucosal iNOS-Producing IgA⁺ Plasma Cells in Helicobacter pylori-Infected Patients
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Background: The mucosal immune system is relevant for homeostasis, immunity, and also pathology in the gastrointestinal tract. Inducible nitric oxide synthase (iNOS) dependent production of nitric oxide (NO) is one of the factors linked to both anti-microbial immunity and pathology. Up-regulation of iNOS has been observed in human Helicobacter pylori infection, the major cause of gastric ulcer, adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma, but the cellular sources of iNOS and NO are ill defined. Methods: To characterize mucosal iNOS-producing leukocyte subtypes in H. pylori-infected patients, antral biopsy specimens from 41 H. pylori-infected patients and 24 H. pylori-negative controls were analyzed by immunohistochemistry, along with flow cytometric analyses of isolated lymphocytes for iNOS expression and activity. Additionally, 24 biopsy specimens from a vaccination trial were analyzed immunohistochemically. Results: Besides macrophages, we newly identified mucosal IgA-expressing plasma cells (PCs) as one major iNOS⁺ cell population detected in H. pylori-infected patients. Since we did not detect iNOS⁺ PCs in four distinct infectious diseases, this finding is not a general feature of mucosal PCs under conditions of infection. By flow cytometry intracellular NO production was detected in live PCs. Furthermore, numbers of iNOS⁺ PCs were elevated in the mucosa of individuals who had cleared experimental H. pylori infection compared to those who had not. Conclusion: IgA⁺ PCs expressing iNOS are described for the first time in humans. iNOS⁺ PCs are induced in the course of human H. pylori infection and may contribute to the clinical course of the infection.

F73. Role of Mucosal Associated Invariant T Cells in Helicobacter Infection
Criselle Dsouza, Zhenjun Chen, Alexandra Corbett, Alison Every, Jean-Pierre Scheerlinck and James McCluskey. University of Melbourne, Melbourne, Australia

Mucosal Associated Innate T (MAIT) cells are innate like T lymphocytes that express a semi-invariant TCR and are restricted by the non-classical MHC class I-related molecule, MR1. These cells are predominantly found at mucosal surfaces and are activated by a novel class of antigens, intermediates of the riboflavin synthesis pathway that are produced by certain class of bacteria and yeasts. We have examined whether MAIT cells play a role in the pathology of chronic H. pylori infection. We have recently developed highly specific MR1 tetramers that have been used to detect and characterize MAIT cells in mice. Our studies show that MAIT cells play a key role in the regulation of gastric inflammation in H. pylori infection. Using a mouse model that first enriches MAIT cells in the lung, we show repopulation of MAIT cells to other mucosal sites including the stomach. On challenge with H. pylori, these mice develop an accelerated inflammatory response leading to atrophic gastritis. In our model, MAIT cells have a pathogenic rather than protective effect. We are working to understand their role with a view to therapeutic intervention.

F74. Mucosal Associated Invariant T Cells Respond *in vivo* to Riboflavin-Derived Ligands and Co-Stimulatory Signals
Zhenjun Chen, Huimeng Wang, Criselle Dsouza, Alexandra Corbett and James McCluskey. University of Melbourne, Melbourne, Australia

We have analyzed the requirements for triggering Mucosal Associated Invariant T (MAIT) cell responses in a mouse model. MAIT cells are a subset of non-conventional T cells, which are restricted by the MHC Class I related (MR1) molecule. Although they have been implicated in the immune response to certain bacteria, their overall role in infection remains unclear. Having recently identified the antigens recognized by these cells as small ring compounds deriving from riboflavin biosynthesis precursors, we now aim to explore the activation and response of MAIT cells in mucosal infection. Here we use an intranasal infection model with gene-deleted mutant bacteria to confirm the requirement for riboflavin-derived ligands in triggering MAIT cells. We also characterize the phenotype, cytokine production and co-receptor expression of responding cells, and show that a full response requires both specific recognition of antigen and co-stimulatory signals.

F75. The Impact of Co-Infection with Citrobacter Rodentium on Mortality and Morbidity During Experimental Malaria Infection

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Malaria is an infectious disease caused by Plasmodium parasites and it is currently considered one of the greatest public health problems worldwide. Epidemiological evidence support the hypothesis that infection with malaria predisposes to infection and mortality from bacterial infections, as patients with concomitant malaria and bacteremia display three to eight times higher mortality than in individuals with malaria alone. However the mechanistic basis for how malaria infection predisposes to bacterial infection is not clear. Aim: To determine the effects of acute intestinal infection with Citrobacter rodentium on concomitant Plasmodium chabaudi infection. Methods: C57BL/6 mice were infected as follows: (1) P. chabaudi; (2) C. rodentium; (3) co-infection; (4) uninfected. The weight and parasitemia of animals were measured daily. Cohorts of each group were euthanized at 7, 14 and 21 days post-infection for collection of liver, spleen, colon, cecum and cecal contents. These were processed and plated to assess Citrobacter rodentium colonization and translocation. Results: Mice infected with P. chabaudi only or co-infected exhibited comparable weight loss with similar kinetics. However, significant mortality was observed in the co-infected group from day 8 post-infection, whereas all single infection groups showed 100% survival. In addition, on 14 post-infection, increased numbers of C. rodentium were found in the spleen, colon and cecum of some co-infected animals, compared to the group infected only by C. rodentium. Conclusion: Our data show that co-infection between P. chabaudi and C. rodentium increases morbidity and mortality of the host and suggest that this model could prove useful in identifying the mechanisms responsible.

F76. Murine Norovirus Infection is Inhibited in Rag2 Gamma Chain- Deficient Mice after Oral Administration

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A major step in murine norovirus (MNV) pathogenesis involves crossing the intestinal epithelial barrier to reach its target cells, macrophages, dendritic cells and B cells, for replication. Recent studies showed a decrease in MNV replication in the intestine after conditionally depleting mice of microfold (M) cells. To define the importance of Peyer's patch M cells during MNV pathogenesis, mice deficient in recombination activation genes (Rag) and common gamma chain (γc) (Rag-$\gamma c^{-/-}$) that do not develop Peyer's patches and Peyer's patch-associated M cells, were used. Rag-$\gamma c^{-/-}$ and immunocompetent Balb/c wild type (WT) controls were challenged intraperitoneally or per-orally with MNV-1 or CR3 for 24 and 72 hr. Both Rag-$\gamma c^{-/-}$ and WT mice showed similar intestinal titers following infection by the intraperitoneal route, which provides direct access to target cells. Although, Rag-$\gamma c^{-/-}$ mice have enhanced percentages of certain MNV target cells (i.e., dendritic cells and monocytes) in the small intestine, Rag-$\gamma c^{-/-}$ mice were not productively infected when virus was administered orally, a route when virions need to cross the intestinal epithelial barrier. These data demonstrate that MNV cannot cross the intestinal epithelium in the absence of Peyer's patch M cells, indicating that they are the sole route for MNV-1 entry into the host interior in Balb/c mice.

F77. IFN-γ Umpires Recovery from Mucosal Inflammation by Regulating Macrophage and γδ T Cell Homeostasis During Microbial Gut Infection

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Acute, self-limiting inflammatory disorders caused by pathogenic bacteria are a critical threat especially to the immunocompromised and elderly. Immune coordination of homeostasis in the gut after such microbial challenges are, however, still poorly understood. Here, we identified a resolving role of tissue resident macrophages and IL-22 released from intraepithelial T lymphocytes of the cecal mucosa in response to Salmonella Typhimurium infection at the single cell level. In detail, we established a remission model of experimental enterocolitis using ciprofloxacin, a fluoroquinolone antibiotic. While formation of persisters was observed in the intestinal wall, treatment upon infection resulted in potent elimination of Salmonella from the gut lumen, and delineated pathological changes of mucosal recovery from early acute inflammation. Defined resolution indices further specified cellular and molecular dynamics of remission during antibiotic therapy. Importantly, composition of the mucosal mononuclear phagocyte compartment, as well as regulation of T cell derived IL-22 levels were substantial to initiate resolution of intestinal

pathology and maintain gut homeostasis in an IFN-γ-dependent fashion. In conclusion, these data revealed a yet unidentified role of cecal IFN-γ and IL-22 within the recovering gut mucosa of infected animals. Understanding the dysregulation of such anti-inflammatory mechanisms upon pathogen invasion and antimicrobial therapy has a decisive importance for the future.

F78. Development of Mucosal Candida Colonization and Dissemination Model in Immunodeficient Mice
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Candida albican is one of common commensals but can become opportunistic pathogen in susceptible hosts. As the similar symptoms to bacterial infection, the Candida infection is difficult to diagnose and causes >40% of mortality in patients due to the delays in antifungal therapy. To investigate the biomarker or therapeutic drugs for systemic Candida infection, we used immunodeficient RAG mice to mimic immune compromised humans. After gastrointestinal infection, a dose-dependent survival was seen in RAG mice with the 100% of survival in 10^6 and 10^5 and 0% in 10^7 of Candida albican strain SC5314 infection. In contrast, none of wild-type C57BL/6 mice died in Candida infection. In 10^7 Candida infected RAG mice, colonization of Candida albican was detected in stool with the fungal burden of $10^{2\text{-}3}$ cfu/ml from day 5-14 and all mice died between day 12-19 due to the disseminated Candida infection. The serological and cellular changes were screened for the surrogate biomarkers represented the transition of Candida albican colonization to systemic dissemination. The significant increase of IL-6 and galectin-3 found in day 9-12 after Candida infection suggested these two molecules have potential as the biomarkers for the life-threaten systemic Candida infection.

F79. The Colonic Cytokine Environment Found During Brachyspira hyodysenteriae Infection Induces Mucus Changes in a Mucus Secreting, Polarized *in vitro* Colonic Mucosal Surface
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Brachyspira hyodysenteriae colonizes the pig colon and causes swine dysentery. We have previously shown that B. hyodysenteriae infection causes changes in the mucin environment characterized by a disorganized mucus structure and a massive mucus induction with de novo expression of MUC5AC and increased expression of MUC2 in the colon, which increases the B. hyodysenteriae binding ability to mucus. The aims of the present study were to determine how the cytokine environment changes in the pig colon during infection, and identify which of these changes are important for mucin production. The cytokine profile in the colon of B. hyodysenteriae infected pigs was characterized by increased expression of IL-1β, IL-6 and IL-8. Mucus producing *in vitro* colonic mucosal surfaces were stimulated with these cytokines, with and without B. hyodysenteriae infection, and mucin type, production rate and turn over were determined. Preliminary data show that the combined effect of these cytokines during infection increased the proportion of MUC2 and MUC5AC secreting goblet cells in the *in vitro* mucosal surface, and increased the mucin turnover rate. Overall, our data suggest that the upregulation of factors from the immune system is at least partly responsible for the mucus induction during B. hyodysenteriae infection.

MUCOSAL TOLERANCE

OR.21. The Influence of Cytoplasmic Nucleic Acid Sensors on the Adaptive Immune Response
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Immune detection of nucleic acids is an essential component of the host response to virus, but how this process influences the adaptive immune response remains incompletely understood. In this study, we analyzed the target tissue response to infection with the enterovirus Coxsackie B virus (CVB4). Studies show that the gut mucosa may be an important CVB4 reservoir influencing dissemination to other target organs such as the pancreas. Strains of mice were compared that were either susceptible or resistant to the development of chronic inflammation following viral clearance. Transcriptome analyses of target tissues (GIT and pancreas) showed that increased expression of RNA sensors correlated with the immune response to RNA virus. However, the propensity to develop chronic inflammation long after viral clearance was distinguished by continued high expression of DNA sensors. The cell intrinsic response to CVB4 was also strongly biased toward interferon regulatory factor (IRF)7, rather than IRF3 mediated pathways.

Cytoplasmic RIG-like helicases (RIG-I and MDA5) use the signaling adaptor mitochondrial antiviral signaling (MAVS) to activate IRF3 and IRF7, which are both regulators of type I IFN gene expression. The role of MAVS in CVB4 infection was explored in a novel MAVS mutant mouse strain that harbors a point mutation in the transmembrane region that influences mitochondrial localization of MAVS. In MAVSsnp mice, MAVS was important for both the type 1 interferon response and antibody production in response to CVB4, suggesting that innate genes involved in antiviral sensing influence adaptive immune responses.

OR.37. GPR15-Mediated Control of Immune Homeostasis in the Large Intestine
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The large intestine is the site most commonly affected in inflammatory bowel disease. Lymphocyte homing, which contributes to inflammation, has been studied extensively in the small intestine, but there is little known about homing to the large intestine. We show here that GPR15, an orphan G-protein coupled receptor known as a SIV/HIV co-receptor, controls the specific homing of T cells, preferentially $FOXP3^+$ regulatory T cells (Tregs), to the large intestine lamina propria (LILP). GPR15 expression is modulated by gut microbiota and TGF-β1, but not by retinoic acid or short-chain fatty acids. GPR15-deficient mice had fewer Tregs in LILP and were prone to develop more severe large intestine inflammation, which was rescued by the transfer of GPR15-sufficient Tregs. Our findings thus describe a T cell homing receptor for LILP and indicate that GPR15 plays a key role in mucosal immune homeostasis, largely by regulating the influx of Tregs. Our study also demonstrates that immune tolerance in the gut is functionally compartmentalized through the differential requirements for Treg homing to the small and large bowel.

OR.38. Divergent Target Specificity of $Foxp3^+$ Regulatory T Cells and Th2 Effector Cells in Patients Allergic to *A. fumigatus*
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$Foxp3^+$ regulatory T cells (Treg) are thought to play a central role in maintaining tolerance against harmless antigens at mucosal sites. However, which antigens are actually recognized by Treg in particular in humans is so far not known. We established a highly sensitive enrichment system to detect antigen-specific conventional T cells (Tcon) and Treg, based on antigen-induced CD154 (CD40L) versus CD137 (4-1BB) expression. We show that the airborne fungus A. fumigatus induces a dominant population of $CD4^+CD25^+CD127^-Foxp3^+Helios^+$ Treg in peripheral blood of healthy donors, with demethylated TSDR and potent *in vitro* suppressive activity. Intriguingly, the strong Treg response contrasts with minimal memory Tcon. This Treg dominance is abrogated in subjects allergic to A. fumigatus, due to a massive expansion of conventional Th2-type memory cells. However, in allergic donors, Treg are still abundant but surprisingly have non-overlapping protein targets with Th2 cells. Our data identify A. fumigatus as a major target of human Treg and provide direct evidence that antigen-specific Treg are potent suppressors of allergy development. Furthermore we provide an explanation how allergen-specific Th2 responses can escape Treg control due to selective targeting of A. fumigatus proteins not protected by a specific Treg response.

OR.39. Food Protein Induced Activation and Death of T Cells Is Required for Normal Development and Homeostasis of the Small Intestine
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Dietary components modulate not only the microbial composition in our body but also seem to be required for the development of the gut-associated lymphoid tissue (GALT). However, the impact of dietary proteins on metabolic state and immune function of the intestinal tissue remains enigmatic. We here show that replacement of food proteins by amino acids not only alters the intestinal morphology but also affects the metabolic activity and T cell reactivity in the small intestine of mice. Pathway analysis of the gene transcripts induced by dietary proteins indicated massive alterations in lipid and fatty acid metabolism, as well as regulation of hormone levels and cholesterol absorption in the small intestine. Further, physiological uptake of dietary proteins generated highly activated $CD4^+CD44^+Helios^+$ T cells, predominantly in Peyer´s patches (PP). Mice fed amino acid-containing, protein-free diet developed a highly impaired intestinal bar rier with atrophic PP and dramatically reduced numbers of activated $CD4^+Helios^+$ T cells

which were increased to normal levels after switching to conventional diet. These findings demonstrate that constant recognition of food proteins is crucial for maintaining the immune homeostasis within the small intestine.

OR.40. Colonic Tolerance Develops in the Iliac Lymph Nodes Independent of $CD103^+CD11b^+$ Dendritic Cells

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Mucosal tolerance to protein antigen relies on the local microenvironment and antigen-carriage by dendritic cells (DCs) to the draining lymph nodes. Previously, we demonstrated that colonically applied OVA leads to antigen-specific T cell proliferation in the iliac lymph nodes (ILN) while oral antigen elicited proliferation in the mesenteric lymph nodes (MLN). Despite the difference in draining site, both small intestinal and colonic antigen administration induced tolerance. Here, we investigated whether distinct locally adapted regulatory mechanisms maintain tolerance in the small and large intestine. Colonic antigen administration increased the number of $CD11c^+MHCII^{hi}$ migratory $CD103^-CD11b^+$ and $CD103^+CD11b^-$ DCs in the ILN. These ILN-derived DCs were nearly devoid of RALDH2 expression and the $CD103^+CD11b^+$ DCs representing the major migratory DC population in the MLN were virtually absent in the ILN. Colonic tolerance was intact in Batf3-deficient mice specifically lacking $CD103^+CD11b^-$ DCs, demonstrating that $CD103^-CD11b^+$ DCs in the ILN are sufficient to drive mucosal tolerance induction after protein antigen encounter in the colon. In agreement, ILN-derived $CD11c^+$ DCs from Batf3-deficient mice were effective in driving TGFβ-mediated $Foxp3^+$ Treg differentiation. Altogether, we identify different inductive sites for small intestinal and colonic T cell responses and reveal that distinct mechanisms are operative to maintain tolerance.

OR.89. Epigenetic Imprinting of Tolerogenic Properties of Stromal Cells in Gut-Draining Lymph Nodes by Micro-Environmental Factors

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A fraction of regulatory T cells (Tregs) are de novo induced in the periphery from naïve precursor cells. These peripherally induced Tregs contribute to tolerance not only to the commensal microflora, but also to food-born antigens. Previous works of others and us has shown that the microenvironment of gut-draining lymph nodes (LN) promotes peripheral Treg induction by shaping the phenotype of non-hematopoietic LN stromal cells. In the present study, we have performed LN transplantations from different settings to unravel the contribution of stromal cells and environmental factors such as infection, chronic inflammation and antibiotic treatment to the high Treg-inducing capacity of gut-draining LN. BisSeq and RNASeq was carried out on stromal cells isolated from skin- and gut-draining LN of colonized vs. germ-free mice to identify genes that are epigenetically and transcriptionally controlled by location- as well as commensal-dependent factors. Our data provide evidence that LN stromal cells are shaped early and stably during ontogeny to contribute to the tolerogenic properties of gut-draining LN. Once established, the tolerogenic properties of gut-draining LN stromal cells are persistent even after infection or chronic inflammation. Thus, tolerogenic properties of stromal cells in gut-draining LN are stably imprinted by microenvironmental factors early during ontogeny.

F80. Intestinal Microbiota Alters Omental Tumor Growth

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The omentum is an adipose tissue that contains milky spots (MS). The MS are similar to secondary lymphoid organs and can generate B and T cell immune responses to peritoneal antigens. Additionally, omentum $CD4^+CD25^+FoxP3^+$ T regulatory cells (Tregs) display an activated phenotype $CD44^{hi}CD62L^{low}$. Moreover, the omentum also collects metastasizing tumor cells in the peritoneal cavity and tumors growing in the omentum are associated with poor clinical prognosis. Here we show that intestinal microbiota

affects Tregs activation profile and impairs tumor growth in omentum. Our transplantable tumor model in wild type (WT) mice shows that peritoneal tumors grow progressively in the omentum and peritoneal cavity in WT mice, whereas in germ free (GF) mice tumors do not grow. Omental tumor growth in WT mice is associated with an increase in PD-1$^+$ Tregs, while tumor-specific CD8$^+$ T are reduced. However, in GF mice numbers of PD1$^+$ Tregs remain unaltered but tumor specific CD8$^+$ T cells are still present. After co-housed GF mice with WT mice, ex-GF mice showed tumor growth and restore the increase in PD-1$^+$ Tregs with low number of specific CD8$^+$ T cells. The induction of tolerance requires PD-1$^+$ Tregs. These data suggest that intestinal microbiota has a role in omental Tr activation and in the tolerance to peritoneal tumors.

F81. Suppressor of Cytokine Signaling (SOCS)-1 in the Cell Nucleus: A Regulator of Local Immunity in the Lung?
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Immune responses are tightly regulated to ensure for effective defense of pathogens but to avoid excessive bystander damage. We focus on suppressor of cytokine signaling (SOCS) 1, an inducible negative feedback inhibitor of JAK/ STAT signaling that is localized in the cell nucleus. To study the role of nuclear Socs1, a BAC transgenic mouse model has been established, expressing only non-nuclear Socs1 (Socs1ΔNLS) termed Socs1$^{-/-}$ Socs1 MGL^{tg}. Socs1$^{-/-}$ Socs1 MGL^{tg} mice are rescued from early lethality as compared to Socs1$^{-/-}$ mice, which die due to excessive interferon signaling within three weeks after birth. Classical interferon gamma signaling in bone-marrow derived macrophages is not altered in Socs1$^{-/-}$ Socs1 MGL^{tg} mice as shown by phosphorylation of STAT1 and by regulation of classical interferon gamma target genes. Microarray analysis verified those results but revealed differential expression of a subset of non-classical interferon gamma target genes in mice lacking nuclear Socs1. Although cytoplasmic Socs1 is crucial for survival, Socs1$^{-/-}$ Socs1 MGL^{tg} mice slowly develop inflammatory lesions and eosinophilia in the lung and a low-grade steatohepatitis, arguing for local immune regulatory functions of nuclear Socs1. Taken together, Socs1$^{-/-}$ Socs1 MGL^{tg} mice present a valuable tool to study the nuclear function of Socs1 *in vivo*.

F82. Expression of the TGFβ-Activating Integrin $\alpha_v\beta_8$ on Dendritic Cells is Important for Regulation of Human T Cell Responses
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A balance of T_H17 cells and regulatory T cells (Tregs) is required to maintain immune homeostasis in the intestine. Activation of the cytokine TGFβ, which is secreted as an inactive complex, plays a crucial role in the induction of both Treg and T_H17 cells. However, while murine studies have highlighted important pathways that regulate TGFβ activity in the intestine, little is currently known about the mechanisms of TGFβ activation in humans. Here we have shown that the TGFβ-activating integrin αvβ8, which we have previously found to play an important role in murine intestinal immune homeostasis, is preferentially expressed on human CD1c$^+$ intestinal dendritic cells (DC). Interestingly, expression on these cells is increased during pathological inflammation. We find that expression of integrin αvβ8 is upregulated on human DC after LPS stimulation, suggesting the enhanced expression of αvβ8 during inflammation could be driven by an infiltrating microbiota. Importantly, we have also shown that TGFβ activation by αvβ8 on human DC promotes CD25$^+$CD127$^-$ Foxp3$^+$ Treg induction *ex vivo*. Together, our data suggest that integrin αvβ8-mediated TGFβ activation by DC may play a role in the regulation of T cell responses in the human intestine, and that this pathway may be perturbed during intestinal inflammation.

F83. Dysregulations of Mucosal and Systemic Immune Responses at Adulthood after Perinatal Exposure to Bisphenol A (BPA): Possible Involvement in Food Adverse Reactions and Inflammatory Diseases
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Oral route is the major route of exposure to the food contaminant BPA, and intestine the first organ exposed. BPA has demonstrated its ability to interfere with the gut immune system, particularly when perinatal exposure occurred. In this study, we report that adult mice perinatally exposed to a low dose of BPA [5µg/kg BW/day] showed a decrease in lysozyme activity and total IgA production in feces. These alterations were associated with a defect in dendritic cells maturation (DC) from lamina propria (LP). Frequency of these cell subsets increased in gut mucosa while it decreased in spleen, suggesting a domiciliation defect.

Concomitantly, a decrease of regulatory and activated T cells in LP and mesenteric lymph node was observed. Interestingly, an alteration in the frequency of innate lymphoid cells ILC3 producing IL-22 occurred in LP associated with dysregulated IgG response against commensal bacteria in plasma. Perinatal exposure to BPA promotes inflammatory secreting profile of T cells in the spleen with a strong increase of IFN-gamma and IL-17 production. Then, BPA treatment impair intestinal immune homeostasis at adulthood, and favored inflammatory systemic immune responses. These dysregulations could participate to the increased susceptibility to food adverse reactions and to the establishment of inflammatory diseases.

F84. Epithelial E-Cadherin and its Receptor KLRG1 Limit the Accumulation of Treg in the Gut

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Intestinal immune responses rely on the tight control of $Foxp3^+$ regulatory T cells (Treg). However, which tissue-specific factors control intestinal Treg populations is not fully understood. Effector Treg, especially gut Treg, express high levels of CD103 and KLRG1, the receptors for the epithelial molecule E-cadherin, suggesting a role for E-cadherin on intestinal Treg. To bypass the lethal effects of intestinal E-cadherin depletion, we use here a model of E-cadherin replacement by N-cadherin. This replacement allows for postnatal mouse survival despite intestinal inflammation. In this model we found a strong accumulation of $KLRG1^+$ Treg in the intestine. The accumulating Treg had a $Foxp3^+$ $GATA3^{int}$ effector Treg phenotype, suggesting that the absence of E-cadherin favors the expansion of this specific Treg subset. We then reciprocally analyzed the effects of KLRG1 on Treg. *In vitro* assays showed that KLRG1 ligation reduces Treg response to TCR signals and limits Treg survival *in vitro*. Analysis of KLRG1-deficient mice showed that lack of KLRG1 confers a competitive advantage on Treg in the gut, but not in lymphoid organs, supporting a direct role for E-cadherin in modulating $KLRG1^+$ Treg. Our findings point to a novel level of interaction between epithelial cells and lymphocytes in shaping gut immunity.

F85. Bet v 1, a Lipocalin-like Allergen, is Capable of Binding to Siderophore-bound Iron Thereby Skewing T Helper Cell Responses

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The major birch pollen allergen, Bet v 1, causes respiratory allergy through yet still elusive circumstances. On the other hand, in the lung the human lipocalin-2, LCN2 is highly expressed and has immune-regulatory properties depending whether it carries iron via siderophores (holo-) or not (apo-). In this respect, we investigated Bet v 1, for its structural and biological resemblance with LCN2. FATCATflex, CE (Combinatorial Extention) algorithm and TM (Template Modeling) Align structural comparison methods indicate that Bet v 1 structure bears a significant resemblance to lipocalins. Prussian blue-staining as well as in silico docking analyses reveal that, similarly to lipocalins, the birch allergen is also capable of binding iron via catechol-based siderophores. Calculated Kd-values of 20nM outpassed affinities to known ligands more than twentyfold. When incubated with activated PBMCs (n=10), only the apo-form of Bet v 1, but not the holo-form, increased CD4+ expression in T cells and the secretion of IL13. Our work supports the claim that Bet v 1 may be considered a lipocalin-like protein capable of binding iron via siderophores. We give for the first time evidence that the form of application (apo- or holo-) is decisive for the subsequent immune response. The apo-form promotes Th2 cells, whereas the holo-form appears immunosuppressive. These results provide for the first time a functional understanding on the allergenicity of Bet v 1.

F86. The Role of Ultraviolet B Light Induced Vitamin D in Gastrointestinal Pathology

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A low vitamin D status is linked to a high incidence rate and a worse outcome of both Ulcerative colitis (UC) and Crohn's disease (CD) - the most common forms of inflammatory bowel disease (IBD). Since the gastrointestinal pathology due to IBD, the major source of vitamin D for patients is generated in the skin after ultraviolet B (UVB) exposure. We would like to characterize the impact of UVB-light induced vitamin D on the development of colitis, as well as its impact on host responses to gut bacteria (both pathogens and commensals). For this study, we use UVB emitting lamps (emission peak at 311 nm) to increase blood circulating vitamin D precursor, $25(OH)_2D_3$, in C57BL/6 mice and vitamin D receptor (VDR) knockout mice. Preliminary results implicate that

UVB radiation protects against bacterial burdens, epithelial barrier disruption and increases expression of anti-inflammatory cytokines in both cecum and colon after oral infection with Salmonella Typhimurium. Furthermore, UVB light induced vitamin D showed similar protective effect against DSS colitis in C57BL/6 mice. Ongoing studies focus on the induction of immunosuppressive of T regulatory cells after vitamin D and gut-homing 103$^+$ dendritic cell signaling in both colitis models. We are the first group to report the interplay between topical UVB light, vitamin D and colitis in mice. These results show that UVB phototherapy is a promising non-invasive application for IBD patients for future therapy.

F87. Symbiotic Microbiota Regulates Type 2 Immunity Through RORγt$^+$ T Cells

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Alteration of the symbiotic microbiota early in life, or absence of it, leads to allergic pathologies. While it is unclear how microbiota regulates type 2 immunity, it is a strong inducer of pro-inflammatory Th17 cells and regulatory T cells (Tregs) in the intestine. We report that microbiota-induced Tregs express the nuclear hormone receptor RORγt, and differentiate along a pathway that also leads to Th17 cells and is regulated by the vitamin A metabolite retinoic acid. RORγt$^+$ Tregs, and more generally RORγt$^+$ T cells, inhibit the generation of Gata3$^+$ T cells, which include Th2 cells and the other major population of intestinal, IL-33-responsive, Tregs. In the absence of RORγt$^+$ Tregs, Th2-driven worm expulsion is more efficient while Th2-associated pathologies are exacerbated. Thus, microbiota regulates type 2 responses through the induction of "type 3" RORγt$^+$ Tregs and Th17 cells, and acts as a key factor in balancing immune responses at mucosal surfaces.

F88. Effect of Low Level Laser on sIgA and Lysozyme Levels in Saliva after Third Molars Surgery

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Third molar extractions in general anesthesia have become a standard procedure in dentistry. There is an effort to shorten healing and decrease the number of complications as well as increase comfort after the treatment. Low level lasers are known for their analgesic, anti-inflammatory and stimulatory effect. The aim of study is to evaluate the effect of low level laser after surgery on sIgA levels in saliva of patients treated by Department of Stomatology, 2nd Medical Faculty. Their diagnosis was third molar retention. The low level laser radiation of 808 nm was applied. Control group was treated using placebo - red light. The exposition time was 11 seconds immediately after the suture, than every day for following 4 days. The sIgA levels in saliva decreased significantly in both groups and into greater extend in laser application group as compared to placebo group. The lysozyme levels in saliva decreased significantly in both groups and into similar extend in laser application group and placebo group. The study confirmed low level laser effect on lysozyme and sIgA levels in saliva.

F89. The Regulation of Oral Tolerance by Intestinal Macrophages Utilizes Two Distinct Pathways Involving Either CD11b or CX3CR1

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Intestinal tolerance requires the priming of FoxP3$^+$ regulatory T cells (Tregs) in the mesenteric lymph nodes (mLNs) by CD103$^+$ dendritic cells and subsequent homing of Tregs to the intestine. Signaling by resident CD11b$^+$CX3CR1^{high} macrophages present in the intestinal lamina propria leads to further Treg expansion. Similarly to our previously published data concerning CX3CR1, we show that the deficiency of CD11b leads to a defect in the development of oral tolerance. Adoptive transfer experiments into CD11b$^{-/-}$ mice showed that, like in CX3CR1$^{-/-}$ mice, Treg cells were readily induced in the mLNs but local Treg expansion in the lamina propria was reduced. As signaling via resident macrophages is critical for the expansion of Tregs in the intestinal lamina propria we investigated the pathways that are altered in intestinal macrophages due to CX3CR1 and CD11b deficiency. Surprisingly, microarray analysis revealed that the lack of CD11b and CX3CR1 lead to distinct transcriptomic changes in the intestinal

macrophage compartment. Our preliminary data suggest a partial deficiency in TLR signaling in macrophages of $CD11b^{-/-}$ mice in contrast to macrophages from $CX_3CR1^{-/-}$ mice which exhibit an impairment of IL-10 signaling. We hypothesize that independent signaling pathways involving either CD11b or CX_3CR1 contribute to the tolerogenic function of intestinal macrophages.

F90. Invariant Natural Killer T Lymphocytes Promote the Development of Intestinal Tumors

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CD1d-restricted natural killer T (NKT) cells are potent immuoregulatory cells. Activation of invariant NKT (iNKT) cells suppresses tumor formation in murine models, and iNKT cells can naturally protect against spontaneous tumors and enhance immune responses to infections. Further, NKT cells promote murine inflammatory bowel disease. Intestinal tumors develop in an environment of constant microbial pressure and inflammatory signals that enhance tumor formation. This raised the question whether NKT cells would suppress, through their anti-tumor function, or promote, by their pro-inflammatory capacity, tumor formation in this tissue. $APC^{min/+}$ mice develop intestinal tumors/polyps due to a mutation in the adenomatous polyposis coli gene, mutated in human colorectal cancer. We show that absence of all NKT cells, or iNKT cells, in $APC^{min/+}$ mice decreased the number of intestinal polyps with 60%. This was associated with reduced FoxP3 expression and increased expression of TH1-associated genes such as IFN-γ and iNOS in polyps. This suggests that iNKT cells promote intestinal polyp formation by enhancing FoxP3 Treg cells and local immunosuppression of anti-tumor TH1-immunity.

F91. Commensal Escherichia coli Induces Systemic Antigen-Specific Tolerance

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Objective: While gut commensal bacteria are locally tolerated, it remains unknown whether they can induce systemic antigen-specific tolerance as it has been described for nutritional antigens. Here we analyses whether commensal-derived antigens mediate systemic immunological tolerance and whether localization of the antigen to different bacterial compartments influences this process. Methods: A 85aa ovalbumin (Ova) fragment, complete Ova or a control protein were expressed in different compartments of E. coli K12 (EC-Ova^{OM}, 85aa membrane-bound; EC-Ova^{SECR}, 85aa secretable; EC-Ova^{CYTO1}, 85aa cytoplasmatic; EC-Ova^{CYTO2}, complete cytoplasmatic; EC-contr, control protein). Western blot analysis confirmed comparable expression levels. Mice were colonized for 20 days with the strains, sensitized with Ova+Alum and exposed to inhalative Ova-challenges. Results: Mice colonized with EC-Ova^{OM} and EC-Ova^{SECR} displayed reduced eosinophil counts in bronchoalveolar lavages, decreased pulmonary mRNA expression of Th2-type cytokines and reduced inflammatory tissue damage compared to mice colonized with EC-contr. Colonization with EC-Ova^{CYTO1} and EC-Ova^{CYTO2} did not efficiently mediate tolerance. Surprisingly, EC-Ova^{OM} and EC-Ova^{SECR}, but not EC-$Ova^{CYTO1/2}$ strongly induced proliferation and cytokine production of Ova-specific TCR-transgenic OTII CD_4 T cells co-cultured *in vitro* with antigen-presenting cells. Conclusion: A model antigen introduced into commensal E. coli triggers systemic oral tolerance upon intestinal colonization. However, tolerance was only induced if the expressed antigen was present at the outer membrane or in a secretable form, but not in the cytoplasm, which correlated with the capacity to present the antigen to T cells. The unexpected differences in presentation of bacterial antigens located in distinct bacterial compartments require further investigation.

F92. CBirTox Selectively Induces $CD4^+Foxp3^+$ T Cells to Microbiota Flagellin

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We have previously defined a protective regulatory T cell (Treg)- Immunoglobulin A (IgA) pathway toward microbiota antigens. Tregs provide critical survival factors to IgA^+ B cells in the intestine in order to maintain mucosal homeostasis. Cholera toxin B (CTB) has previously been demonstrated to induce $Foxp3^+$ Tregs *in vitro* and *in vivo* when conjugated to defined antigens. In order to further study the Treg-IgA pathway, we have generated a construct, termed CBirTox, composed of a distal fragment of CBir1 flagellin fused to the A2 subunit of cholera toxin and expressed with CTB as a GM-1 ganglioside-binding pseudotoxin. $CD11c^+$ dendritic cells (DCs) pulsed with CBirTox for as little as five minutes are capable of activating B6.CBir1 TCR Tg CD_4^+ T cells *in vitro*, while $CD19^+$ B cells require pulse times of approximately one hour. CBirTox, but not CBir1 peptide, pulsed $CD11c^+$ DCs from multiple tissues, including the spleen, MLN, and lamina propria, induced Foxp3 expression in approximately 20-30% of B6.CBir1 TCR Tg

$CD4^+CD25^-$ T cells *in vitro*. Neither Th1 nor Th17 cells are induced with CBirTox, though they can develop under polarizing conditions, demonstrating a selective induction of $Foxp3^+$ Tregs but not inhibition of Th1 or Th17 subsets. While retinoic acid inhibitors had no effect, anti-TGF-β ameliorated Foxp3 expression. CBirTox pulsed B cells downregulate expression of phosphorylated p70S6 kinase, a downstream target of mammalian target of rapamycin (mTOR), suggesting CBirTox acts to induce $Foxp3^+$ Tregs by partially inhibiting antigen presenting cell (APC) mTOR signaling. Collectively, our data demonstrates CBirTox may be used as an effective probe of the Treg-IgA pathway.

F93. Selective Regulation in Expression of Co-Inhibitory Molecule B7-H1/PD-L1 on Masticatory Mucosae

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Purpose: Oral mucosae are covered by stratified squamous epithelia and possess multiple functions as epithelial barrier, masticatory mucosa, and permeabilization. Especially, oral masticatory mucosae receive various dietary and microbial stimuli, they might have protective mechanisms to avoid excess immune responses. B7-H1/PD-L1 (CD274) is one of ligands for co-inhibitory receptor PD-1 (CD279). It's often induced on non-lymphoid tissue cells at the inflammatory condition and B7-H1:PD-1 pathway negatively regulates T cell activation. In this study, we examined expression and regulation of B7-H1 in mouse epithelia. Results and Discussion: B7-H1 was physiologically induced on prickle cells, but not basal cells of masticatory mucosae including dorsal surface of tongue (DST), gingiva, and hard palate. Expression levels were age-relatedly increased, epithelium of non-masticatory oral mucosae and other organs and skin never express B7-H1 in the steady state. Topical painting TPA, DNFB, and OVA on the skin, buccal mucosa (BM), and DST induced activation and proliferation of basal cells assessed by Ki67 expression and induced B7-H1 on both prickle and basal cells. However, the enhanced B7-H1 levels on basal cells of BM and DST were clearly impaired. In OVA-primed DO11.10 T cell-transferred mice, blockade of B7-H1 at the time of topical OVA/DNFB painting on DST dramatically enhanced mucosal inflammation assessed by increased mononuclear cell infiltration and MHC class II expression, suggesting protective roles of B7-H1. Basal cells in masticatory mucosae have conflicting roles; to protect from microbial infection and to maintain metabolism of the epithelium, therefore, B7-H1 expression in the basal cells may be strictly regulated.

F94. The Effects and Mechanism of Tonsil Derived Mesenchymal Stem Cells in a Mouse Model of Eosinophilic Rhinosinusitis with Nasal Polyp

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We sought to evaluate the immunomodulatory effects and mechanism of tonsil derived mesenchymal stem cells (T-MSC) in a mouse model of eosinophilic rhinosinusitis with nasal polyp (ERSwNP). The effect of T-MSCs was evaluated in BALB/c mice that were divided into 4 groups (negative control group nasal polyp group T-MSC group and T-MSC(AD) group (T-MSC incubated with adipogenic differentiated medium)). After induction of OVA- induced ERSwNP model, T-MSCs were administered intravenously (T-MSC and T-MSC(AD) groups) on weeks 5 to 12 (once per week) and subsequent OVA^+SEB challenge was conducted until 12 weeks. Several parameters of inflammation including polyp formation were evaluated including cytokine, chemokine and adhesion molecules in nasal mucosa, spleen and lymph node. Intravenous injection of T-MSCs significantly reduced allergic symptoms, eosinophil, neutrophil, nasal polyp count and serum OVA specific-IgG1 levels. Moreover, the nasal, lymph node and systemic Th2 cytokine profile and innate cytokines such as IL-25 and IL-33, and chemokines (CCL11, CCL24, Cxcl1, CxCl2, ICAM1 and VCAM1) expression were reduced in T-MSCs injected groups, as compared to the nasal polyp group. Usually T-MSC(AD) group showed better inhibitory effects of inflammation than T- MSC group. T-MSCs injected groups have significantly increased Treg cells in cervical lymph node, as compared to the nasal polyp group. Administration of T-MSCs effectively reduced polyp formation, inflammatory cell influx, cytokine profile, chemokine molecule expression, and T cell subset distribution, suggestive of the mechanism of reduced CRS inflammation and less polyp formation in mouse model of ERSwNP. Therefore, T-MSC treatment is potentially an alternative therapeutic modality in CRSwNPs.

F95. Oral Administration of Lactic Acid Bacteria Prevents Steatosis in a Murine Model for Non-Alcoholic Steatohepatitis
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Non-alcoholic steatohepatitis (NASH) is a detrimental process of diabetes to develop into hepatocellular carcinoma. This inflammatory disease is becoming increasingly popular among obese population associated with high-fat diet, especially in developed countries. Therefore establishment of diet-solutions, in addition to symptomatic treatments, are keenly required. We have previously shown that approximately 70% of lactic acid bacteria are able to induce high level of anti-inflammatory interferon-β (IFN-β) from dendritic cells by stimulating endosomal Toll-like receptors. In the present study we show that oral administration of a lactic acid bacterium (LAB, Lactococcus lactis strain C60) prevents inflammation and steatosis in murine liver, using an experimental model of NASH, i.e. non-alcoholic fatty liver disease (NAFLD) activity score including steatosis, lobular inflammation, and hepatocellular ballooning are significantly improved. C60 is a LAB strain, which induce high level of IFN-β and IL-10 from dendritic cells and stabilize oral tolerance. We are currently examining the role of these anti-inflammatory mediators (IL-10/IFN-β) and resultant immune regulatory cells in suppressing liver chronic inflammation.

F96. Oral Dendritic Cells Present Sublingual Antigen and Induce Regulatory T Cells in the Draining Lymph Nodes
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Sublingual immunotherapy is safe and efficient for the treatment of type I allergies, but the underlying immunological mechanisms, particularly induction of regulatory T (Treg) cells, are still unclear. Here we show that sublingual application of ovalbumin induced $Foxp3^+$ Treg cells in the draining submandibular LNs (ManLNs) in mice. In the lingual and sublingual tissues, oral classical dendritic cells (cDCs) were clearly separated from oral macrophages by flow cytometry and expanded *in vivo* by DC differentiation cytokine Flt3 ligand. Oral cDCs showed retinoic acid (RA)-producing activity and converted naive $CD4^+$ T cells to $Foxp3^+$ Treg cells in a TGF-β and RA dependent manner *in vitro*. In the ManLNs, migratory cDCs also showed RA-producing activity. After sublingual application of fluorescent ovalbumin, fluorescence was detected in oral macrophages in the tissues followed by migratory cDCs in the ManLNs, and the sublingual ovalbumin-primed migratory cDCs induced $Foxp3^+$ Treg cell conversion *ex vivo*, suggesting that oral macrophages incorporate sublingual antigens and presumably transfer them to oral cDCs in the sublingual mucosa, and that the antigen-incorporated cDCs migrate to the ManLNs and induce Treg cells. These results highlight the mechanism and pathway by which $Foxp3^+$ Treg cells may be induced by sublingual immunotherapy.

F97. Oral Administration of Neither HSP65-Producing nor IL-10-Producing Lactococcus Lactis Ameliorates Obesity-Related Metabolic Disorders
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Obesity is associated with systemic low-grade inflammation and metabolic disorders such as insulin resistance and dyslipidemia. Frequent consumption of diet with high contents of fat and sucrose contributes to the described lifestyle-related diseases. Many lactobacilli exert immune regulatory effects modulating innate and adaptive responses. It is well established that the gut mucosa is a privileged site for the induction of regulatory T cells and anti-inflammatory cytokines, which are able to suppress inflammatory reactions. Hsp65 (heat shock protein 65) is a protein highly expressed in inflammatory sites and IL-10 is a cytokine important for oral tolerance induction. Recombinant Lactococcus lactis that produces either HSP65 or IL-10 is reported to modulate autoimmune disease models. Herein, we tested the effects of these recombinant bacteria in the development of experimental obesity. Sixty- to eight-week-old C57BL/6 mice were fed either control (AIN93G) or HSF (High Sugar and Fat) diet for eleven weeks. Oral treatment with either HSP65-producing or IL-10 producing L. lactis was given for five days during the seventh week of diet. A control group received oral treatment with either wild type L. lactisor medium. Mice fed HSF diet showed an increase in body weight gain, adiposity index, glycemia, total cholesterol and leptin levels as well as decreased production of IL-10 and TGF-B when compared to AIN93-fed animals. Oral treatment with either HSP65-producing or IL-10 producing L. lactis did not have any effect on these obesity signs.

MUCOSAL VACCINES

PS.2. Targeting the Host to Reverse Infection-Induced Immune Suppression Affords a Novel Approach to Therapy and Vaccine Development Against Neisseria gonorrhoeae

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WHO estimates that there are >100 million new cases of gonorrhea worldwide every year, with the burden of morbidity falling mainly on women. However, gonococcal infection does not induce protective immunity, no vaccine is available, and Neisseria gonorrhoeae is becoming resistant to most available antibiotics. We have demonstrated in mice that the ability of N. gonorrhoeae to suppress Th1/Th2-governed adaptive responses can be reversed by intravaginal (i.vag.) treatment with IL-12 encapsulated in sustained-release biodegradable polymer microspheres (IL-12/ms), resulting in rapid elimination of the infection and protection against re-infection. This promotes Th1-driven responses including anti-gonococcal antibodies in serum and genital secretions, IFN-γ-secreting $CD4^+$ T cells in the genital tract and iliac lymph nodes (ILN), and memory that can be recalled upon re-exposure to N. gonorrhoeae. Protection against re-infection persists for at least 6 months, and extends to antigenically distinct strains of N. gonorrhoeae. Protective immunity is not induced by IL-12/ms in the absence of gonococcal antigen, but gonococcal outer-membrane vesicles (OMV) can substitute for live N. gonorrhoeae to create a vaccine. I.vag. immunization with gonococcal OMV plus IL-12/ms induces similar responses, including serum (IgG) and vaginal (IgG and IgA) anti-gonococcal antibodies, secretion of IFN-γ and IL-17, but not IL-4, by $CD4^+$ T cells isolated from the ILN, and protection against infection. The findings illuminate the mechanisms whereby a significant mucosal pathogen manipulates the host's responses for its own benefit, and suggest new approaches both to treatment of antibiotic-resistant infections, and to developing an effective vaccine against gonorrhea.

OR.73. Mucosal Immune Responses Following Radio Controlled Capsule Delivery to Human Intestines: An Open Label Clinical Trial of An Influenza Vaccine Based on Recombinant Adenovirus

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Background: The development of a tablet vaccine has been hampered by the lack of robust models of human gastrointestinal biology. Stomach acid can destroy pH sensitive vaccines, and enteric coatings that protect vaccines against acid degradation work in a species-specific fashion. For a vaccine embedded in tablets, if no immune response to antigen was observed post administration, it would be unclear on whether the biology or the enteric coating failed. Method: In order to explore vaccine performance independent of enteric coating, a small molecule drug development technique using radio-controlled capsules (RCC) delivered to the intestine was explored in a clinical trial. Mechanical capsules were filled with a recombinant adenoviral vector expressing influenza HA and a radio labeled tracer. These capsules were swallowed by human volunteers, tracked by scintigraphy, and opened remotely in either the ileum or the jejunum for the purpose of determining the best performing location. Results: Substantial numbers of mucosal homing, antigen specific B cells were found 7 days after immunization in the peripheral blood of all immunized volunteers, with up to 15% of B cells having alpha4beta7 high expression compared with 2% at day 0. One location had superior performance, and the HAI seroconversion rate was 67% in this group. Further, fecal samples and nasal washes of subjects immunized by RCC demonstrated increases in the specific antibody responses to influenza. Conclusion: These results demonstrate that the biology of the vaccine can elicit typical influenza vaccine immune responses in serum as well as very potent mucosal antibody responses.

OR.74. Lymphotoxin Signaling is Essential in the Generation of Protective Responses to Mucosal Vaccines

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Although oral vaccination offers many practical advantages, the generation of long-lived protective immunity following inoculation is poorly understood. Using oral vaccination of live, attenuated Salmonella to establish persistent colonization of mice, we demonstrate that the lymphotoxin pathway is essential for the generation of protective immunity to mucosal infection. It was previously shown that LTβR deficient mice have normal colonization of Salmonella in the gut. However, we find that mice deficient in this pathway fail to generate protective immunity following persistent oral vaccination, and succumb to a secondary challenge

with virulent Salmonella. To determine if LTβR$^{-/-}$ animals fail to develop antibody responses, we measured titers of anti-Salmonella IgM and IgG. Although these mice develop normal levels of IgM, they fail to produce anti-Salmonella IgG, implying a role for lymphotoxin in class switching. Animals that lack LTβR have an absence of Peyer's patches and lymph nodes. Surprisingly, we find that lymphotoxin plays an essential role in antibody production independently of its role in lymphoid tissue development, as short-term treatment of WT mice with blocking LTβR-Ig is sufficient to prevent the formation of anti-Salmonella antibody responses. Using genetic approaches we further dissect the contribution of lymphotoxin in generating protective responses to mucosal vaccination.

OR.75. Long-Lived T_{RM} Th17 Cells Develop After Bacterial Immunization

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A defining feature of adaptive immunity is the ability to generate long-lived populations of memory cells. Upon microbial infections, CD8 and CD4 T cells experience clonal expansion followed by a contraction phase and the development of memory. Th17 cells have been revealed as a subset of effector CD4 T cells with an important role in the control of specific pathogens as well as in the development of autoimmune diseases; however their maintenance and stability during memory immune response is controversial. Recent studies suggest that subsets of memory T cells are retained at specific sites as tissue-resident memory T cells (T_{RM}), and may confer an effective in situ first line of defense to tissue-specific infections. CD4-T_{RM} cells are present in several mucosal tissues and they were mainly described as IFN-gamma producers after viral infections. The origin of CD4 T_{RM} cells is still unknown, however CD8 T_{RM} seem to be more closely related to circulating T_{CM} than to T_{EM} based on KLRG1 expression. The maintenance of CD8 T_{RM} involves TGF-b and IL-15, though the factors those keep the longevity of CD4 T_{RM} are an area of extensive research. We are interesting in the study of Th17 T_{RM} and we found that intranasal bacterial immunization induce the differentiation of CD4 T_{RM} Th17 cells that remains as a quiescent population for at least 60 days after immunization. Our preliminary data suggest that Th17 cells include a population of long-lived T_{RM}, which could potentially have important implications for targeting site-specific immunity in vaccines and immunotherapies.

OR.76. Nasal Vaccination Induces Innate Lymphoid Cells (ILC1s) and NK Cells to Initiate the Lung IFN-γ Cascade that Supports Adaptive T Cells Responses to Brucella

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Brucellosis is the most common zoonotic disease worldwide, usually transmitted from Brucella-infected livestock after consumption of contaminated foods or by aerosol exposure. There are no vaccines for humans, and current livestock vaccines are only ~70% efficacious. To address this void, we developed a live, double-mutant Brucella melitensis (BMDM) vaccine that confers sterile immunity after mucosal vaccination. Protection is IFN-γ-dependent since IFN-γ$^{-/-}$ mice succumb to infection. We hypothesize that BMDM's potency is linked to ILC and NK cell stimulation to drive Th1-type responses. To test this hypothesis, mice, nasally vaccinated with BMDM, were evaluated for innate and adaptive immune cell influx into the lungs. By 3 days post-vaccination, IFN-γ-producing ILCs, NK cells, and γδ T cells increased in the lungs by 2-, 3-, and 3-fold, respectively. By 5 days, the NK cells and ILCs contracted, and CD4$^+$ and CD8$^+$ T cells increased 2- and 5-fold, respectively; γδ T cells remained unchanged. By 2 wks, CD8$^+$ T cells were the dominant IFN-γ source, being 15-fold > naive lungs. The CD4$^+$ and CD8$^+$ T cell percentages were also impacted after wild-type BM challenge. Unprotected and RB51 (rough mutant)-vaccinated mice showed no differences in their percentages of lung CD4$^+$ and CD8$^+$ T cells unlike challenged BMDM-vaccinated lungs showing 30% fewer CD4$^+$ T cells and an equal percentage of CD8$^+$ T cells resulting in a net increase in CD8$^+$ T cells. Thus, the vaccine composition and the route of its delivery impact the types of innate and adaptive immune cells responsive to immunization and challenge.

T126. Prime-Boost Immunization Strategy for Induction of Local Genital Immunity in the Minipig Model of Human Genital *Chlamydia trachomatis* Infection

Emma Lorenzen[1,2], Frank Follmann[1], Sarah Bøje[1,2], Karin Erneholm[1,2], Anja Olsen[1], Jørgen Agerholm[2], Gregers Jungersen[3] and Peter Andersen[1]. [1]Statens Serum Institut, Copenhagen, Denmark; [2]University of Copenhagen, Copenhagen, Denmark; [3]Technical University of Denmark, Copenhagen, Denmark

A vaccine is needed to combat the continued worldwide spread of genital Chlamydia and it is crucial for the vaccine to elicit a mucosal immune response to be protective. Until now, it has not been possible to establish a significant mucosal immune response

with traditional vaccination strategies. We have used an advanced minipig model of human genital Chlamydia to evaluate a combined immunization strategy, with an intramuscular (IM) immunization and an intranasal (IN) booster immunization. The vaccine was formulated with CAF01 adjuvant, known to induce a strong Th1/Th17 response. We found that IM priming immunization with CAF01 adjuvant raised a significant systemic and genital IgG response, and a significant cell-mediated IFN-ɣ and IL-17A response from re-stimulated PBMCs and lymph node cells. Mucosal (IN) boosting induced significant levels of IgA in the nasal cavity and following vaginal infection a significant secretory IgA response on the genital surface, which correlated with significant lower bacterial shedding. This study reveals that by combining IM and IN immunization, it is possible to establish a significant systemic and mucosal immune response in the advanced minipig model of human genital Chlamydia. It paves the way for the future Chlamydia vaccine development and induction of genital immunity in general.

F98. Development of Immunization Protocols to Induce Integral (Systemic and Mucosal) Immune Response

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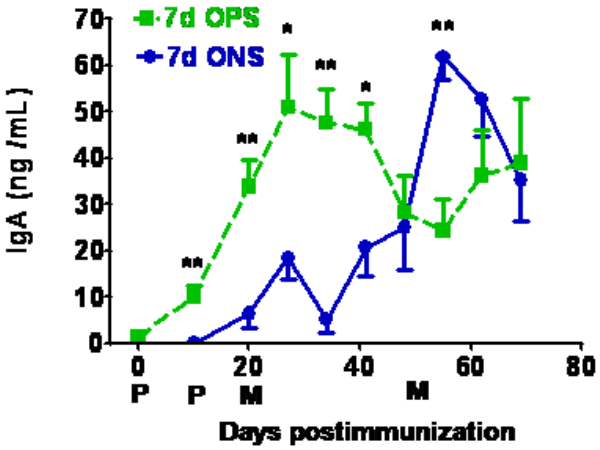

Figure. Nasal anti-OVA IgA response in 7 day-old suckling piglets parenterally (P, days 0 and 10) and mucosally (M, day 20) immunized. A mucosal boost was given on day 50. Each point is the mean of at least 5 animals +/- the SEM. OPS=OVA positive sow´s piglets. ONS=OVA negative sow´s piglets.

Intramuscular (IM) vaccines, although effective in controlling infectious diseases, provide poor mucosal protection, where most of the pathogens gain access into the organism. Mucosal immunization (MI) may prevent the entrance of pathogens to the body interrupting their life cycle and making prophylactic measures more effective. However, direct MI may induce tolerance, which has been avoided using high dose of antigen (Ag), repeated immunization and toxic adjuvants, strategies not for use in human and veterinary medicine. Our group has studied immunization protocols that render integral (systemic and mucosal) immune response (IIR), paying attention to the age, route, site and number of immunizations. Using ovoalbumin (OVA) as Ag in our Vietnamese minipig animal model, before and after weaning, we studied parenteral and mucosal immunization routes at different sites, measuring serum and mucosal anti-OVA (IgG and IgA) responses by ELISA. Our results demonstrated differences between systemic and mucosal immune responses in unweaned animals, the induction of IIR in unweaned and weaned animals, a role for maternal antibodies in perinatal immunization (Figure) and also, the site of immunization had relevance in the effectiveness of the protocol. At the time, three immunizations are needed to induce IIR and a strong memory immune response after Ag boost. In summary, we developed a method to induce integral immunity without the use of high Ag dose, toxic adjuvants or multiple immunizations. Studies on the use of these protocols, with relevant Ags, are under way in our laboratory.

F99. Flagellin is a Strong Vaginal Adjuvant for Therapeutic Vaccine in Genital Cancer

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Cervical cancer is a high incidence female cancer mostly caused by human papilloma virus (HPV) infection in genital mucosa. Immunotherapy targeting HPV-derived tumor antigen has been widely studied in animal models and patients. Because female genital tract is a portal entry site of HPV infection and a highly compartmentalized system, development of topical vaginal immunotherapy in orthotopic cancer model will provide ideal therapeutics. In this regards, we examined whether flagellin can be used as an adjuvant for topical therapeutic cancer vaccine in a genital cancer model. Intravaginal (IVAG) co-administration of E6/E7 peptides with flagellin resulted in tumor suppression and long term survival of the tumor bearing mice. In contrast to IVAG

vaccination, intranasal (IN) or subcutaneous (SC) immunization could not induce significant tumor suppression in the orthotopic genital cancer model. The vaginal adjuvant effect of the flagellin was completely abolished in TLR5 knock out mice. IVAG immunization of E6/E7 peptide with flagellin induced accumulation of CD_4 or CD8 cells and expression of the T cell activation-related genes in draining genital lymph nodes (gLNs). The co-administered flagellin elicited antigen-specific IFN-γ production in gLNs and spleen. The IVAG administered flagellin co-localized with $CD11c^+$ cells in the gLNs and enhances TLR5 expression. These results suggest that flagellin is a strong vaginal adjuvant to induce anti-tumor immune responses in genital cancer.

F100. Bacterial Outer Membrane Vesicles (OMVS) Provide Broad and Immediate Protection Against Influenza Infection
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Influenza has been a serious health problem due to the high mutation and transmission rates as well as the pathogenicity of the influenza viruses. Since the conventional vaccines are type-specific and require several weeks for the induction of protective immunity, novel antiviral agents are needed to overcome the shortage of the current vaccines. In this study, we evaluated the antiviral potential of bacterial outer membrane vesicles (OMVs) using a murine influenza model. OMVs stimulated diverse Toll-like receptors on and in the cells *in vitro*. Additionally, intranasal administration of OMV conferred rapid protection against various subtypes of influenza viruses, whereas the conventional vaccine exhibited subtype or strain-specific efficacy. Pretreatment of OMVs rapidly activated innate immune response in the lungs: production of pro-inflammatory cytokines and recruitment of neutrophils and monocytes. These results demonstrate that OMV could be an active and immediate immunomodulator capable of controlling influenza virus infection.

F101. Gingival Mucosa as an Alternative Vaccination Route: Implications for Elderly Vaccination
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Gingival mucosa (GM) diminishes with age resulting to no keratinized layer, decline in gingival fibroblast cells, and wider gingival crevice which we believe would allow target antigen to easily penetrate and induce an immune response, especially in an elderly host. However, the potential of GM as a vaccination route was never explored. In this study, we used elderly rats (77 week-old) and initially confirmed both gingival entry and optimal xanthan gel:antigen ratio using catechin. We found that higher catechin amounts enter the body through oral-supplementation (via GM) as compared to oral-administration. Moreover, we established that 100 mg mL^{-1} is the optimal amount for oral-supplementation. Subsequently, for vaccine testing, we used influenza H5N1 hemagglutinin (HA) as the target antigen and treated four sets of rats the same optimized antigen amount (via intradermal, oral, sublingual, and gingival route, respectively). Heart blood was obtained 14 days post-vaccination and blood sera were isolated for antibody measurement through a pre-optimized sandwich ELISA method. We found that gingival vaccination was able to induce an antibody response comparable to both oral and sublingual vaccination. This highlights the potential of GM as an alternative vaccination route which we propose can be applicable for elderly vaccination.

F102. New Chimeric NOD2/TLR2 Adjuvant Induces Mucosal Immune Response After Subcutaneous Administration.
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Preventive vaccination is one of the major successes in medicine. However, some obstacles remain for the conception of effective vaccines for challenging pathogens (i.e. HIV) and populations (i.e. elderly). The design of appropriate adjuvants is crucial to solve these issues. Emerging evidences indicate that activation of immune cells through pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) or NOD-like receptors (NLRs) may be critical mechanisms. Most of the adjuvants used in clinical trials target TLRs, but their usefulness in conjunction with NLRs agonists remains poorly studied. In this study, we evaluate a chimeric ligand that targets both TLR2 and NOD2 receptors. We assessed its ability to enhance monocyte-derived dendritic cells (MoDCs) maturation *in vitro* and its effect on systemic and mucosal immune responses in mice. *In vitro*, we showed that the chimeric ligand upregulated the expression of MoDCs maturation markers, of costimulatory molecules, and of pro-inflammatory cytokines. Furthermore, *in vivo* analysis revealed that its co-administration with biodegradable nanoparticles carrying Gag p24 HIV-1 antigen induced high antigen-specific IgA and IgG titers at both systemic and mucosal sites after parenteral immunizations. These findings

demonstrate the potential of chimeric molecules TLR/NOD as adjuvants for vaccines to induce systemic and mucosal immune responses.

F103. Oral Immunization with *Lactococcus lactis* Expressing EspB Enhances Clearance of *Escherichia coli* O157:H7 In Two Murine Infection Models

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Enterohemorrhagic Escherichia coli (EHEC) have been responsible for outbreaks of hemorrhagic diarrhoea and the hemolytic-uremic syndrome (HUS) worldwide. HUS is the most common cause of acute renal failure in children and results in fatalities as high as 50% in the elderly. Currently, neither a specific treatment nor a vaccine is available for EHEC. Lactococcus lactis, a generally regarded as safe bacterium, was constructed to express the EHEC antigen, EspB. Different constructs either produced low cytoplasmic levels of EspB, or secreted EspB constitutively or under nisin-induction. Oral immunization of mice with these constructs resulted in weak to strong responses, respectively, which were correlated with the amount of antigen produced. The EspB-secreting strains successfully induced EspB-specific mucosal and systemic antibodies as well as a mixed Th1/Th2 response with a predominance for Th2. The nisin-induced EspB secreting strain could significantly reduce the amount and/or duration of colonization of EHEC with more than 50%. Our results demonstrate the protective potential of EspB and the efficient delivery of recombinant EspB by L. lactis in mice. In larger species the use of L. lactis will be mortgaged by its low survival in the harsh environment of the gut. We demonstrated the detrimental effect of bile and pancreas enzymes. The presence of aluminum hydroxide (a bile acid binder) and camostat mesylate (a trypsin inhibitor) could significantly improve survival *in vitro* and *in vivo*. In pigs a 38- and 24-fold increase in L. lactis-counts in jejunal and ileal contents of treated animals was obtained.

F104. TSLP-Responsive Mucosal DCs are Critical for the Induction of Pneumococcal Vaccine Antigen-Specific IgA Response in Nasal Immunization

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Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine involved in Th2-type immune responses, in which myeloid dendritic cells (DCs) are known to be its primary target. However, there is no evidence to date for TSLP-mediated induction and regulation of antibody (Ab) production upon mucosal immunization. Here we found that TSLP and TSLP receptor (TSLPR) expressions were highly up-regulated in mucosal DCs of mice nasally immunized with pneumococcal surface protein A (PspA) plus cholera toxin (CT). Interestingly, antigen (Ag)-specific IgA, but not IgG Ab responses in both serum and mucosal secretion were significantly reduced in TSLPR-KO mice compared to WT mice following nasal immunization with PspA plus CT. Furthermore, $CD11c^+$ mucosal DCs isolated from nasally immunized TSLPR-KO mice were less activated and exhibited a remarkable reduction of IgA enhancing factor expressions (e.g., APRIL, BAFF and TGF-β). Finally, DCs from TSLPR-KO mice given nasal PspA plus CT were less effective for supporting IgA productions in a DC-B cell co-culture system. Taken together, these results suggest that TSLP signaling is pivotal to induce Ag-specific IgA Ab immune responses after nasal immunization with PspA plus CT in mice, which is indispensable for eliciting humoral immunity against pathogenic pnemococcal infections.

F105. Interleukin-22 is a Critical Mediator Vaccine-Induced Reduction of Helicobacter Infection in the Mouse Model

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Despite the proven ability of immunization to reduce Helicobacter infection in mouse models, the precise mechanism of protection has remained elusive. In this study, we evaluated the role of IL-22 in the vaccine-induced reduction of Helicobacter infection. We first observed that IL-22 production is increased in the stomach during the vaccine-induced reduction of Helicobacter infection. These high IL-22 levels were associated with an increase production of antimicrobial peptides (AMP) such as RegIIIβ by stomach epithelial cells. FACS analysis revealed that the main source of IL-22 is $CD4^+$ T cells especially IL-17 producing cells. In immunized mice, intraperitoneal injection of anti-IL-22 antibodies significantly impaired the vaccine-induced reduction of Helicobacter infection. Importantly, IL22-Fc injections to mice chronically infected with Helicobacter dramatically reduced bacterial load. Finally

cationic AMP (AMPc) were extracted from stomachs and incubated with Helicobacter to evaluate their bactericidal effects. AMPc extracted from stomachs of vaccinated mice or mice injected with IL-22Fc kill Helicobacter *in vitro*. On the contrary, AMPc extracted from stomachs of non-immunized or immunized mice injected with anti-IL-22 antibodies did not kill Helicobacter. Collectively these results demonstrated that IL-22 plays a critical role in vaccine-induced reduction of Helicobacter infection, by inducing the expression of AMPc capable to kill Helicobacter.

F106. Role of Inflammatory Monocytes In Vaccine-Induced Reduction of Helicobacter Infection

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Despite the proven ability of immunization to reduce Helicobacter infection in mouse models, the precise mechanism of protection has remained elusive. In this study, we evaluated the role of inflammatory monocytes in the vaccine-induced reduction of Helicobacter infection. We first showed by using flow cytometry analysis that $CD11b^{+}CCR2^{+}Ly6Clow$ inflammatory monocytes accumulated in the stomach mucosa during the vaccine-induced reduction of Helicobacter infection. To determine whether inflammatory monocytes play a role in the vaccine-induced reduction of Helicobacter infection, these cells were depleted with anti-CCR2 depleting antibodies. Remarkably, depletion of inflammatory monocytes is associated with an impaired vaccine-induced reduction of Helicobacter infection on day 5 post infection. Finally to determine whether inflammatory monocytes have a direct or indirect role, we studied their antimicrobial activities. We observed that inflammatory monocytes produced TNF-α and iNOS, two major antimicrobial factors. Lastly, by using a Helicobacter *in vitro* killing assay, we showed that inflammatory monocytes kill H. pylori. Collectively, these data show that inflammatory monocytes play a direct role in the immunization-induced reduction of Helicobacter infection from the gastric mucosa.

F107. The Role of Microbiota in CT Induction of Intestinal Homeostatic Th17 Cells

Qing Zhao, Trenton R. Schoeb and Charles Elson. University of Alabama at Birmingham, Birmingham, AL

Cholera toxin (CT) has been long used as a mucosal immunogen and adjuvant to induce both systemic and mucosal humoral responses to itself and co-delivered protein antigens. Additionally, CT oral or intracolonic inoculation has recently been shown to expand non-inflammatory Th17 cells in the mucosal sites. We observed an enlarged CT-induced homeostatic Th17 population in the intestine and a correspondingly 10-fold higher CTB specific serum IgG response in $B6.IgA^{-/-}$ mice compared to wild type (WT) B6 mice after CT immunization. Using 16S rDNA Microbiome Sequencing, we found a difference of intestinal microbiota composition between $B6.IgA^{-/-}$ mice and WT B6 mice, particularly a higher prevalence of segmented filamentous bacteria (SFB) in the ileum of $B6.IgA^{-/-}$ mice. We asked whether the combined effect of CT and the intestinal microbiota leads to the amplified intestinal Th17 population and increased humoral responses in $B6.IgA^{-/-}$ mice after CT immunization. Oral administration of vancomycin, which resulted in the ablation of intestinal gram-positive bacteria including SFB, greatly dampened both CT immunogenicity and adjuvanticity. The differential CT responses in $B6.IgA^{-/-}$ mice and WT B6 mice disappeared when we crossed WT B6 mice back to female $B6.IgA^{-/-}$ mice and immunized littermate F2s, which had the same microbiota composition by 16s rDNA sequencing. Using germ free and Altered Schaedler flora (ASF) gnotobiotic mouse models, we confirmed that SFB and other commensal bacteria are actively involved in CT immunogenicity and adjuvanticity after mucosal immunization, providing us with new insights into mucosal vaccine design and development.

F108. Cathelicidin LL-37 Constructs Immune-Stimulatory Microenvironment in Mucosal Immune System

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Intestinal epithelial cells are exposed to microbes and contribute to establish tolerogenic microenvironment for maintaining mucosal homeostasis. In contrast, Peyer's patch, a mucosal immune inductive site, needs to maintain immunostimulatory microenvironment to induce antigen-specific immune responses, although regulatory mechanism is not clearly defined. We here suggest antimicrobial peptide LL-37 as a mucosal immune-stimulatory molecule. LL-37 is known to have chemotactic and modulatory activity on various cells including monocytes, T cells, and epithelial cells in systemic immune system, but its role in gut mucosal area is unclear. We hypothesized that LL-37 may play a role as an immune modulator by skewing the immune environment toward immune-stimulatory conditions. When LL-37-conjugated antigen was administered orally to mice, we found that cell populations of a tolerogenic Peyer's patch environment was shifted to those containing IL-6-secreting $CD11c^{+}$ cells, $CD11c^{+}CD70^{+}$

cells, and Th17 cells capable of evoking a subsequent antigen-specific immune response in both systemic and mucosal immune compartments. Collectively, we conclude that LL-37 is able to not only act as mucosal stimulator but also be utilized as oral mucosal adjuvant.

F109. Non-Invasive Universal Influenza Vaccine Based on Adenovirus Vector

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Current licensed influenza vaccines including injectable tetravalent inactivated virus (TIV) and nasal live attenuated influenza virus (LAIV) aimed at inducing antibody (Ab) responses against viral surface hemagglutinin (HA) and neuraminidase (NA) provide sterile immunity to infection with the same subtypes. The vaccines need to be reformulated every year to include new virus strains. Vaccines targeting viral conserved determinants shared by the influenza A viruses (IAV) offer heterosubtypic immunity (HSI), a broad protection against different subtypes including newly emerging strains. We generated recombinant adenovirus (rAd) vector encoding HA of H5 virus and M2e (rAdH5/M2e) as a vaccine against H5 and other subtypes. Since adenovirus and influenza virus share natural infection route, the respiratory tract, we proposed an intranasal (i.n.) administration of adenovirus-based vaccine as a non-invasive vaccination for safe and effective induction of cross-protective immunity. We found that single i.n. immunization of mice with the vaccine induced long-lasting protection against challenge with H5 or H1 virus subtypes. The cross-protection is associated with induction of Ab responses directed to conserved stalk HA and M2e that can be boosted upon repeated i.n. immunizations. Importantly, i.n. immunization with live vectored vaccine induced specific Ab responses in the gut. The findings support the development of non-invasive i.n. rAd vector encoding HA and M2e as a universal vaccine against different IAV subtypes and implicate nasal vectored vaccines for control of not only respiratory but also enteric pathogens.

F110. Assessment of Immune Responses in Mice After Intranasal Immunization Using Outer Membrane Vesicles Derived from 3 Different Gram-Negative Pathogens

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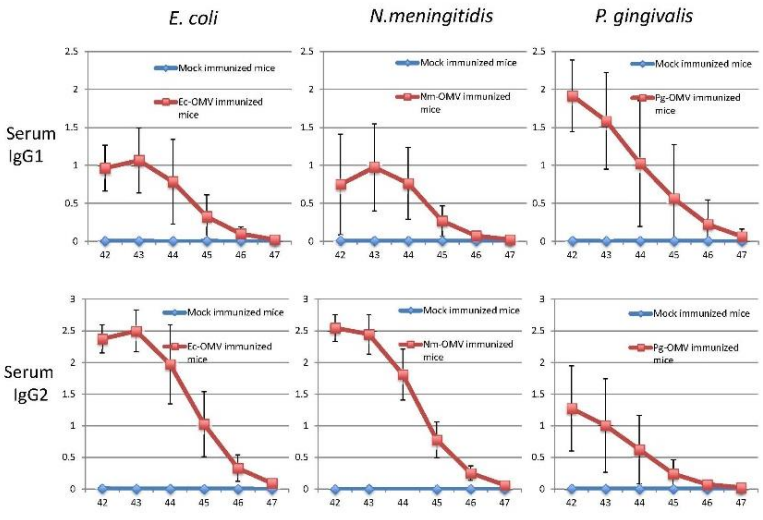

Bacterial outer membrane vesicles (OMVs) were released from the cell surface of Gram-negative bacteria during normal bacterial growth. OMVs contained a wide range of virulence factors and antigens such as LPS and outer membrane proteins. We have already reported strong immunogenicity of OMVs of a periodontal pathogen Porphyromonas gingivalis in an intranasal vaccine mouse model. In the present study, we aimed to compare the immune responses against three different OMVs derived from Escherichia coli, Neisseria meningitidis as well as P. gingivalis. BALB/c mice were intranasally immunized twice by 1 μg of OMVs with Poly(I:C) adjuvant. Serum, nasal wash, saliva, and feces specimens were collected two week after 2nd immunization and analyzed using whole-cell ELISA. Pathogen-specific IgG in sera were successfully elicited in all pathogen OMV models. OMVs of all tested pathogens could also elicit pathogen-specific s-IgA at mucosal surfaces of whole body. In addition, IgG subtype analysis revealed that IgG1 is predominant in P. gingivalis model, while IgG2a is predominant in E. coli and N. meningitidis models. The findings suggest that P. gingivalis OMV vaccine enhances type 2 immunity, in good agreement with the clinical observation that type 2 immunity is predominantly active in periodontal tissues of periodontitis patients. In conclusion, we suggest the general applicability of the intranasal immunization model for eliciting both systemic and mucosal immune responses against pathogens irrespective to the species type, although the type of immunity vary due to the type of OMVs.

F111. Parasite Derived Immunomodulatory Molecules for Prevention and Therapy of Allergy

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The link between reduced incidence of allergic diseases and infection with certain parasites has been repeatedly confirmed in numerous epidemiological and experimental studies. This opens a new field in allergy research aiming to identify immunomodulatory molecules derived from these parasites. We have previously shown that infection with certain protozoa and helminths, such as Toxoplasma gondii or Oesophagostomum dentatum prevented allergic immune responses and airway inflammation in a mouse model of type I allergy. In continuation of these studies we now demonstrate that the application of extracts from these parasites also reduce airway inflammation along with decreased levels of IL-5 and eosinophils in bronchoalveolar lavage. Moreover, we show that upon heat-inactivation the suppressive effect of O.dentatum is stabile, whereas T. gondii extract loses its immunomodulatory potential. Further aim in this study therefore is to identify, characterize and produce T. gondii- and O. dentatum-derived molecules with these immunomodulatory properties. For this purpose the extracts of both parasites are being fractionized and biochemically characterized with different techniques such as normal- and reversed-phase HPLC, 2D gel electrophoresis, followed by MALDI-TOF-MS and ESI-MS/MS in order to identify specific compounds with immunomodulatory/anti-allergic properties. The most promising candidates will be purified/ produced and tested *in vitro* and *in vivo* aiming to use them as adjuvants in future allergy vaccines.

F112. Pathways to Antigen Expression After Oral Delivery of an Adenovirus-Based Vaccine

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Recombinant adenoviruses (rAd) represent an appealing platform for the development of orally delivered vaccines. Nevertheless, the humoral and cell-mediated immune responses elicited by rAd upon oral delivery are currently unsatisfactory. To conceive strategies to optimize their administration by the oral route, a better understanding of the fate of rAd in the digestive tract is a prerequisite. In order to characterize the pathways by which the vector crosses the intestinal epithelium and is captured by sentinel cells, a fluorescent-labelled vector was introduced into ligated intestinal loops of mice. Confocal microscopy revealed that the vector preferentially adheres to M cells in Peyer's patches, and appears to cross the epithelium by transcytosis before being taken up by cells expressing CD11c. To evaluate transgene expression, quantitative RT-PCR was performed in the intestine and in peripheral tissues after intragastric delivery of vector in mice, and revealed that antigen-encoding transcripts were largely confined to the intestine. Finally, in order to provide a global vision of transgene expression in the intestine, whole body bioluminescent imaging after intragastric administration of a rAd encoding firefly luciferase is underway in mice. Identification of the bottlenecks in expression of transgene-encoded antigen should instruct optimization of rAd-based vaccines for oral delivery.

F113. Recombinant Attenuated Salmonella Vaccine (RASV) Vaccination in Neonatal Mice is Enhanced by Antigen Specific Antibodies

Stephen Forbes, Jacquelyn Kilbourne and Roy Curtiss III. Arizona State University, Tempe, AZ

We have developed a live oral recombinant attenuated Salmonella vaccine (RASV) encoding a Streptococcus pneumoniae antigen that is safe, immunogenic, and provides protection to lethal challenge in adult mice and pups (neonates and infants). Vaccinated adult female mice produce antigen-specific antibodies on mucosal surfaces, blood, and, after birthing pups, in their milk. Immunogenicty of the RASV in pups is linked to the mother's immunization state; RASV vaccinated pups of RASV vaccinated mothers resulted in higher levels of antigen-specific antibodies at 3 and 5 weeks post vaccination in fecal pellets and blood compared to RASV vaccinated pups of vector-control vaccinated mothers. The antigen-specific antibodies measured in the pups are produced by the pups, not maternal antibody from the mothers, as demonstrated by the buffer control pups of RASV vaccinated mothers. We evaluated the respective contributions of prenatal and postnatal antibody transfer on the immunogenicity of the RASV in pups by swapping pups from the litters of RASV vaccinated and vector-control mothers. Our findings indicate that immunity in pups is associated more strongly with receiving postnatal antibodies than prenatal antibodies, and we propose that the postnatal SIgA against the RASV is responsible. These findings have several implications for successful vaccination of neonates.

F114. The Role of Nanoparticle Surface Charge in the Generation of Mucosal and Systemic Antibody Responses Following Pulmonary Delivery

Catherine Fromen[1], Gregory Robbins[2], Tojan Rahhal[2], Marc Kai[3], Tammy Shen[2], J. Christopher Luft[2], Jenny Ting[2] and Joseph DeSimone[2,3]. [1]University of Michigan, Ann Arbor, MI; [2]University of North Carolina at Chapel Hill, Chapel Hill, NC; [3]North Carolina State University, Raleigh, NC

Nanoparticles have the potential to be excellent mucosal vaccine carriers, as they can diffuse through mucosa, associate with antigen presenting cells (APCs), and drain to regional lymph nodes, thus enhancing local humoral and cell-mediated mucosal protection. Varying nanoparticle properties, such as size, shape, and surface chemistry, can facilitate pathogen mimicry and co-delivery of relevant antigens and adjuvants. However, optimal nanoparticle properties have not been identified for mucosal vaccine carriers. Using the nano-molding technique Particle Replication In Non-wetting Templates (PRINT), we isolated the role of nanoparticle surface charge in two otherwise identical formulations of nanoparticles, which were covalently attached to a model antigen ovalbumin. Both formulations were readily internalized by lung APCs and drained to mediastinal lymph nodes. However, cationic nanoparticles induced more potent APCs than anionic nanoparticles and upregulated co-stimulatory receptor molecules, cytokines, and chemokines. Pulmonary vaccination using cationic nanoparticles resulted in enhanced systemic and lung antibody titers, stemming from increased germinal center B cell formation and $CD4^+$ T cell activation. Our results indicate that nanoparticle surface charge is a main variable in driving T cell dependent antibody responses *in vivo* and suggest the continued role of cationic nanoparticle platforms for engendering potent mucosal responses.

F115. Targeting Yeast Ghosts to Epithelial CD13 Promotes Mucosal Immunity

Bert Devriendt, Kim Baert, Vesna Melkebeek, Martine De Vos and Eric Cox. University of Ghent, Merelbeke, Belgium

Most pathogens invade the host at mucosal surfaces. Protecting against gut-dwelling pathogens requires intestinal immunity induced by oral vaccination; however the road towards efficient oral immunization remains challenging. Selective targeting of antigens and their encapsulation into microparticles have been envisaged to tackle the main hurdles associated with oral vaccination, i.e. the poor uptake of vaccine antigens, their degradation by the harsh gastrointestinal (GI) environment as well as the tolerogenic microenvironment permeating the GI tract. Recently, our group explored targeting absorptive enterocytes, which vastly outnumber M cells, to promote antigen uptake by the GI tract. Here, we demonstrate that antibody-mediated targeting to the epithelial, endocytotic receptor CD13 on porcine and human small intestinal enterocytes facilitates antigen uptake by these cells and results in an enhanced mucosal immunity. We further expand these findings by combining targeting to CD13 and microencapsulation of antigens through surface decoration of antigen-loaded yeast ghosts with CD13-specific antibodies. Yeast ghosts are comprised of a β-glucan shell and have an inherent mucosal adjuvanticity. CD13 targeting led to an enhanced uptake of these yeast ghosts by enterocytes and dendritic cells and boosted the functional maturation of the latter. This novel combinatorial approach is anticipated to accelerate oral vaccine development.

F117. Dietary Retinol is Required for Both Oral and Intranasal Antileishmanial Vaccine Efficacy

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Cutaneous leishmaniasis is a tropical disease with clinical manifestations ranging from localized skin ulcer to disfiguring chronic lesions. Despite the tremendous effort in research using parenteral vaccination strategies, no leishmanial vaccine has yet been licensed for human use. We have previously shown that mucosal vaccination with an otherwise disease-promoting subcutaneous vaccine composed of whole antigens of Leishmania amazonensis parasites (LaAg) was protective to mice. As a natural source of retinoic acid which is knowingly involved in mucosal T reg cell differentiation, the role of dietary retinol in mucosal vaccine efficacy was investigated. Thus, BALB/c mice subjected for life to dietary retinol restriction ($Retinol^-$) or supplementation ($Retinol^+$) were given two doses of LaAg either by the oral or intranasal routes. One week after the boost immunization, mice were challenged in the footpads with living parasites. Lesion development was monitored for 60 days, when the parasite burden and cytokine profile at the infection site were evaluated. We found that non-vaccinated $Retinol^-$ mice were more resistant to infection than $Retinol^+$ mice, as seen by their smaller lesions and lower parasite burden. Interestingly, only $Retinol^+$ animals responded to vaccination, irrespective of the mucosal vaccination route employed. Protection of $Retinol^+$ mice was accompanied by increased IL-12 and reduced IL-4 at the infection site, compatible with increased Th1 response, and higher levels of TGF-β compared to Retinol-

mice, suggestive of local T regulatory response. Altogether, these results show that dietary retinol influences peripheral immunity to leishmania parasites, and is required for both oral and intranasal vaccine efficacy against leishmaniasis.

F118. A Germinal Center Immunomodulator: The CTA1-DD Adjuvant Acts on Follicular Dendritic Cells and Potentiates Follicular T Helper Cell Functions

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The non-toxic CTA1-DD adjuvant, ADP-ribosylates Gsα in the cell membrane of targeted cells and strongly promotes large and numerous germinal centers (GC) following mucosal or systemic administration. This leads to greatly enhanced antibody responses and memory B cell development. In dissecting the mechanism of activation, we observed that the enhanced GC reaction was critically dependent on activation of complement and subsequent binding to complement receptor 2 on follicular dendritic cells (FDC). Therefore, we developed a mouse model expressing the GFP marker gene under the CD21 promotor to detect the FDC network, which allowed us to analyses the impact of CTA1-DD on GC formation in greater detail. FDC and other stromal cells produce chemokines and molecular binding partners, and in this way promote interactions between B cells and follicular T helper cells (Tfh). After sorting stromal cells populations we conducted a gene-expression analysis. We found that, CTA1-DD induced transcriptional changes in FDC and increased GC responses in adult and infant mice. Thus, CTA1-DD modulated the follicular environment and appeared to circumvent the intrinsic GC B cell and Tfh impairment seen in infants. We, therefore, conclude that cell targeted CTA1-DD adjuvant augments the generation of Tfh and promotes FDC functions to potentiate the GC reaction not only in adults, but also in infants. We think this could be exploited to improve vaccine safety and efficacy in adults as well as young children.

F119. Oral Immunization Induces an Effective Mucosal Immune Response that Protects Mice Against Intravaginal HSV-2 Challenge

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Immunity against genital Herpes Simplex Virus 2 (HSV-2) infection is dependent on local memory immune responses in the genital tract. Oral immunization can induce mucosal immune responses in the genital tract but has not been tested against viral STIs. Here we describe the mucosal immune responses elicited by an oral vaccine that protects mice against intravaginal challenge from lethal HSV-2 infection. Control of HSV-2 was attributed to HSV-2-specific IgG and IgA in the genital mucosa together with CD4 and CD8 T cells recruited to the genital epithelia. Furthermore, combining this vaccine with vaginal application of DNFB to induce transient local inflammation led to the recruitment of CD8 tissue resident memory cells in the genital epithelia that controlled new infection with HSV-2. Thus, this represents the first oral vaccine that can protect mice against lethal intravaginal HSV challenge.

F120. Functional Significance of Antibody Isotype in Protection Against HIV

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The constant heavy chain-domain affects antibody affinity and fine specificity, challenging the paradigm that the only variable regions contribute to antigen binding. Hence, the broadly-neutralizing anti-HIV-1 2F5-IgG1 transformed into IgA2 had higher antigen-affinity, different antiviral activities, although both isotypes acted synergistically to neutralizing HIV-1 transfer from Langerhans to $CD4^+$T cells, one initial step in mucosal HIV-1 entry. Isotype differences correlate with unique epitope specificity (Tudor, PNAS, 2013). Here, we investigate the exclusive role of CH1-domain in antibody specificity/functions. We constructed IgG1-Fabs from two IgA1-Fabs specific for gp41 we previously constructed from vaginal B cells from highly HIV-exposed individuals that remains seronegative (ESN) despite unprotected intercourse with HIV-positive partners (Tudor, Mucosal Immunol, 2009). Fab-IgA2 and IgG1 differ only by their CH1. The two IgA1-Fabs had higher affinity for gp41 clade-B but also clade-A, and C cross-clade, compared to corresponding IgG1-Fabs. Functionally, IgA1-Fabs neutralized more robustly $CD4^+$T cell clade-B-HIV-1 infection and transfer from Langerhans to $CD4^+$T cells. Fabs also strongly neutralized clade-A and C-HIV-1 infection, demonstrating cross-clade antiviral activities. Epitope mapping performed by random peptide library screening and in-silico docking on gp41-envelope indicate that IgAs and corresponding IgGs recognize distinct 3-Dimensional-epitopes. Altogether, these studies clearly

demonstrate that the exclusive CH1-region contributes to shape antibody epitope specificity, affinity and functional activities. More, IgA and IgG can have different but complementary anti-viral activities. We now design a vaccine based on protective (ESN) IgG/A-epitopes for raising a mucosal IgA/IgG-mixed response with complementary activities efficient in blocking sexual HIV-1-transmission. Such strategy should apply to other sexually-transmitted infections.

F121. New Vaccination Strategy Against Helicobacter Pylori
Florian Anderl, Maria Andree, Kathrin Straubinger and Markus Gerhard. ImevaX GmbH, München, Germany

The colonization of the human stomach with Helicobacter pylori represents one of the most common infectious diseases that affects 50% of the world's population. H. pylori infection plays a causative role in the development of chronic gastritis and cancer. Although the infection elicits a strong inflammatory response, the bacterium cannot be cleared by the immune system, and establishes a persistent infection due to potent immune evasion mechanisms. In the era of rising antibiotic resistances targeting these factors by vaccination is a promising strategy to treat H. pylori. We previously described H. pylori gamma-glutamyltranspeptidase (HPgGT) as an immune evasion factor that impairs T-lymphocyte proliferation. Our newly developed vaccine (IMX101) contains recombinant HPgGT combined with the H. pylorisurface protein adhesion A (HpaA), both administered via the mucosal and systemic routes in combination with a potent mucosal adjuvant. Immunization of H. pylori infected mice elicits a strong humoral immune response leading to inactivation of HPgGT. Concomitantly, a cellular immune response is generated against the bacterial surface, reducing bacterial load within the stomach. IMX101 represents an innovative vaccination approach that addresses the immune evasion mechanism of H. pylori. The preclinical results are highly promising, especially with regard to the increasing antibiotic resistances.

F122. Self-Adjuvanting Virus-Like Particles for Sublingual Vaccination Against Group A Streptococcus Elicit Protective Immunity in Systemic and Mucosal Sites
IL Gyu Kong[1,2], Arjun Seth[3], Jin-Young Yang[2], Su-Hyun Lee[2], Yong-Soo Lee[2], Yeji Kim[2], Nani Wibowo[3] and Mi-Na Kweon[2]. [1]Hallym University Sacred Heart Hospital, Anyang, South Korea; [2]University of Ulsan College of Medicine, Seoul, South Korea; [3]University of Queensland, St. Lucia, Australia

Group A streptococcus (GAS) is an important pathogen, which infects primarily in oropharynx and induces mortality and morbidity. Here we introduced virus-like particle (VLP) to GAS vaccine via sublingual route to induced protective immunity. Peptide antigens from GAS M-surface protein can confer protection against infection; however, it still remains a challenge to adapt vaccine candidate peptides for immunogenic mucosal vaccines. In this study, a modular murine polyomavirus (MuPyV) VLP was engineered to display a GAS antigenic peptide, J8i. Heterologous modules containing one or two J8i antigen elements were integrated with the MuPyV-VLP, and produced using microbial protein expression, standard purification techniques and *in vitro* VLP assembly. Sublingual administration with VLP-J8i resulted in high level of IgG antibody in the serum with good balance of Th1 and Th2 immune response. Of note, sublingual vaccination with VLP-J8i induced high level of IgA antibody in saliva. Moreover, saliva isolated from mice immunized with VLP-J8i demonstrated protective immune response against GAS *in vitro*. This study shows that the potential of sublingual vaccination as a favorable mucosal vaccine formula for VLP-J8i which can potentially block horizontal transmission by eradicating pharyngeal colonization of GAS.

F123. A Novel Nasal Multiplex Dna-Gold Nanorod Vaccine Against Human Papillomavirus Infection
Wenqin Li. University of Strathclyde, Glasgow, United Kingdom

Human papillomavious (HPV) causes cervical cancer, which overwhelmingly affects women in many developing countries. Screening is not provided in these regions, plus the two main protein vaccines are unaffordable and undeliverable in their existing health programs. We investigated the possible DNA vaccine adjuvant vehicle for safe and widespread vaccine delivery and the development of integrated systems to screen women for persistent HPV infection through the intra-nasal immunization. As the significant expression of 16L1 viral gene in both cell lines and mouse tissues and their reasonable high immunogenicity compared with VLP in mice model, polymer coated gold nanorods driven DNA vaccine could be considered as a promising nasal vaccine against HPV infection. These advantages will further drive down the costs for vaccination and will make male vaccination affordable in the UK and other developed countries in the future. It will likely persuade health authorities in resource-poor countries

to implement vaccination as well.

RESPIRATORY VIRUS INFECTIONS

OR.57. Type I Interferons Produced by Alveolar Macrophages are Important Drivers of Lung Inflammation During Respiratory Syncytial Virus (RSV) Infection

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Respiratory syncytial virus (RSV) is the leading cause of severe lower respiratory tract infections in infants. Type I interferons (IFNs) are crucial for protection from RSV. To identify the cellular source of type I IFNs following RSV infection, we utilized a mouse strain in which green fluorescent protein (GFP) is expressed under control of the Ifna6 promoter. Upon intranasal RSV infection, GFP was only detected in alveolar macrophages (AMs). We confirmed by mRNA analysis that AM are the major source of type I IFNs following RSV and showed that their response critically depend on MAVS, the adaptor protein for cytosolic RIG-I-like receptors. Like type I IFN receptor-deficient mice, MAVS-deficient mice displayed a loss of viral control that correlated with a marked deficiency in the recruitment of inflammatory monocytes. The latter could be restored by administration of CCL2 to the lungs and, surprisingly, led to nearly normal control of viral infection. Thus, AMs act as the primary initiators of immunity to RSV, secreting type I IFNs that induce the production of monocyte chemoattractants. Recruited monocytes serve as crucial and non-redundant effectors of innate resistance to RSV, indicating a hitherto unappreciated cell-extrinsic mechanism of type I IFN action in antiviral immunity.

OR.58. Innate Lymphoid Cell Are Sequentially Recruited to the Lungs Following Influenza A Virus Infection in Mouse

Isabelle Meunier and Martin V. Richter. Centre de Recherche du CHUS, Sherbrooke, QC, Canada

Influenza A virus (IAV) induces an inflammatory response in the airways that triggers the accumulation of immune cells. Innate lymphoid cells (ILC) of group 2 (ILC2) accumulate in the lungs following IAV infection. However, whether other ILC subtypes are present and the factors contributing to their accumulation in the airways like recruitment and/or proliferation remain unknown. Infection of mice with the PR8 virus (H1N1) induced the sequential accumulation of every ILC subset in the lungs. NK cells began to accumulate at day 3, followed by ILC2 and NCR22 at days 4-6, ILC1 and ILC3 at day 6, and LTi at day 8. These ILCs were activated as they expressed the activation markers CD69 and Sca1 and produced cytokines. Using the X31 virus (H3N2) and the more virulent MAp2009 (H1N1), we demonstrated that viral subtype and virulence has little influence on ILC accumulation. However, viral dose was directly correlated to the magnitude of the accumulation. Analysis of Ki-67 expression revealed that 25% of the different ILC subsets proliferate in the lungs, correlating with high levels of IL-2 and IL-33 transcripts. The number rose to 75% for ILC2, suggesting that cellular expansion is a major factor for their accumulation, while others ILCs are probably recruited. Finally, we detected high transcript levels of CCR2 and CXCR4 in the ILC1 and ILC2 population. Studies are performed to confirm the importance of the corresponding chemokines for ILC migration.

OR.59. Early Interleukin-6 Signaling is a Critical Driver of Immune Regulation During Respiratory Syncytial Virus Infection

Chloe Pyle and James Harker. Imperial College London, London, United Kingdom

The inflammatory cytokine IL-6 is known to have diverse roles in both innate and adaptive immunity. In humans severe infection with Respiratory Syncytial Virus (RSV) is characterized by elevated levels of IL-6, however the IL-6 174-C/C haplotype, which favors lower IL-6 production, is associated with increased risk of both RSV and Rhinovirus infections. Intranasal infection of adult BALB/c mice with RSV A2 resulted in increased IL-6 concentrations in the airways, lung and circulation, peaking at 1 day post infection but detectable for at least two weeks post infection. Treatment of RSV infected mice with a neutralizing anti-IL6 antibody, starting at day -1 for two weeks, resulted in significantly increased disease severity compared to controls, corresponding with increased numbers of virus specific $CD8^+$ T cells in the airways and lungs. Importantly however it did not affect peak viral load or viral clearance. Increased disease severity could be recapitulated by neutralizing IL-6 early (days -1 to +3 post RSV) but not late (days +5 to +14 post RSV). Early IL-6 acted through promotion of both local and systemic levels of the immunoregulatory cytokines IL-10 and IL-27. Loss of IL-6 resulted in reduced numbers of $IL\text{-}27^+$ lung macrophages by 24 hours and $IL\text{-}10^+$ $CD4^+$ T cells by 96 hours post

infection; phenotypes that could be recapitulated by using anti IL-6R blocking antibodies. Overall, we found that while IL-6 is considered a pro-inflammatory cytokine, during RSV infection it plays a key early role in promoting an immune-regulatory response critical in the resolution of inflammation.

OR.91. Inhibition of miR-93 Promotes Interferon Effector Signaling to Suppress Influenza A Infection by Upregulating JAK1
Jin Hou. Institute of Immunology, Shanghai, China

Type I interferon (IFN) plays critical roles in host antiviral innate immune response, and regulation of IFN effector signaling has attracted much attention. microRNAs are important regulators of innate immune response, but microRNAs regulated IFN effector signaling still need further investigation. Here, during influenza A virus (IAV) infection, we found that miR-93 expression was significantly down-regulated in Alveolar epithelial type II cells (AT2) upon IAVs infection through RIG-I/JNK pathway. Inhibition of miR-93 was found to promote host antiviral innate response by facilitating type I IFN effector signaling, and JAK1 was identified to be directly targeted by miR-93. Importantly, *in vivo* administration of miR-93 antagomiR significantly inhibited miR-93 expression and markedly suppressed IAVs infection, which in turn prevented the death of IAVs infected mice. Hence, the inducible down-regulation of miR-93 suppress IAVs infection by up-regulation IFN-JAK-STAT effector pathway, and *in vivo* inhibition of miR-93 bears considerable therapeutic potential for suppressing IAVs infection.

F124. Coadministration of Hedera Helix L. Extract Enabled Mice to Overcome Insufficient Protection Against Influenza A/PR/8 Virus Infection Under Suboptimal Treatment with Oseltamivir.
Eun Hye Hong[1], Bo-Eun Kwon[1], Sang-Gu Yeo[2] and Hyun-Jeong Ko[1]. [1]Kangwon National University, Chuncheon, South Korea; [2]Korea Centers for Diseases Control and Prevention, Cheongju, South Korea

Several anti-influenza drugs that reduce disease manifestation exist, their efficacy is limited by the emergence of drug-resistant influenza viruses. To overcome these limitations, we assessed the therapeutic strategy of enhancing the antiviral efficacy of an existing neuraminidase inhibitor, oseltamivir, by co-administering with the leaf extract from Hedera helix L, commonly known as ivy. Ivy extract has anti-inflammatory, antibacterial, antifungal, and antihelminthic properties. We investigated its potential antiviral properties against influenza A/PR/8 virus in a mouse model with suboptimal oseltamivir that mimics a poor clinical response to antiviral drug treatment. Suboptimal oseltamivir resulted in insufficient protection against PR8 infection. Oral administration of ivy extract with suboptimal oseltamivir increased the antiviral activity of oseltamivir. Ivy extract and its compounds, hederasaponin F, reduced the cytopathic effect in PR8-infected A549 cells in the presence of oseltamivir. Co-administration of the fraction of ivy extract that contained the highest proportion of hedrasaponin F with oseltamivir decreased pulmonary inflammation in PR8-infected mice. Inflammatory cytokines and chemokines, including tumor necrosis factor-alpha and chemokine ligand 2, were reduced by treatment with oseltamivir and the fraction of ivy extract. Analysis of inflammatory cell infiltration in the bronchial alveolar of PR8-infected mice revealed that $CD11b^{+}Ly6G^{+}$ and $CD11b^{+}Ly6Cint$ cells were recruited after virus infection; co-administration of the ivy fraction with oseltamivir reduced infiltration of these inflammatory cells. In a model of suboptimal oseltamivir treatment, co-administration of ivy extract fraction that includes hedrasaponin F increased protection against PR8 infection that could be explained by its antiviral and anti-inflammatory activities.

F125. Investigating the Interaction of SP-A and SP-D with Respiratory Syncytial Virus in Human Bronchial Epithelial Cells
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Respiratory syncytial virus (RSV) is the leading cause of bronchiolitis and hospitalization of infants in developed countries. Surfactant proteins A and D (SP-A and SP-D) are important innate immune molecules expressed throughout the human respiratory tract and present in pulmonary surfactant which covers the alveolar epithelium. Through binding carbohydrates on the surface of pathogens, they enhance their neutralization, agglutination and clearance. They also modulate the lung inflammatory immune response. Previous reports in SP-A and SP-D deficient mice have suggested their importance in promoting clearance of RSV from the murine respiratory tract. The aim of this work was to characterize the interaction of SP-A and SP-D with RSV in a human model and their capacity to prevent infection of immortalized human bronchial epithelial cells (AALEB) by an established clinical strain of RSV-A. RSV infection and replication was determined using RT-qPCR. Pre-incubation of RSV with both SP-A and SP-D reduced levels of infection of AALEB cells in a dose-dependent manner. Pre-incubation with 10µg/ml of SP-A decreased RSV infection by

57.8% (n=3, P<0.01). Pre-incubation with 10µg/ml of SP-D decreased RSV infection by 53.7% (n=3, P<0.01). The capacity of SP-A and SP-D to bind RSV fusion (F) protein has been evaluated by surface plasmon resonance and ELISA. We have shown in this human model that both SP-A and SP-D can directly prevent infection of human AALEB cells with RSV A. We speculate that our recombinant versions of SP-A and SP-D may have therapeutic potential and protect susceptible infants to RSV infection.

F126. Pulmonary Cellular Immune Responses to RSV Infection is Associated with Concomitant Lung Damage and Differential Lymphocytic and Macrophage Activation in Different Age Groups of Hosts

Kainath Durre, Omar Qureshi and Mahboob Qureshi. Touro University Nevada College of Osteopathic Medicine, Henderson, NV

RSV infection causes bronchiolitis and pneumonia in young infants with increased mortality in this age group. Survivors predispose to susceptibility to asthma. In our present study, using a mouse model of BALB/C and $NKT^{-/-}$ mice, we examined the pulmonary responses to RSV infection in the very young hosts as opposed to relatively older hosts. We also examined the effects of exogenous osteopontin (OPN) administration on the alveolar cellular constitution by direct microscopy and flow cytometry and the role of NKT cells in this process. Pulmonary damage was assessed by measuring the Lactic Dehydrogenase (LDH) levels in the BALF by ELISA. Microscopy revealed a decreased lymphocyte response in the bronchoalevolar lavage fluid (BALF) of RSV-infected-4-day- old pups as compared to that of -14- and -20-day-old mice; but an enhanced neutrophil response, which was associated with increased LDH levels. Flow cytometry revealed that administration of OPN had down regulatory effects on lung lymphocyte ($CD4^+$ and $CD8^+$) responses in all age groups with proportional up-regulation of lung macrophage ($CD11b^{med/hi}$, $CD11C^{lo/-}$) responses. This effect was less pronounced in the older age groups. Additionally, activation of macrophages, as indicated by MHCII expression, was NKT dependent. Moreover, RSV- infection significantly inhibited lung $CD8^+$ T cell activation as indicated by decreased CD44 expression, which was recovered by OPN administration. Understanding the underlying mechanisms of RSV-infection induced differential immune responses and OPN-mediated immunomodulations in different age groups may help develop newer therapeutic strategies.

W128. Compensatory roles of $CD8^+$ T cells in immune regulation in the gut with non-functional $CD4^+$ Tregs

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$CD4^+$ regulatory T cells are known as master regulators that maintain the immune suppressive environment in the intestine. $CD4^+$ Tregs need to migrate from the mucosal periphery into the draining lymph node via CCR7 to exert their suppressive effects. Here we investigated whether CCR7 deficiency resulted in failure of immune suppression in dextran sulfate sodium-induced colitis. Intestinal inflammation was not exacerbated in the absence of CCR7. Expression of IL-10, a representative suppressive cytokine, was enhanced in CCR7KO $CD8^+$ T cells. Colon CCR7KO $CD8^+$ T cells reduced the activation of $CD4^+$ T cells. Plasmacytoid dendritic cells were also slightly increased during intestinal inflammation in the absence of CCR7. These results suggest that $CD8^+$ T cells and dendritic cells have compensatory roles in immune regulation in the gut with non-functional $CD4^+$ Tregs.

F.24 Soluble ST2 as a Monitoring Marker of Inflammatory Bowel Disease Evolution

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ST2/IL33 system has been related to IBD. Previously we proposed soluble ST2 (sST2) as a potential severity, inflammation and prognosis biomarker in UC patients. Our aim was to examine whether serum and mucosa ST2 content can be predictive markers of response to treatment, disease activity and outcome.

Prospective study of 28 active IBD patients (24 UC, 4 CD); grouped according to therapy type (conventional or biological (IFX)) and response (responders or non-responders). Colonoscopic biopsy was collected at baseline and 6m (patients with IFX at 14th w), and blood and stool samples at baseline, 1, 3, 6 and 12m. Serum and mucosal ST2, and fecal calprotectin (FC) content were determined by ELISA and correlated to clinical and endoscopic activity. Intestinal ST2 distribution was evaluated by confocal microscopy. Mann-Whitney test and Spearman correlations (Rs) were applied (p<0.05).

Twenty-four patients completed the follow-up year protocol and 4 declined the evaluation at twelve-months. During the study, 5 patients changed therapy towards biological, 3 showed reactivation after 6m, and 2 after switching to IFX. Serum sST2 levels significantly decrease only in responder patients and, in those non-responders rescued with IFX. However, FC levels significantly decreased in responder and non-responder patients. Serum sST2 levels correlate with endoscopic and clinical score ($Rs=0.64$, $p<0.0001$; $Rs=0.51$, $p<0.0001$), mucosa ST2 and FC ($Rs=0.65$, $p=0.0001$; $Rs=0.45$, $p<0.0001$). Changes in ST2 cellular distribution were observed in responder patients.

sST2 can be considered as a useful biomarker in predicting IBD patient outcome in the clinical practice and potentially related to mucosa healing.

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OR.1, OR.2, OR.3, OR.81, OR.82, OR.83, OR.84, W.7, W.8, W.9, W.10, W.11, W.12, W.13, W.14, W.15, W.16, W.17, W.18, W.19, W.20, W.21, W.22, W.23, W.24, W.25, W.26, W.27

Effector T Cells and Cytokines
OR.41, OR.42, OR.43, OR.44, W.28, W.29, W.30, W.31, W.32, W.33, W.34, W.35, W.36, W.37, W.38, W.39, W.40

Epithelial Cells in Innate Immunity
PS.5, OR.5, OR.6, OR.7, OR.8, OR.20, OR.77, OR.78, OR.79, OR.80, W.41, W.42, W.43, W.44, W.45, W.46, W.47, W.48, W.49, W.50, W.51, W.52, W.53, W.54, W.55, W.56, W.57, W.58, W.59, W.60, W.61, W.62, W.63, W.64, W.65, W.66, W.67, F.128

Food Allergy
OR.53, OR.54, W.68, W.69, W.70, W.71, W.72, W.73, W.74, W.75, W.76

HIV
OR.13, OR.14, OR.15, OR.16, W.77, W.78, W.79, W.80, W.81, W.82, W.83, W.84, W.85

Host-Microbiota Interactions
PS.3, OR.29, OR.30, OR.31, OR.32, OR.45, OR.46, OR.47, OR.48, OR.92, W.86, W.87, W.88, W.89, W.90, W.91, W.92, W.93, W.94, W.95, W.96, W.97, W.98, W.99, W.100, W.101, W.102, W.103, W.104, W.105, W.106, W.107, W.108, W.109, W.110, W.111, W.112, W.113, W.114, W.115, W.116, W.117, W.118, W.119, W.120, W.121, W.122, W.123, W.124, W.125, T.1, T.2, T.3, T.4, T.5, T.6, T.7, T.8, T.9, T.10, F.129

Immune Cell Migration
T.11, T.12, T.13, T.14, T.15, T.16, T.17, T.18, T.19, T.20

Immunology of Asthma – Basic
OR.33, OR.34, OR.35, OR.36, T.21, T.22, T.23, T.24, T.25

Immunology of the Eye
T.26, T.27

Immunology of the UG Tract
OR.25, OR.26, OR.27, T.28, T.29, T.30

Inflammatory Bowel Disease - Clinical
T.112, T.113, T.114, T.115, T.116, T.127

Inflammatory Bowel Disease – Basic
PS.1, OR.49, OR.50, OR.51, OR.52, OR.69, OR.70, OR.71, OR.72, W.126, T.31, T.32, T.33, T.34, T.35, T.36, T.37, T.38, T.39, T.40, T.41, T.42, T.43, T.44, T.45, T.46, T.47, T.48, T.49, T.50, T.51, T.52, T.53, T.54, T.55, T.56, T.57, T.58, T.59, T.60, T.61, T.62, T.63, T.64, T.65, T.66, T.67, T.68, T.70, T.71, T.72, T.73, T.74, T.75, T.76, T.77, T.78, T.79, T.80, T.81, T.82, T.83, T.84, T.85, T.86, T.88, T.89, T.90,

T.91, T.92, T.93, T.94, T.95, T.96, T.97, T.98, T.99, T.100, T.101, T.102, T.103, T.104, T.105, T.106, T.107, T.108, T.109, T.110, T.111, F.127

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OR.85, OR.86, OR.87, OR.88, T.117, T.118, T.119, T.120, T.121, T.122, T.123, T.124, T.125, F.1, F.2

MALT + Organogenesis
OR.62, F.3, F.4

Monocytes and Macrophages
OR.17, OR.18, OR.19, OR.64, OR.65, OR.66, OR.67, OR.68, F.5, F.6, F.7, F.8, F.9, F.10, F.11, F.12, F.13, F.14, F.15, F.16, F.17, F.18, F.19, F.20, F.21, F.22

Mucosal B Cells
OR.9, OR.10, OR.11, OR.12, OR.90, T.128, F.23, F.25, F.26, F.27

Mucosal Immunology in the Neonate
OR.93, OR.94, OR.95, OR.96, F.28, F.29, F.30, F.31, F.32, F.33, F.34, F.35

Mucosal Infections
OR.22, OR.23, OR.24, OR.28, OR.60, W.127, F.36, F.37, F.38, F.39, F.40, F.41, F.42, F.43, F.44, F.45, F.46, F.47, F.48, F.49, F.50, F.51, F.52, F.53, F.54, F.55, F.57, F.58, F.59, F.60, F.61, F.62, F.63, F.64, F.65, F.66, F.67, F.68, F.69, F.70, F.71, F.72, F.73, F.74, F.75, F.76, F.77, F.78, F.79

Mucosal Tolerance
OR.21, OR.37, OR.38, OR.39, OR.40, OR.89, F.80, F.81, F.82, F.83, F.84, F.85, F.86, F.87, F.88, F.89, F.90, F.91, F.92, F.93, F.94, F.95, F.96, F.97

Mucosal Vaccines
PS.2, OR.73, OR.74, OR.75, OR.76, T.126, F.98, F.99, F.100, F.101, F.102, F.103, F.104, F.105, F.106, F.107, F.108, F.109, F.110, F.111, F.112, F.113, F.114, F.115, F.117, F.118, F.119, F.120, F.121, F.122, F.123

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