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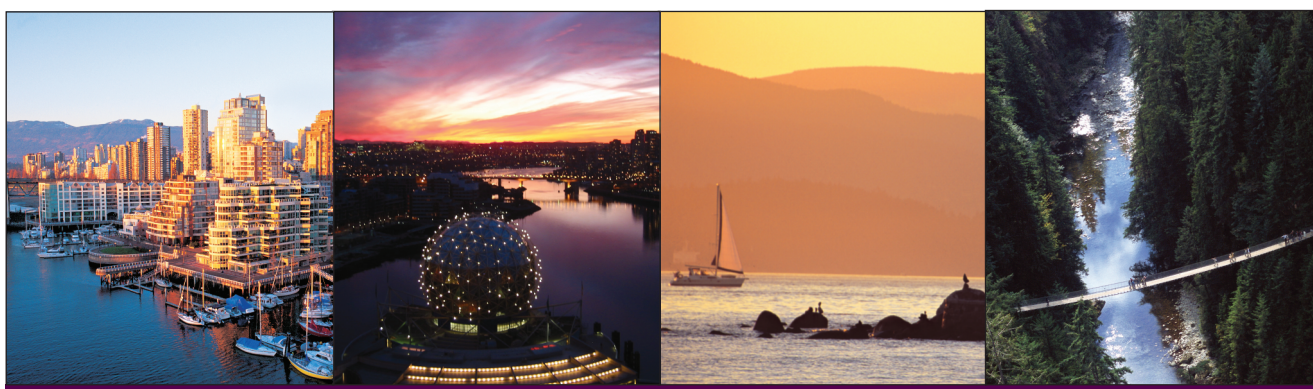
Abstract Supplement



ICMI13
International Congress of Mucosal Immunology

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Oral Presentations: Wednesday, July 17

OR.1. Dendritic Cell Expression of IRF4 is Critical for Intestinal CD103$^+$CD11b$^+$ DCs Survival and Th17 Cell Development

Emma Persson[1], Heli Uronen-Hansson[1], Monika Semmrich[1], Aymeric Rivollier[1], Karin Hägerbrand[1], Jan Marsal[2], Sigurdur Gudjonsson[2], Ulf Håkansson[2], Boris Reizis[3], William Agace[1]. [1]Lund University, Lund, Sweden; [2]Skåne University Hospital, Lund, Sweden; [3]Columbia University Medical Center, New York, NY

The small intestine faces the immunological challenge of maintaining a balance between tolerating commencal bacteria and food antigen and mounting effective immune responses against pathogens. Dendritic cells (DCs) play key roles in regulating innate and adaptive immune responses and as such are centrally involved in regulating gut homeostasis. The majority of conventional DCs in the small intestine (SI) express the integrin CD103. These are migratory DCs, which shuttle constitutively from the intestine to the mesenteric lymph nodes (MLN). In the MLNs they play key roles in priming immune responses to luminal antigens. CD103$^+$ SI DCs can be divided into two subsets based on their expression of CD11b, CD103$^+$CD11b$^+$ DCs being the dominant population in the lamina propria. Both these populations derive from the same precursor of conventional DCs (pre-DC). However, whereas CD103$^+$CD11b- SI-DCs share a distinct differentiation program with CD8α-like DCs, CD103$^+$CD11b$^+$ SI-DCs develop independently of these and the ontogeny of CD103$^+$CD11b$^+$ SI-DCs has not been examined. We have discovered that CD103$^+$CD11b$^+$ LP-DCs share phenotypical characteristics with splenic CD4$^+$ DCs and that they, similar to splenic CD4$^+$ DCs are developmentally dependent on the transcription factor interferon regulatory factor 4 (IRF4). Using a mouse model that depletes IRF4 specifically in DCs, we find that, through a mechanism involving interleukin-6, IRF4 dependent DCs are critical for the generation of intestinal Th17 but not Th1 or Foxp3$^+$ Tregs and for optimal T cell responses to oral antigen in draining MLNs. Finally we identify a major subset of IRF4 expressing CD103$^+$SIRPα$^+$ DCs in the human SI that we propose are the human equivalent of murine SI CD103$^+$CD11b$^+$ DCs.

OR.2. Tissue-Specific Homeostatic Properties of Human Intestinal Antigen Presenting Cells: Dendritic Cells and Macrophages in the Ileum Versus Colon

Elizabeth Mann[1], Hafid Al-Hassi[1], Jonathan Landy[2], David Bernardo Ordiz[1], Nicholas English[1], Simon Peake[2], Ripple Man[2], Gui Han Lee[2], Nuha Yassin[2], Ailsa Hart[2], Stella Knight[1]. [1]Imperial College London, Harrow, United Kingdom; [2]St. Mark's Hospital, Harrow, United Kingdom

Background: Dendritic cells (DC) and macrophages (Mφ) mediate intestinal immune tolerance. However, distinguishing intestinal DC from Mφ is problematic; information in humans is scarce. We compared human gut DC and Mφ within ileum and colon. Methods: Human DC and Mφ were isolated from colonic and ileal biopsies and characterised by flow cytometry. DC generated T cell responses in a mixed leucocyte reaction. Results: Intestinal DC were CD103$^+$CX3CR1-; Mφ were CD103-CX3CR1$^+$. Mφ expressed more CD40, more Toll-like receptors 2/4, and produced more TNFα than DC, which favoured IL-10 production. Mφ were more responsive to LPS stimulation than DC. CD40 on DC/Mφ was increased in the ileum compared to the colon. Ileal DC, compared to colonic DC, displayed an enhanced ability to stimulate T cells and imprint small-bowel homing marker CCR9 on T cells. Numbers of "tolerogenic" CD103$^+$CCR7$^+$ DC were increased in the colon; colonic DC generated Foxp3$^+$CD25$^+$IL-10- and IL-4-producing T cells whilst ileal DC generated IFN-γ-producing/T-bet-expressing T cells. Conclusions: Functional differences exist between human intestinal DC and Mφ. Regulatory properties of colonic DC/Mφ may represent an evolutionary adaptation to the greater bacterial load in the colon. The colon and ileum should be regarded as separate entities, each comprised of DC/Mφ with distinct roles in gut immunity.

OR.3. Dendritic Cell CD83 Homotypic Interactions Regulate Inflammation and Promote Mucosal Homeostasis

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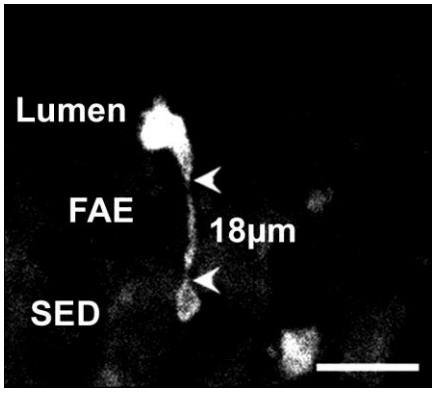
Dendritic cells (DCs) are sentinels of the immune system and form an extensive network in the intestinal

lamina propria, which orchestrates the mucosal immune response towards tolerance or inflammation. Alterations in DC function can perturb mucosal homeostasis and predispose to inflammatory bowel disease (IBD), although by unknown mechanisms. We show that CD83, a highly regulated DC cell surface protein, modulates the immune response to prevent colitis. Mice with a conditional knock-out of CD83 in DCs develop exacerbated colitis and decreased survival following DSS challenge while mucosal overexpression of CD83 inhibits DC inflammatory response and protects against colitis, as evidenced by less histologic lesions and proinflammatory cytokines, including IL-12p40, in serum and in lamina propria DCs. These CD83 perturbations can be modeled *in vitro* where we show CD83 homotypic interaction occurs via cell-cell contact and inhibits pro-inflammatory responses, resulting in decreased secretion of IL-12p40 in DCs. CD83 mediates inflammatory inhibition as CD83 knockdown or cytoplasmic truncation abrogates the effects of homotypic binding. We demonstrate CD83 homotypic interaction regulates DC activation via the MAP kinase pathway by inhibiting p38α phosphorylation, necessary for IL-12p40 production. Our findings indicate that CD83 homotypic interactions regulate DC activation and promote mucosal homeostasis, and targeting CD83 may provide novel IBD therapeutics.

OR.4. LysoDC Sample Antigens by Extending Dendrites Through M Cell-Specific Transcellular Pores Before Migrating in the Peyer's Patch Follicle

Hugues Lelouard, Johnny Bonnardel, Stéphane Méresse, Jean-Pierre Gorvel. The National Centre for Scientific Research, Marseille, France



Peyer's patches (PP) of the small intestine are antigen sampling and inductive sites for the establishment of the mucosal immunity. Luminal antigens are transported from the mucosal surface of PP to the subepithelial dome (SED) through the specialized epithelial M cells of the follicle-associated epithelium. Among the SED resident dendritic cells (DC), which are ideally situated for taking up these antigens, LysoDC express high levels of lysozyme and display a strong phagocytic activity. Here, we investigated the mechanisms by which LysoDC capture luminal antigens *in vivo* using two-photon microscopy on explants of PP from the lys-EGFP transgenic mice, in which LysoDC can be detected by the fluorescence of EGFP. We show that LysoDC extended dendrites through M cell-specific transcellular pores to reach the gut lumen. The M cell adhesion molecules JAM-A and EpCAM were recruited at the site of the transcellular migration. Transcellular dendrites scanned the M cell apical surface and the gut luminal content, and were able to take pathogenic bacteria and inert particles in the lumen before retracting back to the SED and migrating to the follicle region. These data describe a new sampling mechanism that occurs in PP and brings to light cooperation between M cells of the follicle-associated epithelium and DC of the subepithelial dome. It provides alternatives for the specific targeting of mucosal vaccines.

OR.5. Counter-Manipulation of Immune Responses Against Genital Gonococcal Infection: A Novel Strategy for Therapy and Prophylaxis of Mucosal Infections

Yingru Liu, Nejat Egilmez, Michael Russell. University of Buffalo, Buffalo, NY

Gonorrhea remains one of the most common infectious diseases worldwide and it facilitates the transmission of HIV. *Neisseria gonorrhoeae* does not induce a state of specific protective immunity against reinfection and it is becoming resistant to most available antibiotics. We have found that *N. gonorrhoeae* has evolved strategies to suppress host Th1/Th2-mediated adaptive immune responses, by mechanisms dependent upon TGF-β, IL-10, and type 1 regulatory T cells. Systemic administration of anti-TGF-β and/or anti-IL-10 antibodies in a murine model of vaginal gonococcal infection reverses the induced immunosuppression and permits the development of Th1/Th2-driven adaptive immune responses with the establishment of memory, generation of anti-gonococcal antibodies, accelerated clearance of infection, and resistance to reinfection. Intravaginal administration of IL-12 encapsulated in sustained-release polymer microspheres replicated these outcomes and significantly enhanced Th1 responses as well as gonococcus-specific circulating IgG and vaginal IgG and IgA antibodies, with faster

clearance of infection and protection against subsequent reinfection. Similar results were achieved by vaginal administration of microencapsulated anti-TGF-β and/or anti-IL-10 antibodies. Local treatment with sustained-release formulations of IL-12 might serve as a novel therapeutic strategy for treatment of gonorrhea and facilitate the development of an effective vaccine. This approach could also have application in the treatment of other mucosal infections.

OR.6. Associations of Female Genital Tract CD4$^+$ T Cell Populations with HPV Persistence and Clearance

Lyle McKinnon[1], Billy Nyanga[1], Jessie Kwatampora[1], Kamnoosh Shahabi[2], Michael Chitwa[1], Jie Ting[3], Suha Patel[3], Joshua Kimani[4], Nelly Mugo[5], Jennifer Smith[3], Rupert Kaul[2]. [1]University of Nairobi, Nairobi, Kenya; [2]University of Toronto, Toronto, ON, Canada; [3]University of North Carolina, Chapel Hill, NC; [4]University of Manitoba, Winnipeg, MB, Canada; [5]Kenyatta National Hospital, Nairobi, Kenya

HPV is a common sexually transmitted infection and the cause of cervical cancer. HPV infection, and clearance in particular, may be associated with HIV acquisition, perhaps due to the mucosal recruitment of HIV target cells. We conducted cervical cytobrush sampling and T cell immunophenotyping nested within a prospective study of HPV diagnostic methods in Kenyan women. Three participant groups were defined: (1) persistently HPV uninfected (n=77); (2) persistently HPV positive (n=16) at all visits for 1 year; and (3) in the process of HPV clearance at the time of sampling (n=17). Participants clearing HPV showed no significant changes in HIV co-receptors, immune activation, or total cervical T cell numbers, compared to uninfected controls. Persistent HPV infection was associated with increased CTLA4bright CD4$^+$ T cells (p=0.018) and fewer CD39$^+$ CD25- 'inducer' CD4$^+$ T cells (p=0.001); these parameters were inversely correlated (r=-0.439, p<0.0001). The reduction in inducer cells remained associated with HPV persistence (p=0.008) in logistic regression models that adjusted for menstrual cycle phase, contraceptive use, other STIs, and HSV2. Contrary to our hypothesis, HPV clearance was not associated with increased cervical HIV target cells or changes in cervical CD4$^+$ T cell phenotype. However, HPV may induce immunoregulatory changes in mucosal T cells to cause persistent infection.

OR.7. Harnessing Human Vaginal Dendritic Cells (II): Gene Expression Profiles and Immune Responses

Dorothee Duluc[1], Romain Banchereau[1], Julien Gannevat[1], LuAnn Snipes[1], Jean-Philippe Blanck[1], Esperanza Anguiano[1], Muriel Boreham[2], Michael Carley[2], Jack Stecher[2], Jose Rossello-Urgell[1], SangKon Oh[1]. [1]Baylor Institute for Immunology Research, Dallas, TX; [2]Baylor University Medical Center, Dallas, TX

Human vaginal mucosa is the major entry site of sexually-transmitted pathogens. Therefore, understanding the immunology of human vagina is crucial for the design of effective vaccines against such pathogens. We have shown that immune responses in the vagina can be initiated and controlled by four major subsets of antigen-presenting cells (APCs). Langerhans cells (LCs) and CD14- lamina propria dendritic cells (LP-DCs) polarize T cells toward Th2- and Th22-type, whereas CD14$^+$ LP-DCs and macrophages polarize them toward Th1-type. To further understand the immunology of the human vagina, we have examined, for the first time, the transcriptional profiles of the human vaginal APC subsets and then compared these with the transcriptional profiles of APC subsets from human skin and blood myeloid DCs. An unsupervised analysis of the transcriptional profiles shows that approximately 10,000 genes (PALO p<0.01) are differentially expressed in the subsets of vaginal, skin, and blood DCs. Principal component analysis (PCA) reveals that individual vaginal APC subsets express common as well as unique patterns of gene expressions, which is supported by their phenotypes and functional specialty. PCA and Ingenuity pathway analysis also suggest that APCs in the vagina, skin, and blood have distinct functions to induce and control host immune responses.

OR.8. Female Sex Hormones and Hormonal Contraceptives Affect Entry, but not Replication, of HIV-1 into Primary Genital Epithelial Cells

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Women constitute more than half of all people living with HIV/AIDS worldwide yet the early events of HIV-1 infection in the female genital tract (FGT) are poorly understood. The FGT is lined by genital epithelial cells (GECs) which are one of the first cells to encounter HIV-1 during sexual transmission. It is unknown whether endogenous female sex hormones or hormonal contraceptives regulate GEC susceptibility or permissiveness to HIV-1. Primary GEC cultures were prepared from human genital tract tissues and grown in the presence or absence of physiological concentrations of estrogen (E2), progesterone (P4) or medroxyprogesterone acetate (MPA) prior to HIV-1 exposure. Cell-associated HIV was significantly increased within cells grown in MPA and in basolateral supernatants of GECs grown in MPA or P4. Furthermore, heparan sulphate moieties and endocytosis were found to play an important role in HIV entry. Despite this, no early or late reverse transcription products, integrated HIV DNA or spliced HIV RNA transcripts were measured. These results suggest that female sex hormones, particularly MPA, regulate HIV transcytosis across the epithelium and HIV entry into GECs, but not replication, in a non-canonical fashion. Ongoing studies are investigating the significance of MPA-enhanced HIV entry and transcytosis in the absence of productive infection.

OR.9. The Alarmin Interleukin-33 Receptor and its Role in Inflammatory Bowel Diseases

David Díaz-Jiménez[1], Lucía Nuñez[1], Hugo Sepúlveda[2], Marjorie De la Fuente[1], Karen Dubois[1], Jonás Chnaiderman[1], Martín Montecino[2], Rodrigo Quera[3], Marcela Hermoso Ramello[1]. [1]University of Chile, Santiago, Chile; [2]Andres Bello University, Santiago, Chile; [3]Clinica Las Condes, Santiago, Chile

IL-33 extracellular function has attracted attention as a ligand for the Th2-associated ST2 receptor that could critically contribute to IBD pathogenesis. Successful IBD treatment should induce and maintain remission, mucosa healing and prevention of complications. A common IBD aspect is early glucocorticoids (GC) use; however, there is no evidence on its effect on ST2 content and function in patients. We aimed to evaluate how corticosteroid treatment impact on ST2 transcriptional regulation. Blood samples and biopsies of IBD patients undergoing colonoscopy were included. ST2 content in intestinal mucosa cells and plasma were determined by immunofluorescence and ELISA, respectively. Additionally, glucocorticoid receptor (GR) participation was determined using RU486 or a GR mutant lacking DBD, and binding to st2 promoter was assessed by Chip assays in mast cells. A higher expression of ST2 in intestinal $TFF3^+$ and $tryptase^+$ cells was determined in UC patients, and serum ST2 was significantly increased in corticosteroids-treated patients ($p < 0.01$). Furthermore, our results showed that GC-mediated ST2 induction requires GR participation and that distal ST2 promoter is enriched with GR. Our findings indicate that steroid-mediated ST2 induction involves distal promoter transcriptional activity and that intestinal goblet and mast cells mediate inflammation through ST2. FONDECYT 1110381, DA-CLC 2011-014.

OR.10. A Critical Role of the IL-10/IL-10 Receptor Axis in $CX3CR1^+$ Macrophages for Gut Homeostasis

Ehud Zigmond[1], Biana Bernshtein[1], Catherine Walker[3], Simon Yona[1], Gilgi Friedlander[1], Ori Brenner[1], Chen Varol[2], Rita Krauthgamer[1], Robert Jack[4], Werner Muller[3], Steffen Jung[1]. [1]Weizmann Institute of Science, Rehovot, Israel; [2]Tel-Aviv University, Tel Aviv, Israel; [3]University of Manchester, Binyamina, United Kingdom; [4]University of Greifswald, Rehovot, Germany

IL-10 is a pleiotropic cytokine whose activity aims to limit inflammatory responses. It is produced by T and B cells, macrophages as well as by non-hematopoietic cells, usually after activating stimuli. Most hematopoietic cells can also sense IL-10 via the specific IL-10 receptor, IL-10R1; IL-10R1 expression was furthermore reported for certain non-hematopoietic cells, including colonic epithelium. GWA studies revealed a central role of IL-10/IL-10R axis in the pathogenesis of Ulcerative Colitis and loss-of-function mutations of IL10R causes severe early-onset colitis. Moreover, IL-10-deficient mice develop spontaneous enterocolitis. Here we will report on the specific roles of IL-10 production and IL-10 sensing by intestinal $CX3CR1^+$ macrophages. Macrophage-derived IL-10 was reported to be critical for Treg maintenance and oral tolerance; however, its role for the maintenance of gut homeostasis has not been assessed. Specifically we took advantage of new CX3CR1-Cre animals (Yona et al. Immunity 2012) to generate mice that harbor macrophage-restricted IL-10 or IL-10R1 mutations. We will report the comprehensive evaluation of these animals, including their gut condition, macrophage gene expression

profiles and effector T cell distributions in the gut and mesenteric lymph nodes. Collectively, our results define intestinal CX3CR1$^+$ macrophages as central keepers of gut health.

OR.11. Interleukin-25-Mediated Regulation of RORγt$^+$ Innate Lymphoid Cells at the Mucosal Barrier
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RORγt$^+$ innate lymphoid cells (ILCs), such as lymphoid tissue inducer cells and Interleukin(IL)-22 producing NKp46$^+$ cells are required for the development of lymphoid tissues, homeostasis with symbiotic microbiota and defense against pathogens. An equilibrated crosstalk between RORγt$^+$ ILCs, microbiota, pathogens and adaptive immunity is critical for intestinal homeostasis, a loss of which leads to inflammatory immunopathology. We have previously shown that commensal microbiota repress the activity of RORγt$^+$ ILCs through induction of IL-25 expressed by intestinal epithelial cells. The mechanisms by which IL-25 acts on RORγt$^+$ ILCs are unclear, as these cells do not express the IL-25-Receptor (IL-25R). *In vitro* cocultures of purified RORγt$^+$ ILCs in association with stromal and dendritic cells demonstrated that IL-25R$^+$ DCs, found in cryptopatches and isolated lymphoid follicles (ILFs) of the intestinal lamina propria, are sufficient to mediate the repressive activity of IL-25. However, IL-25-mediated repression of RORγt$^+$ ILCs via the DC/ILC axis is only partial. Notably, B cells also express the functional IL-25R$^+$ increasing with ILF maturity along the colonization density. We propose that the spatial accumulation of these cells in organized tertiary structures of the intestine provides a complex network for reciprocal and cooperative regulation.

OR.12. IL-25 Simultaneously Elicits Distinct Populations of ILC2s and Multi-Potent Progenitor Type 2 (MPP^{type2}) Cells
Steven Saenz[1], Mark Siracusa[1], Laurel Monticelli[1], Carly Ziegler[1], E John Wherry[1], Ananda Goldrath[2], Avinash Bhandoola[1], David Artis[1]. [1]University of Pennsylvania, Philadelphia, PA; [2]University of California San Diego, La Jolla, CA

Epithelial cell-derived cytokines, IL-25, IL-33 and TSLP are critical in orchestrating immunity, inflammation and tissue repair at barrier surfaces through the induction of multiple innate immune cell populations. For example, IL-25 and IL-33 were shown to elicit four previously unrecognized innate immune cell populations, termed natural helper cells, nuocytes, innate type 2 cells and multi-potent progenitor type 2 (MPP^{type2}) cells, collectively termed group 2 innate lymphoid cells (ILC2s). However, in contrast to the other ILC2s, MPP^{type2} cells exhibit multi-potent potential and do not express hallmark ILC markers (T1/ST2, IL-7Rα and CD90), suggesting that MPP^{type2} cells represent a distinct cell population from ILC2s. In new studies, we found that while IL-33 elicits ILC2s, IL-25 predominantly promotes MPP^{type2} cell responses and limited ILC2 responses at multiple tissue sites. Critically, MPP^{type2} cells were distinguished from ILC2s by their differential developmental requirements for specific transcription factors, genome-wide transcriptional profile and functional potential. Further, IL-25-induced MPP^{type2} cells could promote Th2 cytokine-associated inflammation in the absence of ILC2s. These findings indicate that IL-25 simultaneously elicits phenotypically and functionally distinct innate lymphoid- and non-lymphoid-associated cell populations and highlights that IL-25-dependent extramedullary hematopoiesis is an additional mechanism that promotes the development of Th2 cytokine responses at mucosal surfaces.

OR.13. Fungal Communities Throughout the Intestine and their Role in Intestinal Inflammation
Iliyan Iliev, Vincent Funari, David Underhill. Cedars-Sinai Medical Center, Los Angeles, CA

Mucosal fungal infections are relatively common in IBD patients, and antibodies against fungal antigens (ASCA) have been used as a clinical marker for disease. We recently found that fungi are common inhabitants of mammalian intestine. However, how fungi are distributed throughout the gastrointestinal tract and whether immunity to fungi might play a role in inflammatory disease is currently unknown. Fungi are sensed by number of innate immune receptors among which Dectin-1 has emerged as a main innate immune receptor for recognition, phagocytosis, and killing of fungi. We found that mice lacking Dectin-1 are more susceptible to experimental colitis characterized by increased infiltration of Th17 and Th1 cells in the colon. Interestingly this pathology was driven by intestinal fungi, and anti-fungal therapy

ameliorated colitis severity in knockout mice. Deep sequencing analysis of gut mycobiome revealed fungal genera that are over-represented during experimental colitis. Using this technology we surveyed the mycobiomes throughout the entire murine gastrointestinal tract, as well as other mucosal sites throughout the body. Our results show specific distribution of certain fungal genera which might be associated with site specific immune responses to fungi and might promote inflammatory conditions at those sites as a result of aberrant immunity to fungi.

OR.14. Development of Novel Humanized Murine Models to Assess Mucosal Homeostasis: Human Anti-CD3 Antibody or TNBS Administration Leads to Small and Large Bowel Inflammation Respectively in Immunodeficient Mice Transferred with Human T Cells

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A critical barrier to understanding human immune cell function in the intestinal mucosa is the lack of a robust *in vivo* model and limitations on experimenting with human subjects. Development of immunodeficient mouse strains has enabled engraftment of human leukocytes into murine hosts, permitting the study of human immune cells in a xenobiotic setting. Using this approach, we have developed two mouse models of intestinal inflammation that are dependent on human CD4 T cells. Results: anti-human CD3 administration resulted in diarrhea and small intestine villous atrophy within 24 hours only in mice reconstituted with human CD4 T cells. T cells recovered form spleen and small intestine LP down regulated cell surface CD3 expression concomitant with increase CD25 expression, a marker of activation. For large bowel disease, TNBS caused colitis in mice receiving HLA matched human CD4 T cells exhibited clinical and histological signs of intestinal inflammation that was absent in mice receiving TNBS but lacking human CD4 T cells. Conclusion(s): Human CD4 T cells adoptively transferred into humanized mice can induce small bowel enteropathy and promote colonic inflammation in mice following exposure to OKT3 and TNBS respectively demonstrating the potential of these models for assessing pre-clinical therapeutic strategies.

OR.15. Rip2-Mediated Control of Pathogenic and Protective T Cell Function During Chronic Colitis

Thierry Mallevaey, Nichole Escalante. University of Toronto, Toronto, Canada

The intestinal mucosa constitutes an unrivaled site of bidirectional exchanges between our immune system and the resident microorganisms. Chronic intestinal inflammation, such as Crohn's disease (CD), likely arises from dysbiosis and/or improper immune defenses. Humans carrying a dysfunctional variant of the bacteria-sensing receptor Nod2 are at high risk for CD. In addition, experimental evidence in mouse models supports a protective function of Nod receptors, although their mechanism of action remains elusive. During CD, T cells seem to trigger a strong inflammatory response that ultimately leads to the destruction of epithelial structures. We have used the T cell transfer colitis model, and generated $Rag1^{-/-}$ $Rip2^{-/-}$ mice (that lack Nod1/2 signaling) to study the function of Nod receptors in T cell function. We found that upon transfer of CD45RBhi effector cells, Rip2-deficient recipient mice have increased number of IFN-gamma-producing colon-infiltrating T cells, and develop a more severe colitis, relative to control mice. In addition, co-transferred CD45RBlow memory T cells were unable to prevent colitis. This points to a dual role of host-derived Nod-mediated signals in the control of effector and regulatory T cell responses. We are exploring the cellular and molecular mechanisms controlling these T cell populations in the gut.

OR.16. The Role of the Cellular Inhibitors of Apoptosis in Colitis and Colitis-Associated Colorectal Cancer

Maryse Dagenais, Jeremy Dupaul-Chicoine, Christian McIntyre and Maya Saleh. McGill University, Montreal, QC, Canada

The cellular inhibitors of apoptosis proteins (cIAPs) were first discovered as inhibitors of the apoptotic cell death program and were reported to be over-expressed in cancer. It has since been shown that the cIAPs modulate apoptosis indirectly via a TNFa autocrine loop. Since then, the cIAPs have also been implicated in multiple pathways including NF-kB pathways, MAPK downstream of TLRs, regulating necroptosis, NOD1/2 signalling and inflammasome activation. Given their role in innate immunity, we hypothesized

that the cIAPs contribute to tissue homeostasis and tumorigenesis. In an experimental model of chronic colitis triggered by chemical injury with DSS, cIAP2$^{-/-}$ mice resulted in defective mucosal regeneration and colitis. This phenotype is caused, in part, by a deficit in IL-18 production, a key cytokine involved in gut repair. Consistently, IEC proliferation was impaired in cIAP2-deficient mice, as revealed by decreased cyclin D1 expression and PCNA staining. Furthermore, using a RIPK1-inhibitor, the susceptibility of the cIAP2-deficient mice was rescued, implicating the role of necroptosis in gut homeostasis. In addition, using AOM-DSS, we found that cIAP deficiency leads to a decreased tumor burden. Given the potential of IAP antagonists as therapeutic targets, understanding the role of the cIAPs in colitis and CRC is crucial.

OR.17. Peyer's Patch Dendritic Cells Constitutively Migrate to the Mesenteric Lymph Node in a CCR7 and S1P Dependent Manner

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Dendritic cells (DCs) are fundamental in controlling intestinal immune responses by migrating to the mesenteric lymph node (MLN) and priming gut-tropic effector or regulatory T cells. Furthermore, DCs direct the immune response in Peyer's Patches (PP). Nevertheless, it is unclear if they contribute to an MLN mediated immune response by migrating and presenting PP derived antigen. Here, we provide evidence that a novel population of DCs migrate from PPs to the MLN. Firstly, after photoconversion of PPs in fluorescent transgenic Kaede mice, converted DCs were found in the MLN, indicating that some DCs migrate from the PP to the MLN. Secondly, we use a novel injection technique to label cells within a PP with FITC, FITC^{+} DCs were found in the MLN. Significantly fewer DCs migrated from the PP to the MLN in CCR7$^{-/-}$ mice and in mice pre-treated with FTY720, but were present in S1P3-deficient mice. We have identified a previously unreported population of DCs that migrate from the PP to the MLN by a CCR7 and S1P dependent mechanism. These cells may play an important role in driving IgA responses in the MLN and their manipulation could lead to significant advances in increasing the efficacy of oral vaccines.

OR.18. Human Gastric Epithelial Cells Contribute to Gastric Immune Regulation by Providing Retinoic Acid to Dendritic Cells

Diane Bimczok, Olga Beliaeva, Natalia Kedishvili, Phillip Smith, Lesley Smythies. University of Alabama at Birmingham, Birmingham, AL

Retinoic acid (RA) is an important immunoregulatory factor in small intestine, where conversion of retinol to RA is thought to occur in epithelial cells, which then enable mucosal dendritic cell (DC) RA biosynthesis and induction of lymphocyte gut homing molecules and Treg responses. We previously showed that human gastric DCs direct the T cell response to *H. pylori*. Here, we investigated whether RA could contribute to gastric DC regulation. Surprisingly, HPLC-analysis revealed significantly higher concentrations of retinol in gastric mucosa compared to small intestinal mucosa from the same patients, although the source of retinol in gastric mucosa is unclear. We also show that human gastric epithelial cells expressed higher levels of RA response genes, including RA synthesis enzymes (RDH10, aldh1a1) and RA receptors (RAR-β/γ), than intestinal epithelial cells. *In vitro*, primary gastric epithelial cells exposed to retinol generated significant amounts of RA. Importantly, isolated human gastric and intestinal DCs had a similar capacity to synthesize RA in the Aldefluor assay, and gastric and intestinal CD4^{+} and CD8^{+} T cells expressed comparable levels of CCR9. These data indicate that stomach and small intestine share the ability to induce RA-metabolizing activity in resident DCs, resulting in CCR9-dependent lymphocyte homing to gastric and intestinal mucosa.

OR.19. Defective Dendritic Cell Migration in Mice with Crohn's-Like Ileitis

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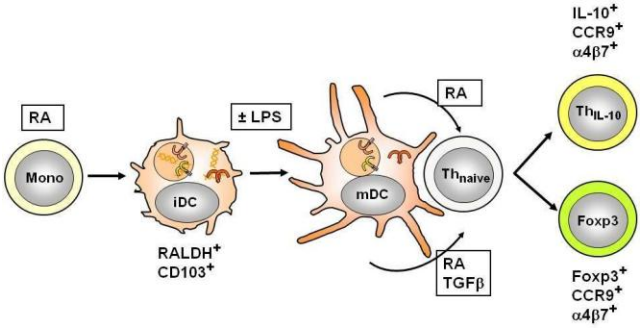
The SAMP1/YitFc mouse model of chronic ileitis has 100% disease penetrance at 10 weeks of age. It is

considered the most relevant model of Crohn's disease, because it responds to treatments that are clinically effective. We tested whether dendritic cells (DC) are altered in chronic ileitis in SAMP1/YitFc. We found severe defects in migratory MHCII^{hi}CD11c^{+}CD11b^{+}CD103^{+} DC in the MLN of SAMP1/YitFc mice (reduced by 75%) and a concomitant accumulation of these cells in the lamina propria (LP) of the terminal ileum. DCs isolated from SAMP1/YitFc MLN had a reduced capacity to produce retinoic acid. The migratory DC defect in SAMP1/YitFc mice resembled that seen in mice lacking CCR7, a chemokine receptor involved in DC migration to secondary lymphoid organs. Although CCR7 expression was normal in SAMP1/YitFc, we found a severe (>98%) defect in expression of one of its ligands, the lymphatic chemokine CCL21. The defects in CCL21 precede the clinical manifestation of ileitis, suggesting a possible causative role. The severe defect of CCL21 and concomitant loss of retinoic acid-producing DC in the MLN of SAMP1/YitFc mice may be a disease-causing mechanism.

OR.20. Retinoic Acid Primes Human Dendritic Cells to Induce Gut-Homing, IL-10 Producing Regulatory T Cells

Ghaith Bakdash, Lisa Vogelpoel, Toni van Capel, Martien Kapsenberg, Esther de Jong. University of Amsterdam, Amsterdam, Netherlands



The vitamin A metabolite all-trans retinoic acid (RA) is an important determinant of intestinal immunity. RA primes dendritic cells to express CD103 and produce RA themselves, which induces the gut-homing receptors α4β7 and CCR9 on T cells and amplifies TGF-β-mediated development of Foxp3^{+} regulatory T (Treg) cells. Here we investigated the effect of RA on human DCs and subsequent development of T cells. We report a novel role of RA in immune regulation by showing that RA-conditioned human CD103^{+} DCs did not substantially enhance Foxp3 but induced α4β7^{+}CCR9^{+} T cells expressing high levels of IL-10, which were functional suppressive Treg cells. IL-10 production was dependent on DC-derived RA and was maintained when DCs were stimulated with TLR ligands. Furthermore, the presence of TGF-β during CD103^{+} DC-driven T cell priming favored the induction of Foxp3^{+} Treg cells over IL-10^{+} Treg cells. Experiments with naïve CD4^{+} T cells stimulated by αCD3 and αCD28 antibodies in the absence of DCs emphasized that RA induces IL-10 in face of inflammatory mediators. The data thus show for the first time that RA induces IL-10-producing Treg cells and postulates a novel mechanism for IL-10 in maintaining tolerance to the intestinal microbiome.

OR.21. The Adjuvant Role of Nanoencapsulated Retinoic Acid in Intranasal Vaccination Against Leishmaniasis

Bartira Rossi-Bergmann[1], Izabella Bezerra[1], Beatriz Souza[1], Guilherme Carmeiro[2], Lucas Ferreira[2]. [1]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; [2]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Mucosal vaccination with disease-promoting antigens of Leishmania amazonensis (LaAg) leads to peripheral protective immunity against leishmaniasis by an as yet unclear mechanism. Since LaAg protection depends on dietary retinol, and retinoic acid (RA) is knowingly required for mucosal Treg differentiation, we investigated the adjuvant effect of RA in vaccination. Thus, BALB/c mice were given two intranasal doses of LaAg alone or associated with solid lipid nanoparticle-encapsulated RA (RA-SLN), free RA or empty SLN. Three days after the second dose, the cervical lymph node cells were phenotyped by FACS, and cytokine expression quantified by qRT-PCR. Four days later, spare animals (n=5) were challenged in the footpad with infective parasites and developing infection was monitored up to 2 months. We found that LaAg association with RA-SLN but not with free RA or empty SLN induced CD4^{+} Foxp3^{+} Treg cell expansion and significantly increased IL-10 expression in nose-draining nodes. IFN-γ, IL-12, IL-4 and TGF-β expression remained unaffected. Association with RA-SLN also led to smaller lesion sizes and lower parasite burden, as compared with LaAg alone. These results show that

RA-SLN ameliorates LaAg efficacy against cutaneous leishmaniasis, and indicates its potential adjuvant use in tolerogenic intranasal vaccines.

OR.22. Asthma Prevention Through Oral Tolerance Induction is Inefficient at Birth: Role of Mesenteric Lymph Node CD103$^+$ Dendritic Cells Retinoic Acid Secretion

Mathilde Turfkruyer[1], Akila Rekima[1], Patricia Macchiaverni[2], Valerie Verhasselt[2]. [1]University of Nice Sophia Antipolis, Nice, France; [2]University of Sao Paulo, Sao Paulo, Brazil

We demonstrated in a mouse model that breastfeeding could prevent asthma by inducing oral tolerance in the breastfed progeny. Breastfeeding-induced tolerance required the presence of both allergen and tolerogenic co-factors, i.e. TGF-ß and/or allergen specific IgG in maternal milk. Recently, we found that full protection was observed only when antigen transfer occurs during the third week of lactation. Here we set out to investigate the mechanism responsible for defective oral tolerance induction during early days of life. We found decreased expression of retinaldehyde deshydrogenase (RALDH) in mesenteric lymph node CD103$^+$ dendritic cells (DC) from 1 and 2 weeks old pups as compared to 3 weeks old. Supplementation of maternal diet with vitamin A during lactation increased RALDH levels in MLN CD103$^+$ DC of pups aged of 1 and 2 weeks up to week 3 levels. *In vitro* ability of MLN cells to drive T cell proliferation was also increased upon vitamin A maternal supplementation. In parallel, we found *in vivo*, that maternal diet supplementation in Vitamin A during lactation decreased TH2 immune response in the progeny while regulatory T cells were not induced. On the contrary, we found increased Th1 immune responses in mice breastfed by mothers under vitamin A enriched diet. These data indicate that, in contrast to what is described in the adult, RALDH increased expression in the neonate is associated with potentiation of Th1 but not regulatory immune responses.

OR.23. Induction of Mucosal Tolerance in the Small and Large Intestine is Mediated by Distinct Immune Processes

Sharon Veenbergen, Fleur du Pré, Lisette van Berkel, Franka Luk, Ytje Simons-Oosterhuis, Janneke Samsom. Erasmus Medical Center, Rotterdam, Netherlands

Mucosal tolerance to protein antigen is a complex process depending on the local environment and draining lymphoid tissue. Here, we investigated whether distinct locally adapted regulatory mechanisms maintain tolerance in the small and large intestine. Ovalbumin (OVA) application to the colon led to antigen-specific T cell proliferation in the iliac lymph nodes (ILN), while oral antigen application elicited a T cell response in the mesenteric lymph nodes (MLN). Despite the difference in draining site, both small intestinal and colonic antigen application induced tolerance. Under steady-state conditions, Foxp3 expression was higher in ILN compared to MLN. Comparison of gene expression profiles of CD11c$^+$ DCs from ILN and MLN revealed that MLN-derived CD11c$^+$ DCs preferentially express RALDH2, a vitamin A-converting enzyme important for Foxp3 induction. In agreement, gut-derived CD103$^+$CD11b$^+$CD8α-DCs known to express RALDH2 were present in MLN and absent in ILN. The ILN-derived CD11c$^+$DCs expressed enhanced levels of IL-10 and cyclooxygenase-2, an enzyme involved in prostaglandin-mediated Foxp3 expression. Despite low RALDH2, ILN-derived CD11c$^+$ DCs were more effective than MLN-derived CD11c$^+$DCs in driving TGFβ-mediated differentiation of Foxp3$^+$IL-10$^+$ Treg cells. Our results demonstrate that within different locations of the gastrointestinal tract tolerance is imposed by local regulatory mechanisms adapted to microenvironmental differences.

OR.24. Passive Mucosal Immunisation with Novel Simplified IgA Antibodies Produced in Seeds Prevents Enterotoxigenic Escherichia Coli (ETEC) Infection in Weaned Piglets

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ETEC related post-weaning diarrhea causes recurrent economic losses to the porcine rearing industry. The embargo on antibiotic prophylaxis has led to a pressing need for a suitable alternative. To evaluate feed based oral prophylactic passive immunisation against ETEC, we produced antibodies in seeds of Arabidopsis thaliana. Antibodies were designed by grafting 4 variable domains of lama heavy chain

antibodies (VHH) against ETEC on the Fc part of porcine IgG and IgA. Transformants producing the 4 VHH-IgG and 4 VHH-IgA antibodies from 0.2% up to 3% of seed weight were obtained. Co-transformation of the VHH-IgA constructs with porcine joining chain and secretory component led to production of assembled dimeric and secretory IgA like antibodies in seeds. *In vitro* analysis of the antibody producing seed extracts were all effective in aggregating ETEC and inhibiting bacterial binding to porcine gut villous enterocytes. In a piglet feed-challenge experiment, the feed containing a milled cocktail of all the VHH-IgA based antibodies (dose 20mg/ pig/ day) protected the piglets against the challenge infection; while feed with the 4 VHH-IgG producing seeds (dose 80mg/ pig/ day) failed to offer similar protection. Piglets receiving the VHH-IgA antibodies had a swift decline in shedding of ETEC, the seroconversion was significantly lower and they had a higher weight gain. Thus these results show a feasibility proof for oral passive immunisation against ETEC.

OR.25. *In vivo* Modeling of Human GALT Development and Pathogenesis Using Xenotransplantation

Tomonori Nochi, Paul Denton, Angela Wahl, J. Victor Garcia. University of North Carolina, Chapel Hill, NC

Abnormal gut-associated lymphoid tissue (GALT) in humans is associated with infectious and autoimmune diseases, which cause dysfunction of the gastrointestinal tract immune system. Unfortunately, to date there are no *in vivo* preclinical models with human GALT for research on these conditions. To aid in investigating GALT pathologies *in vivo*, we bioengineered a human-mouse chimeric model where human GALT is created via xenotransplantation of human hematopoietic stem cells and autologous thymic and liver tissues. This model is characterized by the development of human GALT-like structures composed of $CD4^+$ and $CD8^+$ T cells, B cells, dendritic cells and macrophages originating in mouse cryptopatches. This novel observation represents a fundamental breakthrough in our mechanistic understanding of the role of cryptopatches in human GALT genesis and emphasizes the evolutionary conservation of this developmental process between humans and mice. Immunoglobulin class switching to IgA occurs in the GALT-like structures leading to numerous human IgA-producing plasma cells throughout the intestinal lamina propria. $CD4^+$ T cell depletion within the GALT-like structures results from HIV infection, as in humans. This human-mouse chimeric model represents the most comprehensive experimental platform currently available for the study and the pre-clinical efficacy testing of therapeutics designed to repair disease-damaged GALT.

OR.26. IL-23 and IL-25 Regulate Colonic Isolated Lymphoid Follicle Development

David Donaldson, Neil Mabbott. University of Edinburgh, Edinburgh, United Kingdom

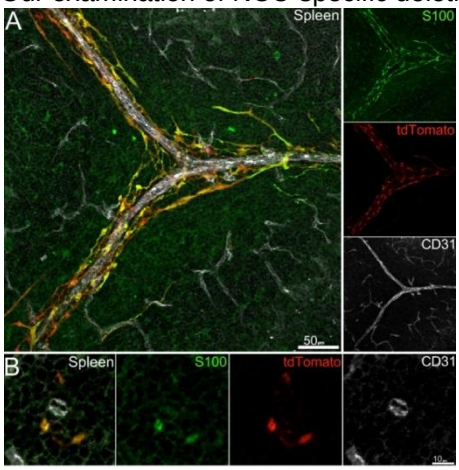
Isolated lymphoid follicles (ILF) are single B cell follicles that develop post-birth in small and large intestines (SI and LI) and are dynamically regulated by the intestinal microbiota. The development of SI and LI-ILF differs in timing, developmental requirements and microbial dependence. Using conventionalised germ-free mice, we show that unlike in the SI, the microbiota negatively regulate the development of ILF in the LI. This change in ILF numbers paralleled changes in IL-23 expression and we have shown that $CD11c^+IL\text{-}23p19^+$ cells are specifically enriched in colonic but not SI-ILF. Furthermore, $IL\text{-}23p19^{-/-}$ mice have a colon-specific reduction in ILF. Microbial colonisation is associated with increased IL-25 expression and $IL\text{-}25^{-/-}$ mice have a colon-specific increase in ILF, suggesting that ILF development in the colon is reciprocally regulated by microbiota induced changes in IL-23 and IL-25 expression in the steady state. This novel regulatory feedback loop has important implications for the pathogenesis of colonic disease in which IL-23 and IL-25 have central roles.

OR.27. A Novel Role for the Neural Crest in Splenic Development

Ankush Gosain, Christopher Erickson, Amanda Barlow, Miles Epstein. University of Wisconsin, Madison, WI

Hirschsprung's disease (HSCR) results from the inability of neural crest cells (NCC) to completely colonize the hindgut, leading to absence of ganglia. Despite surgical removal of the aganglionic bowel, patients may suffer recurrent Hirschsprung's-associated enterocolitis (HAEC). Investigations into HAEC

using an Endothelin receptor B (Ednrb) knockout mouse model of HSCR identified splenic lymphopenia. Our examination of NCC-specific deletion of Ednrb revealed smaller Peyer's Patches (PP) and spleens with decreased cellularity, primarily B-lymphocytes. Since the spleen is the primary source of mature B-lymphocytes in the PP and our mice contain an absence of Ednrb only in NCC, we hypothesized that defective NCC development or migration could underly these changes. Expression of the fluorophore TdTomato in NCC enabled identification of these cells in our animals (Wnt1-Cre$^{+/-}$ R26RtdTomato^{+} Ednrbflex3/flex3). We noted entry of TdTomato^{+} NCC into the spleen from embryonic day E15-18. By post-natal day P21, these cells were associated with blood vessels and expressing proteins indicative of astrocytes (Sox10^{+}, S-100^{+}, PDGFRβ-). Our results support a novel role for NCC in splenic development and may provide an embryological basis for our post-natal observations on HAEC. The role of NCC-derived astrocyte-like cells in the splenic inflammatory response remains to be determined.



OR.28. SFB Can Elicit Adaptive Immune Responses by Inducing the Formation of Gut Tertiary Lymphoid Tissue

Emelyne Lécuyer[1], Sabine Rakotobe[2], Hélène Lengliné-Garnier[1], Corinne Lebreton[1], Nadine Cerf-Bensussan[1], Valérie Gaboriau-Routhiau[2]. [1]INSERM U989, Paris, France; [2]INRA, Jouy-en-Josas, France

Using gnotobiotic mice, we have shown that Segmented Filamentous Bacterium (SFB), in contrast to many other intestinal bacteria, can drive the coordinated maturation of innate and adaptive intestinal immune responses. However the mechanisms underlying the immunostimulatory properties of SFB are not known. Herein we show that: i) mono-colonization by SFB induces a strong stimulation of T cell responses in Peyer's patches and a marked expansion of germinal centers which are not observed in mice colonized by a commensal strain of E. coli. Ii) Inhibiting the development of Peyer's patches by lymphotoxin β receptor-immunoglobulin abolished IgA responses to E. Coli but not to SFB. Iii) In mice lacking Peyer's patches and cryptopatch-derived follicles, SFB, but not E coli, induced gut tertiary lymphoid follicles able to partially substitute Peyer's patches as inductive sites for IgA responses. Iiii) IgA responses to SFB were abolished in mice lacking constitutive and inducible lymphoid tissues. These data indicate that gut lymphoid tissues are instrumental to develop intestinal IgA responses to the microbiota. That SFB can initiate adaptive immune responses not only in Peyer's patches but also via the novo induction of gut lymphoid tissue may explain its outstanding immunostimulatory properties.

OR.29. Nlrp3 Inflammasome Activation in the Intestinal Epithelium Protects Against a Mucosal Pathogen

George Song-Zhao[1], Naren Srinivasan[1], Johanna Pott[1], Dilair Baban[1], Gad Frankel[2], Kevin Maloy[1]. [1]University of Oxford, Oxford, United Kingdom; [2]Imperial College London, London, United Kingdom

Polymorphisms in the intracellular pattern recognition receptor gene NLRP3 have been associated with susceptibility to Crohn's disease, a type of inflammatory bowel disease (IBD). Following tissue damage or infection, NLRP3 triggers the formation of inflammasomes, containing NLRP3, ASC and caspase-1, which mediate secretion of IL-1β and IL-18. NLRP3 inflammasome activation in macrophages has been implicated in immunity to several pathogenic infections, but whether NLRP3 activation in tissue cells contributes to protective immunity against bacterial pathogens is not known. Here we show that upon infection with the attaching/effacing intestinal pathogen *Citrobacter rodentium*, Nlrp3$^{-/-}$ mice displayed increased bacterial colonization and dispersion, more severe weight loss and exacerbated intestinal inflammation. Bone marrow chimera experiments revealed that Nlrp3 activation in non-hematopoietic compartment was required for protection against *C. rodentium*. We further demonstrate that epithelial Nlrp3 inflammasome activation acts rapidly after infection to limit bacterial replication and penetration, and inhibits the development of inflammatory pathology in the gut. We also show that this Nlrp3-mediated protection is independent of the classical inflammasome cytokines IL-1β and IL-18. Thus, Nlrp3

inflammasome sensing in intestinal epithelial cells regulates early defense against a mucosal pathogen and limits inflammation in the intestine, potentially explaining the link between NLRP3 polymorphisms and IBD.

OR.30. Identification of a *C. Albicans* Protein Critical for the Epithelial Cell 'Danger' Response

David Moyes[1], Celia Murciano[7], Shirley Tang[1], Craig Murdoch[6], Oliver Bader[4], Ernesto Cota Segura[5], Duncan Wilson[2], Sarah Hoefs[2], Gary Moran[3], Stephen Challacombe[1], Bernhard Hube[2], Julian Naglik[1]. [1]King's College, London, London, United Kingdom; [2]Hans Knoell Institute, Jena, Gabon; [3]Trinity College Dublin, Dublin, Ireland; [4]University of Göttingen, Göttingen, Germany; [5]Imperial College, London, London, United Kingdom; [6]University of Sheffield, Sheffield, United Kingdom; [7]University of Valencia, Valencia, Spain

A host's ability to discriminate between commensal and pathogenic microbes is key to homeostasis and immunity. Previously, we have demonstrated a MAPK-specific mechanism enabling epithelial cells (ECs) to discriminate between pathogenic and commensal *C. albicans* by responding specifically to hyphae. Here, we show this EC 'danger' response mechanism is targeted against the hyphal protein, Ece1p. Unlike wild-type *C. albicans*, infection of ECs with ece1Δ/Δ does not activate the 'danger' response (p38/c-Fos, ERK1/2/pMKP1, cytokines) or cause damage, despite growing as hyphae and invading ECs. In contrast, overexpression of ECE1 in an avirulent non-filamentous mutant restores activation of the 'danger' response. A direct role for Ece1p was confirmed by stimulating ECs with recombinant Ece1p, which induced damage and the 'danger' response (at sub-lytic concentrations). Further analysis identified the active regions of Ece1p and stimulation with the active peptides resulted in activation of EC 'danger' responses. To identify the clinical relevance of these data we infected zebrafish with ece1Δ/Δ demonstrating increased survival compared with wild-type infected fish. These data provide evidence that EC discrimination of pathogenic *C. albicans* and activation of the MAPK 'danger' response occurs via Ece1p recognition, suggesting possible uses in treating chronic mucosal infections.

OR.31. IL-28 Signaling in Intestinal Epithelial Cells via STAT-1 Contributes to an Accelerated Wound Healing Process

Heike Dornhoff[1], Konstantin Fietkau[1], Sean Doyle[2], Jürgen Siebler[1], Markus Neurath[1]. [1]University Nuremberg-Erlangen, Erlangen, Germany; [2]ZymoGenetics, Inc., Seattle, WA

IL-28 signals through a unique receptor consisting of the IL-28Rα and IL-10Rβ Subunits via STAT-1. It belongs to the IL-10 superfamily and has antiviral activity similar to Type I IFNs. The aim of this study was to determine the functional role of IL-28Rα expression on epithelial cells in the pathogenesis of experimental colitis. Staining of IL-28Rα expression on human gut sections revealed an upregulation of the receptor expression during colitis on epithelial cells. Additionally, we found an upregulation of IRF7 a transcription factor for IL-28 in colitis patients compared to controls in specific CD11c populations. Further analysis in the mouse system confirmed the upregulation of the IL-28 signaling in the gut during experimental colitis. Gut sections from DSS WT and IL-28RKO mice showed a diminishment of epithelial cell proliferation suggesting a delayed wound healing process. Additional wound healing experiments underpin our findings regarding the decelerated wound closure after injury of the gut epithelium via STAT-1. Interestingly, topical administration of IL-28 onto the wound induces a better and faster closure of the lesion. Treatment with type III interferons may serve as a potential new therapeutic method to protect against chronic intestinal inflammation and promote wound healing processes in the context of colitis.

OR.32. Intestinal Epithelial Cell-Intrinsic Expression of Set-7 Controls Intestinal Homeostasis and Immunity Through the Hippo Pathway

Menno Oudhoff[1], Spencer Freeman[1], Amber Couzens[2], Frann Antignano[1], Fabio Rossi[1], Michael Gold[1], Anne-Claude Gringas[2], Colby Zaph[1]. [1]University of British Columbia, Vancouver, BC, Canada; [2]Mount Sinai Hospital, Toronto, BC, Canada

Post-translational lysine methylation is emerging as a regulatory mechanism to control protein function. However, the relevance of these modifications remains unclear. Set-7 is a lysine methyltransferase that has been shown to methylate and alter the function of a wide variety of proteins *in vitro*. To determine the

role of Set-7 *in vivo*, mice with an intestinal epithelial cell (IEC)-specific deletion of Set-7 were infected with the intestinal helminth Trichuris muris. We found that IEC-intrinsic deletion of Set-7 renders mice more resistant to infection with Trichuris. Surprisingly, the increased resistance observed was not associated with heightened immune responses but rather increased IEC proliferation and turnover. In the absence of Set-7, IECs displayed dysregulated Hippo pathway signaling, with increased nuclear accumulation of the Hippo transducer Yes-associated protein (Yap). Activation of the Hippo pathway-a regulator of proliferation and survival-results in the cytoplasmic sequestration of Yap. We show that Set-7 interacts with Yap and is required for its cytoplasmic retention. We have determined that Yap is mono-methylated at lysine 494 and this residue is critical for its subcellular localization. Together, these results identify a novel methylation-dependent checkpoint in the Hippo pathway that regulates IEC homeostasis and response to infection.

Oral Presentations: Thursday, July 18

OR.33. Vitamin A Controls the Fate and Function of Mucosal Innate Lymphoid Cells
Christoph Wilhelm, Sean Spencer, Jason Hall, Yasmine Belkaid. National Institutes of Health, Washington, MD

Innate lymphoid cells are important mediators for maintaining barrier integrity and are critically involved in repair mechanisms following infection induced tissue destruction. However, how these cells are regulated and controlled by local mucosal cues remains unclear. Increasing evidence suggests that the mammalian intestinal tract is tuned to use dietary signals to guide barrier immunity. We find that mice deprived of the essential dietary nutrient vitamin A show impaired numbers and function of Rorγt^{+} ILC (ILC3) and are consequently highly susceptible to infections with Citrobacter rodentium, a mouse model of attaching-effacing diarrheal disease. Conversely limiting vitamin A dramatically enhanced the accumulation of Th2 like innate lymphoid cells (ILC2) in the intestine. Further, *in vivo* and *in vitro* evidence demonstrates that retinoic acid directly enhances both the function and proliferation of ILC3 while actively suppresses the development of ILC2. All together our data propose that retinoic acid represents a key mucosal metabolite controlling the reciprocal fate and function of ILC subsets in the intestine and strengthens our understanding of the role of vitamin A as an essential dietary component and critical mediator of intestinal immunity.

OR.34. Innate Lymphoid Cells Regulate Mucosal Tissue Repair Through an Amphiregulin-EGFR Pathway
Laurel Monticelli, Lisa Osborne, Gregory Rak, Jerome Molleston, Elia Wojno, Gregory Sonnenberg, Theresa Alenghat, Carly Ziegler, E John Wherry, David Artis. University of Pennsylvania, Philadelphia, PA

While recent studies identified a role for Group 2 innate lymphoid cells (ILC2) in promoting pathologic type 2 inflammation in the skin, lung and intestine, the tissue-protective roles of ILCs at mucosal sites remain poorly understood. Using genome-wide transcriptional profiling, we identified that lung-resident ILC2s express multiple genes associated with wound healing and tissue repair, including the epidermal growth factor family member amphiregulin. Expression of amphiregulin was increased following influenza virus-induced lung injury in mice and depletion of ILCs during infection resulted in decreased amphiregulin expression, reduced lung function and impaired tissue repair in the lung. IL-33 promoted expression of amphiregulin in lung ILC2s and administration of exogenous amphiregulin effectively restored tissue remodeling in ILC-depleted mice, indicating that ILC-intrinsic amphiregulin is a central mediator of lung repair. Supporting this, chemical or genetic inhibition of the EGFR-amphiregulin pathway following viral infection resulted in severely impaired lung function and a failure to restore tissue homeostasis, leading to increased host mortality. Taken together, these data indicate a critical role for ILC2s in mediating lung tissue homeostasis though the IL-33-amphiregulin-EGFR axis and suggest that ILC2s provide a functional link between the signaling pathways that promote type 2 inflammation and tissue repair.

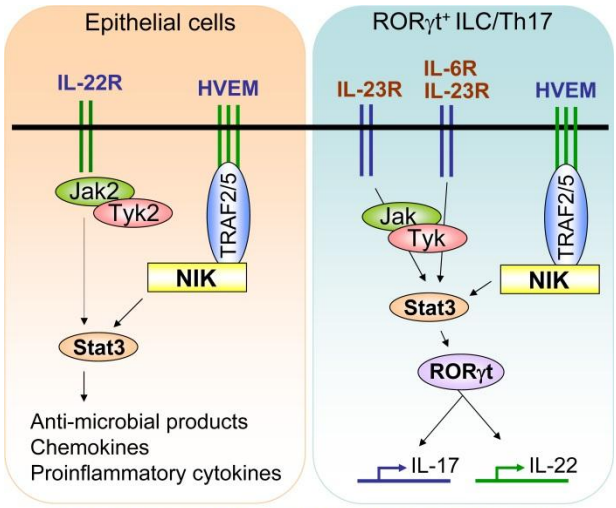
OR.35. Lyn Activity Protects Against Colitis and Colon Cancer and Regulates Innate Lymphoid Cell Function

Morgan Roberts, Jennifer Bishop, Jennifer Beer, Kenneth Harder. University of British Columbia, Vancouver, BC, Canada

The Lyn tyrosine kinase is a critical regulator of immune cell function and its dysregulation has been linked to cancer, yet the role of Lyn in regulating gastrointestinal inflammation and colon cancer has not been investigated. Utilizing immunosufficient and immunodeficient ($Rag^{-/-}$) Lyn knock-out and Lyn gain-of-function ($Lyn^{up/up}$) mice, we have identified a protective role for Lyn in innate immune cells in mouse models of colitis and colitis-associated cancer using the chemical irritant dextran sulfate sodium (DSS). Hypersensitivity to LPS, leading to increased production of protective factors including IL-22, may be an important mechanism of protection in $Lyn^{up/up}$ mice. In response to DSS or LPS treatment, IL-22 levels are increased in the blood and colon in $Lyn^{up/up}$ compared to wild-type mice. Studies using depletion strategies targeting DCs, innate lymphoid cells (ILC), and commensal flora showed that DCs, ILCs, and pattern-recognition receptor agonists are all required for increased IL-22 production by $Lyn^{up/up}$ mice. Furthermore, IL-22 production by ILCs correlated with Lyn activity in DCs. Overall, these data indicate a novel role for Lyn in the regulation of ILC biology and suggest that Lyn activity influences TLR-dependent activation of DCs and/or ILC populations that may limit intestinal inflammation and reduce colitis-associated cancer.

OR.36. HVEM is an Unusual TNF Receptor that Regulates Mucosal RORgt$^+$ Innate Lymphoid Cells and Th17 Cells During Intestinal Bacteria Infection

Jr-Wen Shui, Mitchell Kronenberg. La Jolla Institute for Allergy & Immunology, La Jolla, CA



HVEM, an unusual TNF receptor, regulates epithelial immunity and mucosal RORγt$^+$ cells via NIK-Stat3 signaling

We have recently shown that the herpes virus entry mediator (HVEM), a TNF receptor superfamily, plays an essential role in epithelial immunity and host defense against mucosal infections including intestinal Citrobacter rodentium and pulmonary Streptococcus pneumoniae (Nature 2012). We further identified that HVEM mediates mucosal host protection by inducing epithelial NIK (NFkB inducing kinase)-Stat3 activation. Here, we report a role for HVEM-Stat3 signaling in regulating mucosal RORgt$^+$ cells including CD3- innate lymphoid cells (ILCs) and Th17 cells, during intestinal bacterial infection. HVEM is expressed by various subsets of mucosal ILCs and HVEM deficiency significantly affects steady-state IL-22 production from RORgt$^+$ ILCs in the small intestine and, to a lesser degree, in the colon. Furthermore, HVEM deficiency has negative impact on mucosal IL-22 production from ILCs and Th17 cells during infection. HVEM co-stimulation promotes Th17 differentiation *in vitro* and $Hvem^{-/-}$ mice had impaired Th17 cell expansion at the later stage of infection. Therefore, our preliminary results indicate that HVEM-NIK-STAT-3 signaling, a novel pathway recently identified in epithelial cells, is also operative in mucosal RORgt$^+$ cells and is important for their mucosal functions during bacterial infection. Taken together, these findings establish HVEM as a crucial regulator of mucosa with diverse functions.

OR.37. Lung Dendritic Cells Induce Homing of Protective T Cells to the Gastrointestinal Tract

Darren Ruane[1], Lucas Brane[1], Bernardo Reid[2], Cheolho Cheong[4], Jordan Poles[1], Yoonkyung Do[5], Hongfa Zhu[1], Jae-Hoon Choi[6], Lloyd Mayer[1], Ed Lavelle[3], Ralph Steinman[2], Daniel Mucida[4], Saurabh Mehandru[1]. [1]Mount Sinai School of Medicine, New York, NY; [2]Rockefeller University, New York, NY; [3]Trinity College Dublin, Dublin, Ireland; [4]Institut de Recherches, Montreal, QC, Canada; [5]Ulsan National Institute of Science, Seoul, Republic of Korea; [6]Hanyang University, Seoul, Republic of Korea

Better vaccines for enteric infections are a global health priority. Our approach to developing more efficacious mucosal vaccines includes a study of dendritic cell (DC) induced cellular recruitment dynamics at mucosal surfaces. To explore alternative pathways of cellular homing to the GI tract, we have screened the ability of DCs from various tissues to recruit lymphocytes to the GI tract. Unexpectedly, we are able to confirm that lung DCs (LDC), like $CD103^+$ MLN DC, induce the expression of α4β7 *in vitro* and *in vivo*. i.n. vaccination significantly increased the expression of α4β7 on these transferred cells compared to s.c. immunization. LDCs enhanced the migration of adoptively transferred syngeneic cells to the GI tract *in vivo*, which was α4β7 dependent. α4β7 induction in the mediastinal LN and lung by day 4 post i.n. immunization corresponds to the appearance of transferred cells in the GI sites. LDCs, both $CD103^+$ and $CD11b^+$ subsets, express RALDH and induce α4β7 expression. Induction of α4β7 by LDCs was dependent on RA and TGF-β signaling. Consistent with a role of this pathway, targeting of LDC by i.n. immunization induced protective immunity against enteric challenge with a highly pathogenic strain of Salmonella. This present report defines an unexpected pathway of lymphocyte migration to the GI tract. Additionally, these data demonstrates a hitherto underappreciated cross talk between two mucosal compartments.

OR.38. Human Pathogen-Reactive MAIT Cells are Highly Enriched in the Lung, Suggesting a Unique Role in the Control of Respiratory Intracellular Infections

Marielle Gold[1], Prabhat Sharma[1], Sue Smyk-Pearson[1], Yvonne Eberling[1], Emily Wong[2], Deborah Lewinsohn[1], David Lewinsohn[1]. [1]Oregon Health & Science University, Portland, OR; [2]Massachusetts General Hospital, Boston, MA

Human MAIT cells express the semi-invariant TRAV1-2 TCR, are restricted by MR1, and detect infection with bacteria and fungi. MAIT cells were coined "mucosal" due to their presence in the gut lamina propria. However, their distribution, phenotype, and function, which presumably correlates with their role is poorly defined. We evaluated the distribution of $TRAV1\text{-}2^+$ and pathogen-reactive $TRAV1\text{-}2^+$ cells at peripheral and mucosal sites. On average, 5% of $CD8^+$ T cells from human blood and mediastinal lymph nodes expressed TRAV1-2 and on average 5% of these were pathogen-reactive. At mucosal sites, $CD8^+$ T cell frequencies were double that in the periphery. Moreover, the proportion of CD8s expressing $TRAV1\text{-}2^+$ was also doubled highlighting the enrichment of $TRAV1\text{-}2^+$ cells at mucosal sites. In spite of this enrichment, pathogen-reactive MAIT cell responses were rarely detected from the tissues of the small intestine including the mesenteric lymph nodes. In contrast, 20% of lung resident $TRAV1\text{-}2^+$ cells were pathogen-reactive, an overall 10-fold increase over T cells from peripheral sites. Finally, peripheral and mucosal MAIT cell subsets expressed unique phenotypic markers associated with their location and function. These results suggest pathogen-reactive MAIT cells are uniquely positioned to respond to respiratory infections.

OR.39. Immunity Against Respiratory Syncytial Virus Infection is Affected by Microbiome Composition in Mice

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Objective: Early bacterial colonization is necessary for development of neonatal immunity. Inadequate balance between microbiome composition and innate or adaptive immune system might contribute to RSV induced disease severity. To investigate if microbial composition affects RSV induced immune responses, microbiome alterations were studied in a mouse model for primary RSV infection and FI-RSV induced vaccination model for enhanced disease. Methods: Microbiome composition was altered in C57BL/6 mice using either dietary intervention with specific oligosaccharides (scGOS/lcFOS/pAOS) or broad spectrum antibiotic treatment during FI-RSV vaccination. Fecal taxonomic composition and lung RSV-specific immune responses were determined. Results: During primary RSV infection, dietary intervention increased the number of IFN-ɣ producing $CD4^+$ T cells 8 days post infection compared to control diet. Moreover, in the FI-RSV model, dietary intervention decreased lung total cell influx, eosinophilia and the number of IL-4, -5 and -13 producing $CD4^+$ T cells. Lower microbial diversity induced

by broad spectrum antibiotics during FI-RSV vaccination correlated with decreased IFN-ɣproducing $CD4^+$ and $CD8^+$ T cells six days after viral challenge. Conclusions: Specific modulation of microbial composition and diversity correlate with different host immunity responses against RSV and suggests that optimizing early microbial implementation may have an impact on the susceptibility to RSV induced disease.

OR.40. Why Pandemic Flu-Vaccines Based on M2e are Feasible and Could be Successful

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We generated a universal flu-vaccine candidate based on the CTA1-DD adjuvant genetically fused to the conserved matrix protein 2 ectodomain (M2e) of influenza A virus; CTA1-3M2e-DD. Following intranasal immunizations in Balb/c mice complete protection was achieved. M2e was found to be I-Ad restricted and despite strong M2e-specific antibody responses Balb/b congenic mice were poorly protected and had no M2e CD4 T cells. Thus, CD4 T cells appeared to contribute to protection in Balb/c mice. B cell-deficient JhD-mice also exhibited significant protection, supporting that M2e CD4 T cells had a protective function. The M2e CD4 T cells in Balb/c mice were oligoclonal and dominated by IL-17- and polyfunctional IFN-g and TNFa producing cells. M2e-tetramer binding studies revealed dramatic accumulation of M2e CD4 T cells in the lungs. Finally, IL-17A-deficient mice exhibited poor protective ability, while having strong serum M2e-antibody titers. Intranasal CTA1-3M2e-DD effectively stimulated memory CD4 T cells that after 1 year contributed to complete protection against live challenge infection. Protection was associated with greatly enhanced anti-hemagglutinin (HA) IgG antibody titers, suggesting exceptional helper functions of M2e CD4 T cells to naïve HA-specific B cells, suggesting an antigen-presenting function. Thus, M2e CD4 T cells exert both direct and indirect protective capacity

OR.41. The Crucial Role of Spi-B Transcription Factor in M cell Differentiation

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Gut-associated lymphoid tissue including Peyer's patches (PPs) is the inductive site for intestinal immunity. To evoke the antigen-specific immune responses, PPs incorporate luminal antigens. This task is accomplished by specialized epithelial cells, known as M cells, within the follicle-associated epithelium covering the lymphoid follicles of PPs. M cells possess a high capacity for transcytosis, which allows the rapid transport of antigens to dendritic cells. Thus, M cell-mediated antigen transport is important for the initiation of mucosal immune responses; however, the mechanisms of M cell differentiation have been poorly understood. Recently, exogenous treatment of mice with receptor activator of NF-κB ligand (RANKL) was shown to induce ectopic M cell differentiation in the villous epithelium of small intestine. We took advantages of RANKL treatment to identify M cell lineage-specific transcription factors, and found that Spi-B, an Ets family transcription factor, was highly upregulated shortly after RANKL treatment. Its expression was clearly observed in the nuclei of GP2-positive M cells. In $Spib^{-/-}$ mice GP2-positive M cells were completely absent. Furthermore we found that translocation of orally administered *Salmonella Typhimurium* was markedly decreased in PPs of $Spib^{-/-}$ mice. Consistent with the defect in transcytotic capability, *S. Typhimurium*-specific immune response was severely impaired in $Spib^{-/-}$ mice.

OR.42. Epithelial E-Cadherin Controls Gut Effector Treg

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Foxp3-expressing regulatory $CD4^+$ T cells (Treg) are essential to prevent damaging inflammation at the gut mucosa, but the mechanisms mediating the crosstalk between the intestinal epithelium and local Treg are still poorly understood. Here we identify E-cadherin, a component of epithelial intercellular junctions, as a key molecule regulating intestinal Treg. E-cadherin is recognised by two heterotypic receptors on T cells, the integrin CD103 and the lectin KLRG1. We used a mouse model where E-cadherin is replaced on gut epithelial cells by the related molecule N-cadherin. The mice developed colitis and accumulated effector $KLRG1^+$ Treg in the intestinal lamina propria. Germ-free mice lacking intestinal E-cadherin

remained free of inflammation but still showed an increased KLRG1^{+} Treg population in the gut, showing that the accumulation of KLRG1^{+} Treg is not a mere consequence of colitis. Accordingly, we did not find increased KLRG1^{+} Treg in two unrelated models of intestinal inflammation. KLRG1-deficient bone marrow chimeras showed a specific accumulation of KLRG1$^{-/-}$ Treg in the intestine but not in spleen or mesenteric lymph nodes. Together, these data show an essential role for E-cadherin in intestinal homeostasis and indicate that E-cadherin is involved in the epithelial-immune crosstalk in the gut.

OR.43. Intestinal M Cells are the Principal Means of Antigen Sampling for Initiation of Mucosal IgA Production to Commensal Enteric Bacteria

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RANKL is necessary and sufficient for inducing M cell differentiation by RANK-expressing intestinal epithelial precursor cells. To investigate how the specific loss of intestinal M cells perturbs host mucosal immunity, we generated conditional knockout mice with a floxed allele of RANK deleted only in intestinal epithelial cells (IEC) by a villin-cre transgene. In these RANKΔIEC mice, intestinal M cell differentiation was completely abrogated based on loss of staining for GP2^{+} M cells in the Peyer's patch (PP) dome and lack of expression of GP2 mRNA by the follicle-associated epithelium. Loss of M cells in RANKΔIEC mice was associated with a marked deficit in PP uptake of particulate antigens introduced by oral gavage including 0.2 µm fluorescent microspheres and FITC-labeled Lactobacillus rhamnosus bacteria. RANKΔIEC mice also had fewer lamina propria IgA^{+} plasma cells than control littermates, produced significantly less fecal IgA at all-time points after weaning and exhibited significant deficits in PP germinal center formation. To assess IgA responses to a model antigen expressed by a commensal enteric bacterium, we created a model system using an isolate of Enterobacter cloacae expressing ovalbumin (OVA) under the control of the anaerobically inducible nirB promoter. RANKΔIEC mice were unable to make fecal IgA specific for OVA after repeated intragastric gavage of OVA-producing *E. cloacae.* We conclude that particulate antigen sampling by intestinal M cells is a nonredundant mechanism required for efficient secretory IgA responses to antigens originating from the commensal enteric flora.

OR.44. A20/TNFAIP3 as a Brake on Intestinal Inflammation and Tumorigenesis

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Intestinal immune homeostasis is established through complex interaction between commensal bacteria, the intestinal epithelium and mucosal immune cells. A defective interaction between these compartments can result in intestinal pathology. A crucial mediator in establishing this homeostasis is the transcription factor NF-Ƙß, since it regulates multiple protective mechanisms in intestinal epithelial cells (IEC's), and pro-inflammatory responses in mucosal immune cells. NF-kappaB activation is tightly controlled by its negative feedback regulator A20, which in addition has pronounced anti-apoptotic functions. Genetic studies identified polymorphisms in the A20 locus associated with multiple inflammatory and auto-immune pathologies, including coeliac and Crohn's disease. To investigate the physiological role of A20 in intestinal immune homeostasis, we generated tissue specific A20 knockout mice. We found that A20 has predominant anti-apoptotic functions in IEC's and predominant anti-inflammatory functions in myeloid cells. By deleting A20 in both IEC's and myeloid cells, we generated a novel mouse model of intestinal inflammation which is characterized by early Paneth and goblet cell loss. Older mice suffer from ileitis and severe colitis which often progresses to colorectal cancer development. These findings suggest that defects in proper A20 function may contribute to the development and progression of inflammatory bowel disease and cancer in humans.

OR.45. Epigenetic Regulation of Gut Macrophages in Inflammatory Bowel Disease
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Introduction: Intestinal macrophages (IMACs) are an integral part of first-line gut defence. A major feature of IMACs 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 cell samples (LPMCs) were obtained from gut tissue resections of healthy control (HC) and Crohn's disease (CD) patients. Gut macrophages were purified using positive selection with anti-CD33 antibodies and analysed using chromatin immunoprecipitation (ChIP-PCR) assays 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. Additionally, the repressed state of tnfa promotes in HC macrophages was associated with increased binding of H3K9me3 and H3K9me1. 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 proinflammatory cytokines.

OR.46. HDAC Dependent Regulation of the IL-6 Receptor Expression in Experimental Colitis
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While several histone deacetylase (HDAC) inhibitors are currently in clinical cancer studies, an additional anti-inflammatory potency has been demonstrated in various murine colitis models. In the presence of ITF2357, the generation of $FoxP3^+$ cells could be enhanced, the polarization to the pro-inflammatory Th17 cells suppressed. In parallel, we demonstrated a dose-dependent down-regulation of the IL-6 receptor on naïve CD4 T cells treated with ITF2357 on the mRNA expression level and on the protein level via flow cytometry. Furthermore, ChIP assays revealed changes in the chromatin acetylation at the IL-6R gene. Consequently, HDAC inhibition resulted in a reduced STAT3 phosphorylation as well as RORgammaT expression, identifying the IL-6/STAT3/IL-17 pathway as an important target of HDAC inhibitors. These results were confirmed in murine colitis models, where the IL6R expression was diminished on naïve T cells, paralleled by a significant reduction of Th17 cells in the lamina propria of ITF2357-treated animals resulting in reduced inflammation signs. The present study demonstrates that inhibition of HDAC exerts an anti-inflammatory potency in experimental colitis by modulating T helper cell polarization via targeting the IL-6 pathway.

OR.47. B Cell-Derived Cholinergic Signaling Regulates Local Innate Immune Responses
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Background: Inhibition of immune response occurs through several mechanisms, including neuro-transmitters like acetylcholine (Ach). Previous studies demonstrated that Ach is produced by specific $CD4^+$ T cells and reduces mortality during septic shock. Aim: To evaluate Ach signaling in other immune cells, including B cells, and their role in mediating innate and adaptive immunity. Results: We determined that choline acetyltransferase (ChAT) is expressed and Ach is produced by B cells. ChAT expression in immune cells is dependent on microbial colonization, and requires MyD88-dependent signaling. Unlike T cells, $ChAT^+$ B cells release Ach after stimulation with sulfated cholecystokinin but not norepinephrine. Interestingly, despite the requirement for MyD88 signaling to induce ChAT expression, TLR agonists were not capable of inducing Ach release. In addition, $ChAT^+$ B cells were able to reduce peritoneal neutrophil recruitment during sterile endotoxemia, independent of the vagus nerve, without impacting cytokine or chemokine production. Endotoxemia was induced in mice with conditional ablation of ChAT in B cells causing increased TNFα production versus WT. Immune inhibition by Ach is limited to innate immunity, as Ach released from immune cells did not suppress effector T cell responses *in vivo*. Conclusion: Ach

produced by lymphocytes has specific context dependent functions, with ChAT$^+$ B cells controlling the local neutrophils recruitment.

OR.48. Epigenetic Licensing of T_H17 and T_{reg} Cell Differentiation During Intestinal Inflammation
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Naïve CD4$^+$ T helper (T_H) cells acquire a range of cellular fates and functions depending upon the cytokine milieu and anatomical location. For example, interleukin-17-producing T_H17 and Foxp3$^+$ regulatory T (T_{reg}) cells are dependent upon transforming growth factor-β1 (TGFβ1) and are critical for intestinal homeostasis. Although the molecular mechanisms associated with T_H17 and T_{reg} cell differentiation are becoming clearer, the epigenetic regulation of these subsets is unknown. We demonstrate that the histone lysine methyltransferase G9a restricts T_H17 and T_{reg} cell differentiation *in vitro* and *in vivo*. G9a-mediated dimethylation of histone H3 lysine 9 (H3K9me2), a repressive histone modification, is found at high levels in naïve T_H cells and is lost following T_H17 and T_{reg} cell activation. In the absence of G9a, T_H cells display heightened sensitivity to TGFβ1 and fail to induce intestinal inflammation. We conclude that G9a-dependent H3K9me2 is a homeostatic epigenetic checkpoint that controls the magnitude of T_H17 and T_{reg} cell responses.

OR.49. G9a is Required for Natural Helper Cell Dependent Lung Inflammation
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Allergic asthma is a pulmonary inflammatory disease dependent on eosinophil infiltration into the lung. Allergic asthma is thought to be driven by Th2 cell cytokines including IL-4, IL-5 and IL-13; however, recent studies have found lung natural helper (LNH) cells are a source of Th2 cell-type cytokines after protease allergen challenge. G9a is a histone lysine methyltransferase with di-methyl activity towards H3K9 that is linked to transcriptional repression. We have previously shown that G9a is required for efficient Th2 responses. We hypothesized that G9a may also be required for efficient Th2-type cytokine production from LNH cells and for allergic lung inflammation. We isolated LNH cells from WT and G9a$^{-/-}$ mice and stimulated them with TSLP and IL-33. G9a$^{-/-}$ LNH cells were defective in their ability to produce IL-5 and IL-13. In addition, when we treated G9a$^{-/-}$ mice intranasally with papain, eosinophil influx into the BAL was reduced and production of IL-15 and IL-13 mRNA in the lung was attenuated. Lastly, we employed a mouse model of house dust mite allergy and found a significant protection from disease in the G9a$^{-/-}$ mice. In summary, our data suggest that G9a enhances LNH cell responsiveness, which contributes to the development of allergic inflammation.

OR.50. CD103 in the Regulation of Inflammation in Allergic Airway Inflammation
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The regulatory mechanisms of airway inflammation are not clearly understood. CD103 is expressed by dendritic cells (DCs) and regulatory T cells (Tregs) and recently emerged as a regulator of inflammation in the gut. To verify if this molecule plays a role in allergic airway inflammation, asthma was induced in CD103$^{-/-}$ and wild type (wt) controls using two classic mouse models: the ovalbumin (OVA) model and the house dust mite (HDM) model. The severity of inflammation was analyzed via total cell and differential counts, histology and flow cytometry. Interestingly, CD103$^+$ DCs and Tregs (natural and inducible) were largely increased in wt mice exposed to both antigens. Also, CD103$^{-/-}$ mice showed striking exacerbated inflammation in both models characterized by increased total inflammatory cells and eosinophils in the bronchoalveolar lavage (HDM model total cells: 1.0 ±0.49x10^6 in wt vs 1.9 ±0.45x10^6 in CD103$^{-/-}$; $p = 0.03$ n= 4-5 mice/group), increased tissue inflammation and increased *ex-vivo* cytokine production. Furthermore, a delay in the resolution of airway inflammation was observed in CD103$^{-/-}$ mice. Our results

support that CD103 plays an important role in the regulation of inflammation in the lung by promoting the resolution phase of allergic inflammation in response to OVA and HDM.

OR.51. Group 2 Innate Lymphoid Cells (ILC2) Facilitate the Development of Th2 Responses to Local but not Systemically Delivered Antigens

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Introduction: Group 2 Innate Lymphoid Cells (ILC2s) have been characterized as strong producers of Th2 cytokines and may represent a link between innate and adaptive immunity that directs Th2 responses. ILC2s express high levels of the retinoic acid receptor-related orphan nuclear receptor-α (Rora), and ROR-α deficient mice lack ILC2s. We intend to examine the potential role of ILC2s in the development of allergic airway inflammation (AAI) using wild-type (WT) and ROR-α deficient mice. Methods: Mice were evaluated in either a house dust mite (HDM) model of AAI that consisted of intranasal priming and repeated intranasal challenges of HDM antigen, or through an Ovalbumin/Alum model of AAI consisting of systemic priming with Ova/Alum followed by intranasal challenges with Ova. Results: ROR-α deficient mice were protected from the development of HDM-induced AAI, displaying reduced accumulation of eosinophils into the airways and lungs, and reduced serum IgE levels. Interestingly, in the Ova/Alum model of AAI, ILC2-deficient mice developed normal airway eosinophilia and Th2 responses but had impaired production of Th17 responses. These results suggest to an important role for ILC2s in the local priming of Th2 responses in the lung but are dispensable for systemically immunized responses.

OR.52. Pulmonary Over-Expression of Oncostatin M (OSM) Promotes iBALT Formation Independently of IL-6 and Induces a Th2 Skewed Antibody Response at Mouse Lung Mucosa

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Responses to respiratory infections and diseases with pulmonary inflammation are associated with the increased presence of induced Bronchus-Associated Lymphoid Tissue (iBALT). Formation of iBALT can be induced in animal models of IL-6 over-expression, and we have assessed the role of IL-6 family member Oncostatin M (OSM) in B cell activation, antibody levels and iBALT formation in mouse lungs. Pulmonary overexpression of OSM in C57Bl/6 mice using adenovirus vector (Ad-mOSM) caused marked increases in Th2 cytokines (IL-4, IL-5), chemokine levels (Eotaxin-2, KC and MCP-1) and inflammatory cells in lungs. iBALT formation (IF using B220, CD3, and FDCM-1), B cell, T cell and dendritic cell number elevation and increased activation markers (FACS analysis) were induced by Ad-mOSM but not control vector (Addel70) in wt and in IL-$6^{-/-}$ and $STAT6^{-/-}$ mice. Serum neutralizing antibody to Adenovirus was elevated after primary challenge with Ad-mOSM (not Addel70) and increased 10-fold further upon subsequent challenge with Addel70. Furthermore, anti-Adenovirus isotypes detected in BAL showed high IgG1/IgG2A ratios by Ad-mOSM. Collectively, these results support the ability of OSM to induce B cells and iBALT formation independently of IL-6 and STAT6 and to cause a skewing of mucosal immunity toward Th2 antibody profile in mouse lungs.

OR.53. RELM-β Contributes to Host Defense During Citrobacter Rodentium Infection by Recruiting $CD4^{+}$ve T Cells to the Infected Colon

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RELM-β is a goblet cell specific mediator, known to contribute to the expulsion of lumen dwelling parasites from the intestine, however, its role in host defense against enteric bacterial pathogens has not yet been studied. RELM-β deficient $^{(-/-)}$ mice were infected with Citrobacter rodentium, a natural bacterial pathogen of mice. By day 8 post-infection (pi), RELM-$\beta^{-/-}$ mice suffered severe colonic ulcers, in concert with impaired induction of intestinal epithelial cell (IEC) proliferation and reduced recruitment of $CD4^{+}$ T

cells to the infected colon. T cell deficient RAG1$^{-/-}$ mice are normally impaired in IEC proliferation during C. rodentium infection, whereas reconstitution of CD4^{+} T cells led to increased IEC proliferation, suggesting CD4^{+} T cells are sufficient to promote IEC proliferation during infection. *In-vitro* Transwell™ migration assays revealed a direct chemotactic role for RELM-β (50ng/ml) in recruiting CD4^{+} T cells. Remarkably, intra-rectal reconstitution of recombinant RELM-β (2μg/ml) in RELM-β$^{-/-}$ mice completely protected the mice from infection induced ulceration, in concert with increased CD4^{+} T cell recruitment and increased IEC proliferation within the infected colon. Together, these results indicate that RELM-β plays a novel role in protecting the host intestine during a bacterial infection, by driving both CD4^{+} T cell recruitment, and promoting IEC homeostatic responses.

OR.54. Constitutive Induction of Intestinal Tc17 Cells in the Absence of MHC Class II is not Sufficient to Protect Against *C. Rodentium* Infection

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The enteric pathogen Citrobacter rodentium induces a mucosal IL-17 response in CD4^{+}T helper (Th17) cells that is dependent on the Nod-like receptors Nod1 and Nod2. Here, we sought to determine whether this early Th17 response required antigen presentation by major histocompatibility complex (MHC) class II for full induction. At early phases of C. rodentium infection, we observed that the intestinal mucosal Th17 and Th22 response was fully blunted in irradiated mice reconstituted with MHCII-deficient (MHCII$^{-/-}$→WT) hematopoietic cells. Surprisingly, we also observed a substantial increase in the number of IL-17^{+}CD8^{+}CD4-TCRβ^{+} cells (Tc17 cells) and FOXP3^{+}CD8^{+}CD4-TCRβ^{+} cells in the lamina propria of MHCII$^{-/-}$→WT mice compared to WT→WT counterparts. Moreover, MHCII$^{-/-}$→WT mice displayed increased susceptibility, increased bacterial translocation to deeper organs and more severe colonic histopathology after infection with *C. rodentium*. Finally, a similar phenotype was observed in mice deficient for CIITA, a transcriptional regulator of MHCII expression. Together, these results indicate that MHCII is required to mount early mucosal Th17 responses to an enteric pathogen, and that MHCII regulates the induction of atypical CD8^{+} T cell subsets, such as Tc17 cells and FOXP3^{+}CD8^{+} cells, *in vivo*.

OR.55. Vaccination Does not Deter the Early Invasion of Peyer's Patches by Salmonella

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For many infectious diseases, vaccination has proven a powerful mean to protect the host from clinical symptoms. Still, we often lack the tools to reliably track the course of the infection in immunized individuals and consequently an understanding the mechanisms of vaccine-mediated protection. Here we report a novel approach to quantify the number of *Salmonella enterica serovar Typhimurium (S. Typhimurium)* invading host tissues and to track bacteria population dynamics. An inoculums consisting of 23 genetically tagged Salmonella strains of identical pathogenicity was used for infection, where after the representation of individual clones in different compartments was determined by next generation sequencing. The stochastic variation of clonal frequencies was used to quantify the number of bacteria founding the infection in Peyer's patches, mesenteric lymph nodes, liver and spleen. Interestingly, our analysis implied that vaccination did not significantly reduce the number of bacteria invading Peyer's patches even though the total colony forming units were largely reduced in vaccinated compared to non-vaccinated mice. Furthermore, the representation of individual clones was similar in Peyer's patches and mesenteric lymph nodes but differed from systemic compartments, indicating different routes of Salmonella dissemination. Thus, our data suggest that Salmonella directed humoral responses are insufficient to protect intestinal tissues from Salmonella invasion. Our approach provides a new way to resolve pathogen population dynamics.

OR.56. Hyperglycemia-Induced Enteric Dysbiosis and iNOS Contribute to Klebsiella Pneumonia Liver Abscess

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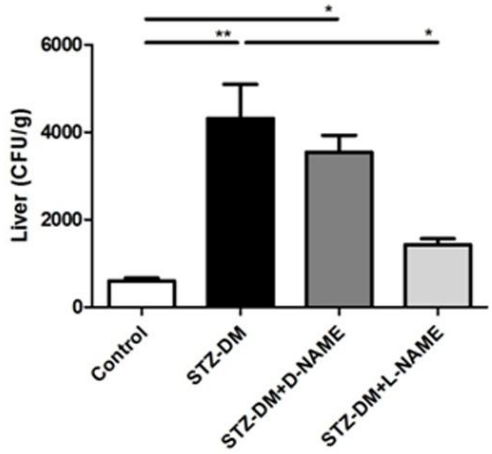
Klebsiella pneumonia (KP) is the most common pathogen of pyogenic liver abscess in East and Southeast Asia and diabetes mellitus (DM) is a major risk factor. The effect and mechanism of hyperglycemia on KP liver abscess was examined in streptozotocin-induced diabetic mice and diabetic Akita mice (C57BL/6J-Ins2Akita). KP translocation to liver and plasma alaine transaminase levels were increased and hepatic clearance of KP was decreased in DM mice. Diabetic mice exhibited overgrowth of Enterococcus and Enterobacteriaceae in intestine, increased intestinal iNOS activity as well as nitrite in portal vein, and increased activation of Kupffer cells. Germ-free mice showed decreased nitrite production and Kupffer cell activation. Oral antibiotic treatment decreased the growth of Enterococcus and Enterobacteriaceae in intestine and reversed hyperglycemia-induced nitrite production and KP translocation in DM mice. Moreover, L-NAME but not D-NAME given in drinking water decreased intestinal iNOS activity, nitrite production, as well as Kupffer cell activation and increased liver clearance of KP in DM mice. Collectively, the balance of intestinal microflora is important for preventing KP liver abscess. Hyperglycemia induces intestinal dysbiosis, iNOS activity, Kupffer cell activation, and KP liver abscess. These findings suggest that increased KP liver abscess in DM could be prevented by iNOS inhibitors.



OR.57. Modeling the Distinct Roles for IL-23 and IL-17 in Inflammatory Bowel Disease Using Mdr1a$^{-/-}$ Mice

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Th17 cells are implicated in a number of autoimmune diseases including IBD. IL-23 drives expression of IL-17 in Th17 cells. IL-23 and IL-17 are elevated in IBD and SNPs in the IL-23R show strong disease association. We compared inhibition of IL-23p19 and IL-17RA in the Helicobacter bilis-infected mdr1a-/- mouse model of colitis. IL-23 inhibition provided strong efficacy while IL-17RA antagonism exacerbated disease. To understand which IL-17 cytokines are responsible, we compared inhibition of IL-17RA with inhibition of IL-17A, IL-17F or IL-25. IL-17A inhibition alone resulted in disease exacerbation similar to IL-17RA inhibition. IL-23 inhibition decreased expression of cytokines while IL-17RA inhibition increased expression of many genes including IFNγ and TNFα. Inhibition of IFNγ was highly efficacious; however, IFNγ blockade did not affect disease exacerbation with anti-IL-17RA treatment suggesting exacerbation is not due to a shift from Th17 cells to Th1 cells. IL-17RA inhibition did not exacerbate disease in uninfected mdr1a$^{-/-}$ mice suggesting a possible role for IL-17A in maintenance of barrier integrity. These data are similar to what is emerging from Crohn's disease clinical trials: IL-12/23p40 inhibitors are showing promise while inhibition of IL-17A exacerbated disease in a subset of patients.

OR.58. Hsp65-Producing Lactococcus Lactis Prevents Experimental Colitis by IL-10- and TLR2-Dependent Pathways

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To maintain intestinal health, the immune system must faithfully respond to antigens from pathogenic microbes, while maintaining a state of tolerance to commensals and food antigens. However, disruption of this delicate balance can cause inflammatory bowel disease (IBD) in genetic susceptible hosts. Heat shock proteins (Hsp) are conserved proteins that are highly expressed in inflammation and, thus, good therapeutic antigenic targets for oral tolerance in inflammatory diseases. The aim of this study was to investigate the effects of Hsp65-producing *Lactococcus lactis* in murine colitis. We used *L. lactis*

genetically engineered to deliver Hsp65 in the gut. Mice received oral treatment with Hsp65-*L. lactis* during four days. Ten days thereafter, colitis was induced by 1.5% dextran sodium sulfate (DSS). Oral administration of Hsp65-*L. lactis* completely prevented colitis. Protection was accompanied by diminished levels of TNF-α, IL-6 and IL-4 and by enhanced IL-10 levels in the colonic mucosa. Moreover, IL-10 deficient mice Hsp65-*L. lactis* show no improvement in the histological score. The same was observed in TLR2$^{-/-}$ but not in TLR4$^{-/-}$ mice. Interestingly, frequency of lamina propria B cells expressing IL-10 enhanced in mice treated with Hsp65-*L. lactis*. In conclusion, Hsp65 delivered by *Lactococcus lactis* prevents intestinal inflammation via IL-10 and TLR2 pathways.

OR.59. Heat Shock Protein 90 Inhibition Attenuates Murine Colitis via Suppression of TNF

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While antibody-based inhibition of tumor necrosis factor (TNF) has proven beneficial in many inflammatory bowel disease (IBD) patients, these therapies lose considerable efficacy within 12 months leaving an unmet need for alternative approaches. In studies using both small and large intestinal murine models of IBD we demonstrate an anti-inflammatory effect of selective inhibition of the C-terminal ATPase of heat shock protein 90 (HSP90) using novobiocin. Treatment with novobiocin attenuated both DSS-induced colitis and CD45RB^{high} adoptive transfer colitis via suppression of inflammatory cytokine secretion including TNF. *In vitro* assays demonstrate the CD4^{+} T cells treated with novobiocin produced significantly less TNF measured by flow cytometry and ELISA. Finally to verify the anti-TNF action of novobiocin, 20-week-old TNF$^{\Delta ARE}$ mice were treated for two weeks with subcutaneous administration of novobiocin 40mg/kg/day delivered by osmotic pump. This gene-targeted model expresses over-stabilized TNF mRNA resulting in spontaneous ileitis reminiscent of human Crohn's disease. Novobiocin significantly reduced histological evidence of inflammation and inflammatory cell infiltration as confirmed by flow cytometric analysis of leukocytes in the ileal lamina propria. In conclusion, HSP90 inhibition with novobiocin offers a novel method of inflammatory cytokine suppression without potential for the development of tolerance that limits current antibody-based methods.

OR.60. Adoptive Transfer of CD4^{+} TNFα-KO T Cells Exacerbate the Ulcerative Uolitis in the Wrag Mouse Model

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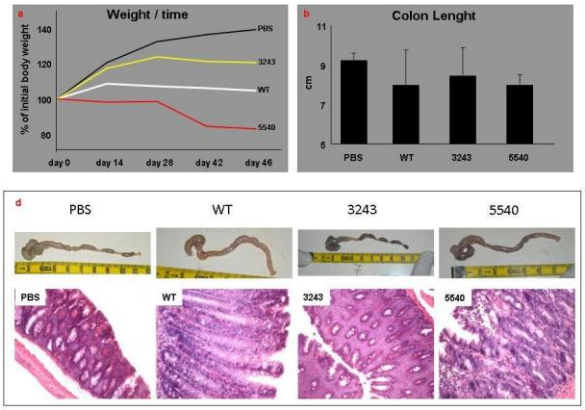

The inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are intestinal multi-factorial disorders with complex genetic and environmental etiology, commonly associated with aberrant mucosal immune response, resulting from epithelial cell defects. The epithelial monolayer is a barrier between lumenal antigens and the intestinal immune system. Recent data demonstrated the importance of the inner mucus layer, extremely rich in Muc2, in maintaining a bacteria-free epithelial cell surface. Mutated "Winnie" mice are characterized by a missense mutation in the Muc2 gene. In these mice, the defect in the mucus layer generate the epithelial exposure to the luminal content that progress in chronic intestinal inflammation equivalent to the human ulcerative colitis. Our collaborator previously described that loss in T and B lymphocyte (Wrag mice) did not prevent the spontaneous inflammation. This observation suggests that it is not the adaptive immune response to initiate the inflammatory response. To evaluate the role of TNFα in the inflammatory cascade, we adoptively transferred naïve CD4^{+} T cells obtained from C57/Bl6, TNFα-KO or TNFαR-KO mice into Wrag recipient. Mice were observed for the following 6 weeks. As previously described, inflammation and fibrosis were

present in the terminal colon following WT $CD4^+$ T cells adoptive transfer. Interestingly, the adoptive transfer of TNFα-KO $CD4^+$ cells severely increased inflammation and fibrosis while TNFαRKO $CD4^+$ cells appeared to play a protective role. By intracellular staining, TNFαR-KO T cells appeared high TNFα producers but no difference in IFNγ, IL-4, IL-10, IL-17a or Foxp3 was detected as compared to WT or TNFαKO. This study suggests that T cells derived TNFα may explicit a protective rather than pathological role in the UC.

OR.61. Genetically-Determined Cytokine Programs of Human NK Cells Uncover a Novel Mechanism of NK Cells and Killer Cell IgG-like Receptor (KIR) Genes in Crohn's Disease (CD) Susceptibility

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Mucosal innate lymphocyte subsets have emerged as an important new factor in CD pathogenesis, but the role of NK cells is poorly understood. A recent genetic study identified the KIR gene KIR2DL2/3 as a risk factor for CD in the genetic context of its ligand HLA-C1. However, the mechanism of this genetic contribution is unknown. NK cells are mainly known for their cytolytic function, but recent work indicates that strong KIR/HLA interactions that induce NK "licensing" augment NK cytotoxicity and IFN-γ production. We therefore tested the hypothesis that NK licensing by KIR2DL2/3 and HLA-C1 induces a broader cytokine program that modifies the threshold of pro-inflammatory T cell activation. Single cell multiplex functional proteomics revealed that licensed and unlicensed NK cells had strikingly distinct cytokine programs. Licensed NK cells were distinguished by high-output, proinflammatory, poly-cytokine expression. Specific cytokines among this output fully accounted for their unique capacity to efficiently induce $CD4^+$ T cell proliferation and IL-17A production. In conclusion, NK licensing mediated by KIR2DL2/3 and HLA-C1 elicits a novel cytokine program of NK cells that activates and induces pro-inflammatory $CD4^+$ T cells, and therefore provides a predisposing biologic mechanism for this KIR-associated susceptibility to CD and other chronic inflammatory syndromes.

OR.62. Fetal Programming of the Intestinal Epithelium Towards Inflammation-Driven Pathology

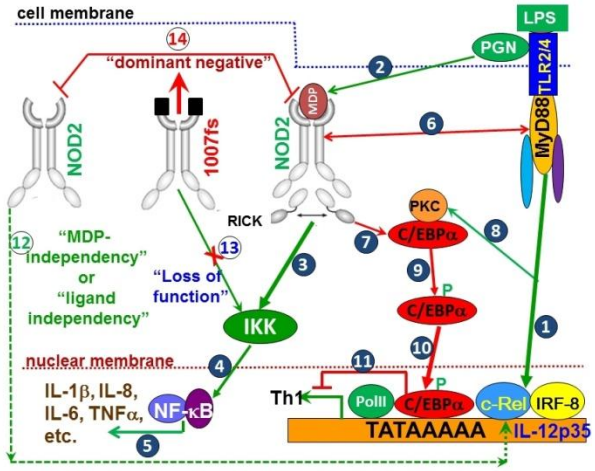
Jana Hemmerling[1], Katharina Heller[1], George Kollias[2], Dirk Haller[1]. [1]Technische Universität München, Freising- Weihenstephan, Germany; [2]Biomedical Sciences Research Centre, Vari, Greece

Chronic inflammatory disorders increase in incidence over the past decades following geographical patterns of industrialization. Fetal exposure to inflammation may alter organ function and the offspring's disease risk. We studied the role of TNF-driven maternal inflammation in programming the fetal gut epithelium towards Crohn's disease like-ileitis. Female $Tnf^{+/+}$ mice (WT) were bred with male $Tnf^{\Delta ARE/+}$ mice (ARE) and vice versa, generating offspring from healthy WT and inflamed ARE dams (iWT and iARE). Microarray and gene ontology analyses were performed on laser microdissected intestinal epithelial cells 17.5 days post conception, demonstrating that maternal inflammation differentially regulated 2174 (iWT) and 3345 (iARE) genes in fetal tissue. Reg3b and Fabp6 were top regulated genes. Fetal transcriptional profiles were overwritten in the postnatal environment dominated by tissue inflammation. Ileal pathology was evaluated in $Tnf^{\Delta ARE/+}$ mice deriving from high and low disease-susceptibility conditions, demonstrating that maternal inflammation accelerates Crohn's disease like ileitis. Loss of REG3B significantly correlated with the grade of tissue inflammation (R2=0.98) and was most importantly affected by fetal exposure to maternal inflammation. In conclusion, maternal inflammation highly impacts the fetal transcriptional program in the intestine and accelerates the pathology of TNF-driven ileitis in a mouse model for Crohn's disease-like ileitis.

OR.63. Crosstalk Between TLR4- and NOD2-Mediated Signaling in Inflammatory Bowel Disease: A Novel "Gain of Function" of the Crohn's Disease-Associated 1007fs Mutant

Xiaojing Ma[1], Hajeong Kim[2]. [1]Weill Cornell Medical College, New York, NY; [2]Shanghai Jiao Tong University, Shanghai, China

TLR-mediated signaling plays a major role in distinguishing self from non-self at the mucosal surface whereas NOD-mediated signaling is believed to supplement the surface defense by providing a second tier, intracellular resistance mechanism. Largely unappreciated but no less important, however, is the crosstalk between TLR4- and NOD2-mediated signaling in maintaining the equilibrium between a strong capacity to fight off pathogenic invasions and a delicate ability to control exacerbated inflammation and prevent tissue damage. Our recent work in this area has uncovered a novel interaction between these two pathways and identified an important inter-connecting transcriptional regulator, C/EBPa, that converge on the control of the production of Interleukin-12 (IL-12), a cytokine not only critical for T cell responses against infectious agents but also detrimental to the host when inappropriately produced. Furthermore, how the CD-associated NOD2 mutants contribute to the development and pathogenesis of the disease is a very controversial subject. We have revealed an "acquired activity" of the 1007fs NOD2 mutant, which disrupts the crosstalk between TLR4- and NOD2-mediated signaling in a previously unappreciated regulatory circuitry for cytokine response, mucosal defense, and homeostatic preservation. These findings will likely lead to novel biological concepts, and innovative ways for therapeutic intervention in CD.



OR.64. The Inflammatory Response of Human Lamina Propria Myeloid Cells to Loss of Epithelial Cells

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Loss of the intestinal epithelial layer (LEL) is associated with an inflammatory response in resident human lamina propria immune cells including myeloid cells (LPMO) as documented by the up-regulation of pattern recognition receptors (e.g. CD14, TLR2), costimulatory molecules (e.g. CD80/86) as well as soluble mediators (e.g. IL-1β, IL-6, MIP-1b). To further characterize this inflammatory response as well as to identify associated signaling pathways global gene expression analysis was performed in LPMO emigrated from cultured mucosal specimens denuded of epithelial cells. In comparison to autologous blood monocytes (PBMO), these LEL-activated LPMO expressed high levels of genes known to be associated with a dendritic cell phenotype: FLT3, LAMP3, CD80/CD86, CCR7. With regard to functional properties, high transcript numbers of CCL19, CCL20, IL-23 p19, and EBI3 are consistent with an important role of these LPMO in the regulation of an intestinal immune response. Preliminary signal pathway analysis hints at a potential contribution of the OX40/OX40L pathway to the activation of LPMO by LEL.

Oral Presentations: Friday, July 19

OR.65. The Microbiota Regulates the Trans-Epithelial Delivery of Luminal Antigens via Goblet Cells

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The mucosal immune system must maintain tolerance to innocuous antigens, such as those derived from the diet while remaining poised to mount inflammatory responses to potential pathogens. Using intravital two-photon imaging to better understand how these antigens are delivered across the epithelium we recently showed small intestine (SI) goblet cells (GC) formed goblet cell-associated antigen passages (GAPs) and delivered small soluble antigens to tolerogenic lamina propria dendritic cells. Further studies revealed GAP formation results when GCs secrete in response to muscarinic acetylcholine receptor 4 (mAChR4) signaling and that mAChR4 signaling and GAP formation was inhibited in colonic GCs in SPF

housed mice. We observed inhibition of GAP formation in the colon was GC intrinsic involving TLR trans-activation of the EGFR, p42/p44 MAPK activation, and inhibition of mAChR4 signaling. Impairing microbial sensing via antibiotic treatment, gnotobiotic housing, or deletion of MyD88 reversed the inhibition on mAChR4 signaling and allowed colonic GAPs to form. Additionally, colonic GAPs develop in SPF housed neonatal mice through the weaning period, a time characterized by quantitative and qualitative changes to the microbiota. Like SI GAPs, colonic GAPs recruited dendritic cells to the epithelium to acquire luminal antigens to stimulate T cell proliferation. This regulation of GAPs would allow sampling of luminal antigens when the environment is permissive, such as in the homeostatic small intestine, but would prevent sampling luminal antigens in a hostile environment, such as the high microbial load in the colon or during infection.

OR.66. A Human Microbiota-Associated Mouse Model of Asthma

Marie-Claire Arrieta[1], Shannon Russell[1], Eric Brown[1], Manish Sadarangani[2], Brett Finlay[1]. [1]University of British Columbia, Vancouver, BC, Canada; [2]British Columbia Children's Hospital, Vancouver, BC, Canada

Recent data indicate that the intestinal microbiota has a profound impact on the immune system, and of allergic diseases such as asthma. One of the challenges of studying the microbiota in mice is that it differs from humans, and using traditional murine models may not generate relevant clinical information. Thus, we generated a mouse model to study asthma by colonizing 3 to 5-week-old germ-free mice with infant human feces. Mice were sensitized systemically with ovalbumin (OVA) and later challenged with OVA intranasally to induce asthma. Disease severity was determined by measuring cytokine and humoral profiles, and the bronchial alveolar fluid assay. Although a better microbiota transfer was achieved with mouse feces, a large proportion of human microbiota was successfully transferred. There was an overall decrease in systemic immune response towards the human microbiota, as well as a significant decrease in IgA secretion in the gut. Colonization of human microbiota induced less severe lung inflammation in mice, although different human microbiotas induced different degrees in asthma severity. ‘Humanizing' mice with human microbiota resulted in a model that develops lung inflammation characteristic of asthma. Our laboratory is currently studying the effect of inoculating germ-free mice with feces from severe asthmatic vs. normal children.

OR.67. Commensal Bacteria Induce a Barrier Protective Response to Prevent Sensitization to Food Allergens

Taylor Feehley[1], Andrew Stefka[1], Prabhanshu Tripathi[1], Kathy McCoy[2], Sarkis Mazmanian[3], Goo-Young Seo[1], Betty Theriault[1], Dionysios Antonopoulos[1], Eugene Chang[1], Cathryn Nagler[1]. [1]University of Chicago, Chicago, IL; [2]University of Bern, Bern, Switzerland; [3]California Institute of Technology, Pasadena, CA

Environmentally-induced alterations in the commensal microbiome have been implicated in the increasing prevalence of food allergy. We found that antibiotic treatment of neonatal mice leads to reduced proportions of regulatory T cells (Tregs) in the colonic lamina propria (LP), impaired production of intestinal IgA, and elevated peanut (PN) specific IgE/IgG1 in response to sensitization. Selective colonization of gnotobiotic mice linked the Treg/IgA inducing capacity to a consortium of bacteria within the Clostridia class. Introduction of either a conventional SPF microbiota or a mixture of Clostridia strains to antibiotic-treated mice restored the Treg and IgA compartments and blocked the induction of a food allergic response. Clostridia colonized gnotobiotic mice displayed increased expression of IL-23 and IL-22 in the colonic LP compartment and the induction of anti-microbial RegIIIβ expression in the epithelium. Collectively, these results suggest that the maintenance of oral tolerance to dietary antigens relies on bacterial populations that induce a barrier protective response, which includes activation of the IL-23/IL-22 axis and the expansion of intestinal Tregs and IgA-secreting B cells. Our findings hold promise for the development of approaches to prevent or treat food allergy based on modulation of the composition of intestinal microbiota. Supported by the Food Allergy Initiative.

OR.68. A Single Strain of *Clostridium Butyricum* Promotes IL-10-Producing Regulatory Macrophages in Colitic Conditions

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Recent study demonstrated that *Clostridium (C.) species*, clusters IV and XIVa (a cocktail of 46 strains of *C. coccoides* and *C. leptum*), induce IL-10-producing Foxp3^{+} regulatory T cells, and protect the development of colitis in mice. In this study, we examine anti-inflammatory ability of single gram-positive strain of *C. butyricum* (CB) that belongs to cluster I. First, colitic WT LPMC stimulated with CB irrespective of DSS and oxazolone models produced significantly higher amounts of IL-10 compared to control Enterococcus faecalis. Second, production of IL-10 was canceled in colitic LPMC derived from colitic MyD88$^{-/-}$ and TLR2$^{-/-}$ mice. Third, FACS analysis revealed that colitic WT CD11b^{+}F4/80^{+} cells were major IL-10-producers. Consistently, CB significantly increased IL-10 mRNA expression in CD11b^{+} cells, but not in CD4^{+} T cells, in colitic conditions. Fifth, CB treatment significantly suppressed the DSS-induced inflammatory symptoms in both SPF and GF conditions. Finally, the IL-10-mediated anti-inflammatory effect of CB was retained in Rag2$^{-/-}$ and Treg-depleted immunocompetent mice. Our results demonstrated that a single strain of CB converted colitogenic macrophages into IL-10-producing CD11b^{+}F4/80^{+} regulatory macrophages via PAMP-TLRs pathway, resulting in the suppression of development of colitis, concluding that CB may have potentials as immunomodulatory probiotics for intestinal inflammation.

OR.69. Early Exposure to Secretory IgA in Breast Milk Shapes the Composition of the Gut Microbiota and Epithelial Gene Expression in the Colon Throughout Life

Eric Rogier, Aubrey Frantz, Maria Bruno, Donald Cohen, Arnold Stromburg, Leia Wedlund, Charlotte Kaetzel. University of Kentucky, Lexington, KY

Breast milk supplies the first source of secretory IgA (SIgA) for suckling mammals. Locally synthesized IgA in the mammary gland is transported across epithelial cells into milk by the polymeric immunoglobulin receptor (pIgR). Using a novel breeding scheme with pIgR-sufficient and -deficient mice, we studied development of the gut microbiota and host intestinal immunity in the presence or absence of maternal SIgA. Failure to receive SIgA in breast milk was associated with translocation of aerobic bacteria from the intestine into mesenteric lymph nodes of neonatal mice, including the opportunistic pathogen *Ochrobactrum anthropi*. The composition of the gut microbiota varied significantly due to exposure to SIgA in breast milk, and these alterations persisted into adult life. Microarray analysis of gene expression in colonic epithelial cells from adult mice revealed significant differences due to early exposure to SIgA in breast milk. Responses to the colitis-inducing agent DSS were also altered, including genes associated with intestinal inflammatory diseases in humans. Our findings suggest that SIgA in breast milk shapes the intestinal environment by inhibiting opportunistic bacterial pathogens in neonates, causing permanent changes in the composition of the gut microbiota, and establishing intestinal epithelial gene expression patterns that persist throughout life.

OR.70. Fucose-Dependent Interkingdom Communication Between Mutualist Microbiota and the Mammalian Gut is Mediated by a Unique Form of TLR4

David Newburg[1], Di Meng[2], Nanda Nanthakumar[1]. [1]Boston College, Chestnut Hill, MA; [2]Massachusetts General Hospital, Boston, MA

Mammalian toll-like receptors (TLRs) are activated by characteristic microbial molecules and precipitate a cascade of signaling pathways that generally result in an appropriate inflammatory response. The critical signaling events of the inflammatory response are activation of nuclear factor kappa B (NF-kB) and interferon regulatory factor 3 (IRF3). We have discovered a novel form of TLR4 that is fucosylated in the absence of gut microbiome. This posttranslational modification allows TLR4 to be activated by fucose-specific ligands, stimulating a non-inflammatory (NF-kB and IRF3 independent) signaling pathway that mediates communication between a component of the gut microbiota and the intestinal epithelium. Activation of this pathway leads to specific transcription of the secretor (fucosyltransferase 2) gene resulting in fucosylation of the mucosa, facilitating colonization by a distinct microbial community. This represents a novel form of interkingdom communication between gut microbiota and intestinal epithelium. It seems central to succession of normal mammalian microbiota by facilitating mutualistic relationships with putative pioneering or keystone species that utilize fucose of the mucosal glycocalyx. Activation of this pathway is essential to recovery from the mucosal injury of chemically-induced colitis. This may underlie the association between secretor gene expression and intestinal inflammatory diseases such as

necrotizing enterocolitis and Crohn's disease.

OR.71. The Cross-Talk Between Fungi and the Mammalian Host via Microbiota's Metabolism

Rossana Iannitti[1], Zelante Teresa[1], Cunha Cristina[1], Antonella De Luca[1], Giuseppe Pieraccini[2], Francesca Fallarino[1], Agostinho Carvalho[1], Paolo Puccetti[1], Luigina Romani[1]. [1]University, Perugia, Italy; [2]University, Florence, Italy

Diseases caused by opportunistic fungi have traditionally been viewed as the gross result of a pathogenic automatism which makes a weakened host more vulnerable to microbial insults. The occurrence of fungal diseases in primary immune deficiencies suggests the pivotal contribution of the underlying deregulated inflammatory immunity to susceptibility to fungal diseases. However, humans have evolved intimate symbiotic relationships with a consortium of gut microbes, including fungi. The interplay between the fungal biota with the host and its microbiota might render this process more elaborate than previously appreciated. We have defined an immune-metabolic phenotype (metabotype) underlying multilevel host−fungus interactions in experimental candidiasis. We identified a metabolic pathway whereby tryptophan metabolites from the microbiota, by acting as agonist ligands of the host's aryl hydrocarbon receptor, balance mucosal reactivity to fungi. Our study confirms the important role played by tryptophan and its derivatives in gut immune homeostasis, extends this role to antifungal mucosal reactivity, describes the role of gut microbiota in linking tryptophan metabolism to local host reactivity and point to immunometabolism as an emerging and challenging field of investigation at the interface between fungi and the mammalian hosts. This work is supported by the SP2-Ideas, ERC-2011-ADG_20110310. FUNMETA, Grant Agreement 293714.

OR.72. SIGIRR Limits Colitic and Epithelial Homeostatic Responses, but Promotes Microbiota Dependent Colonization Resistance to Enteric Bacterial Pathogens

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Enteric bacterial pathogens such as Enteropathogenic Escherichia coli (EPEC) frequently target the intestinal epithelial cells (IEC) lining the mammalian gastrointestinal (GI) tract. Despite expressing innate Toll like receptors (TLRs), IEC are generally hypo-responsive to invading bacterial pathogens and their products. One reason is Single Ig IL-1 Related Receptor (SIGIRR), a negative regulator of interleukin IL-1/ TLR signaling expressed by IEC. To address whether SIGIRR expression impacts on enteric host defense, Sigirr deficient $^{(-/-)}$ mice were infected with the EPEC related, mouse specific pathogen Citrobacter rodentium. Sigirr $^{-/-}$ mice responded with accelerated IEC proliferation and strong pro-inflammatory and antimicrobial responses, but were highly susceptible to infection (100x heavier pathogen burden at Day (D) 6 and D10 post infection (pi)). Their exaggerated epithelial responses were primarily dependent on IL-1R signaling. The exaggerated antimicrobial responses in the Sigirr $^{-/-}$ mice led to rapid loss of competing commensal microbes (~80%) from the infected intestine reducing colonization resistance by D1 pi. Sigirr $^{-/-}$ mice were also surprisingly susceptible to EPEC colonization, displaying attaching and effacing lesions on infected IECs. Despite SIGIRR's suppression of IEC responses to infection, SIGIRR in fact aids host defense by promoting commensal based resistance to pathogen colonization of the GI tract.

OR.73. Altered Responses of CD8αα^{+} TCRαβ^{+} Intestinal Intraepithelial Lymphocytes During Chronic Intestinal Inflammation

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

Intestinal CD8αα^{+} CD8β- TCRαβ^{+} intraepithelial lymphocyte (IEL) are a predominant unconventional T cell subset in mice. They are believed to be strictly resident in the intestinal epithelium under homeostatic conditions, to contain cells with self-specific T cell receptors, and to be reportedly in a hyporesponsive state. Here we monitored CD8αα^{+} TCRαβ^{+} IEL during chronic intestinal inflammation. To this end, we

used transgenic mice that contain a monoclonal population of self-specific CD8αα^{+} TCRαβ^{+} IEL, but no conventional CD4^{+} and CD8^{+} T cells (318xH8xRAG$^{-/-}$ mice) and induced colitis by transfer of CD4^{+}CD45RBhi T cells. Compared to RAG$^{-/-}$ recipients, 318xH8xRAG$^{-/-}$ recipients exhibited a slightly attenuated, yet still severe colitis. Intriguingly, we observed a substantial increase in CD8αα^{+} TCRαβ^{+} T cells in peripheral blood, lymph nodes, spleen and colon of colitic 318xH8xRAG$^{-/-}$ mice. The appearance of CD8αα^{+} TCRαβ^{+}T cells at extraintestinal sites in colitic mice was accompanied by vigorous proliferation and changes in homing marker expression. Moreover, CD8αα^{+} TCRαβ^{+} T cells showed reduced expression of the regulatory genes TGF-β3, fgl2 and LAG3, and gained the ability to produce IFN-γ and TNF-α after restimulation *in vitro*. Hence, these results indicate a so far unanticipated plasticity and an unexpected capacity of CD8αα^{+} TCRαβ^{+} T cells to respond to stimulation under inflammatory conditions.

OR.74. Mucosa Associated Invariant T Cells Role in Intestinal Homeostasis

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Mucosa-associated Invariant T (MAIT) cells are evolutionarily conserved T cells expressing an invariant TCR, restricted by the MHC class Ib molecule, MR1. MAIT cells are abundant in human blood (0.1-10% of T cells), the intestinal mucosa, mesenteric lymph nodes and liver. MAIT cells are specifically activated by molecules derived from bacteria or yeasts, presented on MR1. The reactivity of MAIT cells towards microbes, including commensal species, begs the question of their implication in the intestinal mucosal homeostasis. We show that MAIT have different impact on the intestinal inflammatory state depending on their phenotype. Sorted MAIT cells transferred into RAG-deficient recipients induced strong colitis. MAIT cells with different phenotypes (ie, CD4^{+}, CD8^{+} or CD4-CD8- (DN)) have been previously observed and hence were sorted and transferred. CD4^{+} and DN MAIT cells induced a strong colitis in recipient mice while CD8^{+} MAIT cells were unable to do so. This inflammation was strictly MR1 dependent as MAIT cell did not induce colitis in RAG- MR1-deficient hosts. We observed that CD4^{+} and DN MAIT cells, isolated from the lamina propria, were in greater numbers and produce more cytokines than CD8^{+} cells. In humans, MAIT cells, as defined by the expression of Va7.2 TCR segement and high epxression of CD161, also contain different subsets. The numbers and functions of these MAIT subsets are currently under investigation in patients with inflammatory bowel diseases. Altogether, these data indicate that MAIT cells sensing of commensal microbes have a significant impact on the intestinal homeostasis.

OR.75. T Cell Lymphoma in Celiac Disease Arise from a Subset of Induced-T-to-NK Like Intraepithelial Lymphocytes

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A rare but most severe complication of celiac disease is the emergence of lymphomas from intraepithelial lymphocytes (IEL). In the majority of the patients, the first step of the transformation is characterized by the appearance of IEL containing clonal TCR rearrangements and displaying dual T/NK features but no surface CD3. Herein we show that i) clonal malignant IEL resemble murine induced-T-to-NK (ITNK) cells in which defective Bcl11b expression leads to an early block in T cell differentiation and to reprogramming into NK cells, ii) the normal epithelium contains a subset of innate lymphoid cells displaying an I-TNK-like phenotype. These cells represent 1-10% of IEL in adult controls and 40-70% IEL frequency in children under the age of one year. iii) Interleukin (IL-15), can act on NOTCH-stimulated T cell precursors and induce their conversion into ITNK-like cells which have lost Bcl11b. We therefore suggest that malignant clonal IEL arise from a novel subset of intra-epithelial innate lymphoid cells (IE-ILC) differentiating in the gut epithelium under the combined influence of NOTCH and IL-15 signals. Future work will be necessary to defining the role of IE-ILC and the mechanism by which IL-15 fosters their transformation.

OR.76. Mucosal Associated Invariant T Cells: What Do They See?

Onisha Patel[1], Lars Kjer-Nielsen[2], Jerome Le Nours[1], Bronwyn Meehan[2], Alexandra Corbett[2], Ligong Liu[3], Zhenjun Chen[2], Rangsima Reantragoon[2], David Fairlie[3], James McCluskey[2], Jamie Rosshjohn[1]. [1]Monash

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Mucosal associated invariant T cells (MAIT) are innate-like T cells that are preferentially located in the gut lamina propria. MAIT cells are activated by antigens bound to monomorphic non-classical Major Histocompatibility Complex (MHC) related molecule, MR1. It is well documented that T cells can be activated by peptide-based and lipid-based antigens presented by classical MHC and Cluster of differentiation 1 (CD1) molecules respectively. However, to date the identity of the antigen that can bind to MR1 and activate MAIT cells was unknown. Here we present a crystal structure of MR1 bound to 6-formyl pterin, a folic acid (vitamin B9) metabolite. The structure of MR1 has revealed how its binding cleft is ideally suited to present small organic compounds. Additionally, we provide further insight into microbe-derived ligands that can activate MAIT cells. Insight into identification of MAIT cell antigens will provide further understanding of the function of these cells within the immune system. This study provides a first example of how T cells can recognize antigens that are not peptide-based or lipid-based in nature.

OR.77. NLRP3 Inflammasome is Activated by Integrin Binding an RGD-Containing Protease of the Enteric Parasite Entamoeba Histolytica

Leanne Mortimer, France Moreau, Kris Chadee. University of Calgary, Calgary, AB, Canada

Entamoeba histolytica (Eh) is an enteric invasive parasite that elicits a severe inflammatory response. Inflammasomes are intracellular sensors that have emerged as major pathways of inflammation and host defense. We hypothesized Eh activates an inflammasome. THP-1 macrophages were stimulated with live Eh and caspase-1 and IL-1β activation were detected by Western blotting and ELISA. Eh triggered the NLRP3 inflammasome via ATP-$P2X_7R$ signaling: ATP hydrolysis and blockade of $P2X_7R$, K^+ efflux, reactive oxygen and caspase-1 abrogated inflammasome activation. We identified the major virulence factor, Eh cysteine protease 5 (EhCP5), activates NLRP3 by ATP release via binding of its RGD site to a β_1-containing integrin and proteolysis of an unidentified protein. Predictibly, EhCP5- Eh and pharmacological inhibition of Eh cysteine protease (CP) activity impaired inflammasome activation. We measured ATP release and found Wt Eh and rCP5 triggered ATP, while EhCP5- Eh and CP inhibition induced no ATP. Mutating rCP5 RGD to RAD, addition of integrin blocking peptide GRGDSP and src kinase inhibitors, to block upstream integrin signaling, all prevented ATP release and inflammasome activation. Specific blockade of β_1-integrin with a mAb prevented ATP release and subsequent inflammasome activation. This study has unraveled a novel pathway where Eh protease targets integrins to trigger NLRP3 activation in disease pathogenesis.

OR.78. A New Mouse Model of *Salmonella Typhi* Infection

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Typhoid fever affects more than 20-million individuals and causes more than 220,000 deaths annually. Interestingly, typhoid disease in humans is caused by the *Salmonella enterica serovars Typhi* and, which do not infect mice, therefore, no effective mice model accessible to investigate *S. typhi* infection in mice. Recently, our report (Mathur etal., Cell 2012), reveals the contribution of Toll-like receptor-11 (TLR11) in *Salmonella spp*. infection of the mouse and shown that flagellin from *Salmonella spp*. is recognized in mouse intestine by TLR11. We have purified the ligand from *S. typhimurium* and found that, like TLR5, TLR11 recognizes flagellin. Absence of TLR11 renders mice more susceptible to infection by *S. typhimurium*. TLR11-deficient mice are severely compromised in innate epithelial responses to *S. typhimurium* and exhibit poorly controlled and widely disseminated infection, resulting in enhanced lethality. TLR11 is expressed in mice, but not in humans, and remarkably, $TLR11^{-/-}$ mice are efficiently infected with orally administered S. typhi. We also find that $TLR11^{-/-}$ mice can be immunized against *S. typhi*. Therefore, $TLR11^{-/-}$ mice represent a small-animal model for the study of the immune response to *S. typhi* and for the development of vaccines against other human pathogen.

OR.79. The Role of IL-23 Production by Monocyte-Derived Lamina Propria Cells in Gut

Homeostasis and the Host Defense Against Citrobacter Rodentium

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Strategically positioned intestinal lamina propria mononuclear phagocytes are considered crucial for maintaining gut homeostasis and immune defense. This includes: monocyte-derived $CX3CR1^+$ $CD11c^+$ cells displaying macrophage (MAC) features and migratory $CD103^+$ $CD11c^+$ cells that are derived from dedicated dendritic cell (DC) precursors and display DC hallmarks. Understanding differential contributions of these two cell types and their intercellular communication should provide critical insights into the mechanisms that maintain the gut homeostasis or lead to IBD. Here we focused on the cytokines IL-12 and IL-23 that are considered to play a key role in the development of Th1 and Th17 cell responses. To define the importance of cytokine production by the specific mononuclear phagocyte populations, we used a cell ablation strategy combined with a challenge by the murine Attaching & Effacing (A&E) pathogen Citrobacter rodentium. Chimeras generated with CD11c-DTR and $IL\text{-}23p19^{-/-}$ BM allowed us to show that DC/MAC-derived IL-23 is required for the induction of IL-22 and anti-microbial peptides (AMPs). Moreover, also when the IL-23 deficiency was restricted to $CX3CR1^+$ cells using newly established CX3CR1Cre:iDTR mice AMP and IL-22 production were impaired. Surprisingly, *C. rodentium*-challenged mice carrying IL-23 deficiencies died from the challenge. Our results suggest that in an IL-23 deficient environment, IL-12 secreted by $CD103^+$ DCs drives uncontrolled IFN-γ production by T cells leads causing severe immunopathology. In support of this notion, the immunopathology was prevented by neutralization of IFN-γ that rescued the mice.

OR.80. Phenylbutyrate Treatment Induces Mucosal Cathelicidin and Reduces Diarrhea due to Enteropathogenic *Escherichia Coli*

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Antimicrobial peptides are effectors of innate immunity. Treatment of shigellosis in rabbits with phenylbutyrate (PB) reduces clinical severity and counteracts down-regulation of antimicrobial peptide cathelicidin (CAP-18) in the large intestine. We aimed to further evaluate whether CAP-18 is down-regulated in the small intestinal mucosa in a rabbit model of enteropathogenic *Escherichia coli* (EPEC) diarrhea, and if oral PB treatment modifies CAP-18 expression and improves clinical and microbiological features of diarrhea. EPEC-induced diarrhea resulted in down-regulation of CAP-18 in the small intestinal mucosa of rabbits as revealed by immunohistochemistry. PB treatment alleviated clinical illness, reduced histological inflammation and up-regulated CAP-18 in the mucosa. Release of active CAP-18 peptide in the stool was noted in Western blot analysis. Multiplex PCR analysis of bacterial DNA in the stool showed absence of EPEC specific genes eae and bfp after PB treatment. However, 4 of 5 PB-treated rabbits shed rough EPEC strains still harboring eae and bfp genes, which were less potent in binding to HeLa cells and induced delayed onset of diarrhea in rabbits. In conclusion, EPEC down-regulates CAP-18 in the small intestinal mucosa, which is counteracted by PB treatment. Treatment with Phenylbutyrate reduces bacterial shedding and virulence properties of EPEC and reduces diarrhea due to EPEC.

OR.81. Association of IL-23 Receptor Gene (IL-23R) Single Nucleotide Polymorphisms (SNPs) with the Intestinal Microbiome in Healthy First Degree Relatives (FDRs) of Crohn's Disease (CD) Subjects

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We assessed the microbiome in a cohort of 578 FDR of CD patients enriched for the genetic variations in the IL23R previously shown to be associated with an increased risk of CD. Bacterial DNA was extracted from stool samples and 16S rRNA genes were sequenced using 454 pyrosequencing. The sequences were clustered, aligned and assigned to operational taxonomic units (OTUs) using QIIME pipeline, USEARCH and RDP core sequences. We analyzed the relationship of OTU composition with 30 CD-associated SNPs genotyped using Sequenom Gold iPlex or TaqMan platforms. At the genera level, the dominant genera in stool samples were Bacteroidetes, Faecalibacterium and Roseburia (mean ± standard error are 23.9% ± 0.9, 18.0% ± 0.6, and 12.3% ± 0.4 of total OTUs, respectively). Heterozygotes for IL-23R SNPs, rs11209026 or rs11465804 were each associated with higher proportions of Bacteroides, Odoribacter and lower proportions of Clostridium, Faecalibacterium, Lachnospira, and Subdoligranulum (ranging from $p<10\text{-}10$ to $p<0.004$). These results indicate microbiota differences in asymptomatic FDR carrying IL-23R SNPs. These SNPs have confirmed association with CD (Odds Ratio=2). Funded by CCFC Michael J. Howorth GEM Project Team and CIHR.

OR.82. The Involvement of IL-34 in Intestinal Inflammation of Crohn's Disease

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Background and Aim: IL-34 acts as a regulator of macrophage differentiation and proliferation. However, the involvement of IL-34 in pathophysiology of IBD remains unclear. The aim of this study is to evaluate the role of IL-34 in murine and human IBD. Method: To detect the IL-34 expressing cells in colonic mucosa of C57/BL6 mice, immunohistochemistry was performed. Gene expression of IL-34 was evaluated in intestinal mucosa of C57/BL6 mice, IL-10 KO mice and IBD patients (Control, active Crohn's disease (CD), active Ulcerative Colitis (UC) by real-time PCR. Gene expression of IL-34 in TNF-α stimulated murine fibroblast cells (NIH3T3) was evaluated. Results: In C57/BL6 mice, IL-34 positive cells were positive for α-SMA, suggesting that fibroblast cells mainly produce IL-34. Gene expression of IL-34 in colonic mucosa of IL-10 KO mice with colitis was significantly higher than those without colitis. The gene expression of IL-34 was also significantly higher in intestinal mucosa of CD patients than control and UC patients. TNF-α strongly induced IL-34 expression on NIH3T3 cells. Conclusion: Our data suggested that IL-34 derived from fibroblasts might be involved in intestinal inflammation of CD, and be one of new therapeutic targets for patients with CD.

OR.83. X-Linked Inhibitor of Apoptosis Protein in Early-Onset Inflammatory Bowel Disease

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Background: The genetic contribution to inflammatory bowel disease (IBD) has only been partially unraveled and it has been suggested that rare variants contribute to the genetic risk in IBD. Methods: Exome-sequencing and functional studies were performed in cases of familial and sporadic early-onset IBD. Results: In a three-year-old patient with colonic, fistulizing, treatment-refractory CD, exome-sequencing revealed a novel hemizygous nonsense mutation (E99X) in XIAP, the gene encoding for the X-linked inhibitor of apoptosis protein. XIAP was not detected by flow cytometry in peripheral blood mononuclear cells (PBMCs). Relative and absolute frequencies of PBMC subsets, T cell proliferation, apoptosis, and cytokine production were unaltered in the patient. However, while XIAP-deficient PBMCs and monocytes exhibited unaltered responses to toll-like receptor 2, 3, 4, 7 and 9 stimulation and unimpaired NLRP3-dependent IL-1β secretion, NOD2-dependent IL-8 and IL-1β release were not detected upon muramyl dipeptide (MDP) stimulation. Furthermore, wildtype but not E99X XIAP associated with NOD2 in a RIP2-dependent manner as revealed by co-immunoprecipitation. Lentiviral XIAP reconstitution in primary dendritic cells restored NOD2 signaling. Conclusion: Our studies confirm a critical role of XIAP in the pathogenesis of early onset IBD and support the notion that a subset of IBD cases may results from immunodeficiency.

OR.84. *In vitro* Generated Regulatory T Cells from Crohn's Disease Patients' Blood Home to Inflamed Human Small Bowel *in vivo*

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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) by restoring mucosal Treg numbers and function. Tregs generated *in vitro* from FACS-sorted $CD4^+CD25^{hi}CD127^{lo}CD45RA^+$ precursors from CD peripheral blood (PB) are phenotypically stable and suppress activation of MLN and LP $CD3^+$ from inflamed CD mucosa. Homing to gut and MLN may be required for Treg-mediated immune modulation in inflamed CD mucosa. However, the capacity for *in vitro* expanded Tregs to home to human gut is unknown. Tregs generated in the presence of rapamycin expressed gut homing integrins $\alpha_4\beta_7$ (median=21.4% [IQR 16.4-24.0%]) and receptors CD62L, CXCR3, CCR5 and CCR6. To determine whether these Tregs migrate to inflamed gut a C.B-17scid human intestinal xenograft model was then established. Human gut xenograft inflammation was triggered by intraluminal injection of enteropathogenic *E. coli*. Human $CD45^+CD3^+CD4^+$ cells were seen in inflamed xenograft LP, 24 hours after IV adoptive transfer of *in vitro* generated CD Tregs (n=2 independent experiments). In conclusion, Tregs expanded *in vitro* from CD PB express homing receptors that enable migration into inflamed human gut mucosa. These data add further weight to the therapeutic promise of expanded Tregs as a novel cellular therapy for human IBD.

OR.85. Loss of the TGFβ-Activating Integrin αvβ8 on Dendritic Cells Protects Mice from Chronic Intestinal Parasitic Infection via Control of Type 2 Immunity

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The cytokine TGFβ plays a central role in regulating immune responses at mucosal surfaces. However, the role and regulation of TGFβ during responses to infection is not well understood. Here we find that during development of chronic infection with the intestinal parasite Trichuris muris, TGFβ signalling in $CD4^+$ T cells is induced early during infection. This enhanced TGFβ signalling involves expression of the TGFβ-activating integrin αvβ8 by dendritic cells (DCs), which we have previously shown is highly expressed by a $CD103^+$ DC subset in the gut. Importantly, mice lacking integrin αvβ8 on DCs are completely resistant to chronic infection with T. muris. This protection appears independent of $Foxp3^+$ Tregs, but requires early induction of the protective cytokine IL-13 by $CD4^+$ T cells. Our results therefore provide novel insights into how type 2 immunity is controlled in the intestine during gastrointestinal parasite infection, and may help contribute to the development of new therapies aimed at promoting expulsion of gut parasites.

OR.86. IL-1β Maintains Helminth Chronicity by Suppression of Innate IL-25 and IL-33 Production

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Approximately 2 billion people currently suffer from intestinal helminth infection, which are typically chronic in nature. The host typically raises a strong type 2 immune response against the helminths, however the majority of helminthes have developed the capacity to escape effective immunity and can remain chronically within the intestine for years. Such chronicity results from co-evolution between helminths and their mammalian hosts, however the molecular mechanisms by which helminthes avert

immune rejection are not clear. Using the natural murine helminth, *Heligmosomoides polygyrus bakeri* (Hp) we have found that Hp elicits the secretion of IL-1β *in vivo* and *in vitro* and that this cytokine is critical for shaping a mucosal environment suited to helminth chronicity. Indeed in mice deficient for IL-1β-/-, or treated with the soluble IL-1βR antagonist, Anakinra, helminth infection results in enhanced type 2 immunity and accelerated parasite expulsion. IL-1β acts to decrease production of IL-25 and IL-33 by intestinal epithelial cells at early time points following infection and the consequent expansion of Type 2 innate lymphoid cells. Taken together these data indicate that Hp maintains its chronicity by promoting the release of host-derived IL-1β which counter acts innate and adaptive Th2 responses, thus thwarting host immunity.

OR.87. Viral Pathogens Mediate Necroptosis Directly via the TLR3-TRIF Signaling in Caspase-8ΔIEC Mice

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We have recently described intestinal epithelial necroptosis as a potential pathogenic mechanism driving the development of ileitis in Caspase-8ΔIEC mice and Crohn's Disease patients. However, the mechanism triggering necroptosis is still unknown. Now we could demonstrate that programmed necrosis of intestinal epithelial cells is not mediated via the TNF-receptor. To examine the impact of Toll like receptors (TLR) in necroptosis, we injected Poly (I:C) and LPS into mice. TLR-ligands induced a fast and dramatic villous atrophy and severe destruction of the intestine of Caspase-8ΔIEC mice as compared to control littermates, leading to the death of the former mice within six hours. Immunohistochemistry revealed an excessive number of TUNEL positive but caspase-3 negative dying epithelial cells after TLR-stimulation in Caspase-8ΔIECmice, but not in $Rip3^{-/-}$Caspase-8ΔIEC, indicating that this form of cell death is due to Rip3-mediated necroptosis. Moreover we discovered that PIC triggered necroptosis was directly mediated via the TLR3-TRIF pathway, whereas LPS-induced programmed necrosis was prevented in $Tnf\text{-}R1^{-/-}$Caspase-8ΔIEC mice, demonstrating the influence of TNF-α in this setting. Our data demonstrate that dsRNA activation of the TLR3 induces epithelial cell necroptosis in a Tnf-independent manner. This indicates that viral pathogens are the main triggering effectors for small intestinal inflammation in Caspase-8ΔIEC mice.

OR.88. Virus and Host Genes Involved in Murine Norovirus Intestinal Persistence

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Murine norovirus (MNV) establishes persistent intestinal infection and triggers inflammatory bowel disease. We used a representative persistent strain (CR6) and non-persistent strain (CW3) to identify virus and host genes involved in persistence. A screen of viral genes identified NS1/2 as necessary and sufficient for persistence. NS1/2 also promoted MNV growth in the intestine, and persistence was restricted to the intestine with clearance from systemic sites. These data suggest that replication in the intestine is dependent on NS1/2 and is critical for MNV persistence in WT mice. We found that deletion of the interferon (IFN) α/β receptor (IFNAR1) in LysM- or CD11c-expressing myeloid cells but not villin-expressing intestinal epithelial cells allowed persistence of CW3 at systemic sites with minimal persistence in the intestine. CR6 replication in the intestine was also unchanged in IFNAR1 as well as IFNγ receptor knockouts. However, signal transducer and activator of transcription 1 (STAT1), which is critical for both IFNα/β and IFNγ signaling, was found to be important for restricting intestinal replication. Therefore, IFNAR1 is important for clearance at systemic sites but surprisingly not in the intestine, suggesting that either IFNα/β acts synergistically with IFNγ or a distinct STAT1-dependant pathway is important for preventing intestinal persistence.

OR.89. The Immunoregulatory Factor Transforming Growth Factor β1 is Compartmentalized Between Blood and Seminal Plasma of HIV-Positive Men and its Levels are Reduced Following Anti-retroviral Therapy

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TGF-β1 is an immunoregulatory factor found in high levels in seminal plasma (SP). We previously showed that HIV-positive, chronically infected, antiretroviral therapy (ART)-naïve men contained significantly higher levels of TGF-β1 in their SP compared to acutely infected and HIV-negative men. To determine whether high TGF-β1 levels in these men could be playing a role in naturally controlling chronic immune activation, we examined TGF-β1 levels in SP and blood plasma (BP) of HIV-positive men who were on ART and determined whether TGF-β1 correlated with sCD14, an immune activation marker. BP and SP were collected from HIV-positive ART-naïve and ART-treated men, and HIV-negative men. TGF-β1 and sCD14 levels in BP and SP were measured by ELISA. TGF-β1 in HIV-positive men was compartmentalized between SP and BP. As well, no correlations between SP and BP TGF-β1, and sCD14 were observed. TGF-β1 levels were significantly lower in SP from ART-treated men compared to ART-naïve men. Furthermore, men on protease inhibitors (PIs) had significantly decreased TGF-β1 levels in SP compared to men not on PIs. Our results suggest that increased TGF-β1 expression in SP is a mucosal response to local HIV-1 infection compartmentalized to the male genital tract which returns to normal levels with ART.

OR.90. Dysbiosis of the Colonic Mucosal-Adherent Microbiota is Associated with HIV Disease Progression and Chronic Inflammation

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HIV infection is characterized by dysregulation of the intestinal barrier, translocation of immunostimulatory microbial products, and chronic systemic inflammation that is thought to drive the progression to AIDS. Elements of this process persist despite viral suppression during highly active anti-retroviral therapy (HAART) and drivers of these phenomena remain poorly understood. Disrupted intestinal immunity can precipitate dysbiosis that induces chronic inflammation in the mucosa and periphery. However, putative microbial drivers of HIV-associated immunopathology versus recovery have not been identified. Using high-resolution bacterial community profiling, we identified a dysbiotic mucosal-adherent community enriched in Proteobacteria and depleted of Bacteroidia members that was associated with markers of mucosal immune disruption, T cell activation, and inflammation in HIV-infected subjects. Furthermore, this dysbiosis was evident among HIV-infected subjects undergoing HAART, and the extent of dysbiosis correlated with established markers of disease progression. These results demonstrate a link between mucosal-adherent colonic bacteria and immunopathogenesis during progressive HIV infection, which is apparent even in the setting of viral suppression during HAART. The findings described herein suggest that therapies aimed at restoration of intestinal homeostasis may constitute novel strategies for the management of HIV disease.

OR.91. HIV-Associated Alterations in Gut Th17 Cell Function Correlate with Microbial Translocation and Immune Activation

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Th17 cells maintain the gut epithelial barrier and prevent invasion by *luminal bacteria* through the balanced production of immunosuppressive and pro-inflammatory cytokines; however, HIV infection is characterized by increased bacterial translocation. We therefore assessed the functional capacity of blood and sigmoid Th17 cells in: HIV-uninfected individuals (n=9), anti-retroviral therapy (ART)-naïve individuals during early (N=24) and chronic (N=12) HIV infection and long-term ART-treated men (N=15). A subset of HIV-infected individuals (N=10) was re-sampled after 12 months of ART. Th17 cells were defined as IL-17a^{+} CD4 T cells, and their pro-inflammatory polyfunctional capacity was assessed by the co-production of cytokines IL-22, TNF-α and IFN-γ. The polyfunctional capacity of gut Th17 cells greatly exceeded that in the blood of HIV-uninfected participants, but was dramatically reduced during all HIV

stages. Immunoregulatory skewing of gut Th17 cell function, characterized by IL-10 production, was uniquely seen during early HIV infection and correlated with reduced microbial translocation and decreased immune activation. Mucosal Th17 cell numbers were restored soon after ART initiation, but Th17 polyfunctionality and normal levels of immune activation and microbial translocation only recovered after long-term ART. Overall, HIV infection was associated with a rapid loss of mucosal Th17 polyfunction, with delayed restoration on standard ART.

OR.92. How do Novel IL-13Rα2 Adjuvented Vaccines Modulate HIV-Specific Mucosal Immunity, CD8 T Cell Avidity and Protective Immunity?

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We have shown that unlike systemic immunisation, mucosal (intranasal) immunisation can induce cytotoxic T cells (CTL) of higher avidity with lower IL-4/IL-13 activity and better protective immunity against HIV-1. Our recent studies, indicate that transient inhibition of IL-13 at the vaccination site, the lung mucosae (by co-expression of IL-13Rα2 soluble or membrane bound receptors together with HIV-1 vaccine antigens) can dramatically enhance HIV-specific mucosal immunity, CTL avidity/ multi-functionality and protective immunity against a surrogate mucosal HIV-1 challenge (similar to an IL-13 gene knockout animal). When the mechanisms by which these IL-13Rα2 adjuvented vaccines modulate CTL avidity were investigated, data revealed that CD8 T cell avidity is primarily defined at the vaccination site according to the antigen presenting cell subsets they recruit. Also following vaccination, unlike other IL-4/IL-13 receptors, down-regulation of IL-4Rα densities on effector CD8 T cells and up-regulation of CD8α/β co-receptors play a critical role in modulating CD8 T cell avidity. Our findings not only offer exciting prospects for a future HIV-1 vaccine development but also many other chronic mucosal infections where high avidity CTL are required for protection.

OR.93. IL-36 Ligands Induce IL-23/IL-17A Responses and Enhance Intestinal Inflammation

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The CX3CR1/CX3CL1 axis is important for maintaining intestinal macrophage homeostasis, bacterial translocation, and limiting colitogenic Th17 responses. CX3CR1 deficiency leads to a loss of resident intestinal macrophages in the steady state, however during colitis the intestine is populated by $Ly6C^+$ inflammatory macrophages. In a microarray comparison between $Ly6C^+$ and $CX3CR1^+$ colonic macrophages, we identified the IL-1 family member IL-36g as the top most preferentially expressed cytokine in the $Ly6C^+$ population. While several members of the IL-1 family of cytokines are associated with the pathogenesis of experimental and human IBD, the expression and functions of IL-36 ligands during intestinal inflammation are unclear. We demonstrate here that IL-36g is expressed by $Ly6C^+$ colonic macrophages during acute and chronic experimental colitis and during human IBD. IL-36g is induced by bacterial ligands and activates intestinal DCs to express IL-23 and augment Th17 and Th1 responses while inhibiting $Foxp3^+$ Treg differentiation. Importantly, inhibition of IL-36R signaling ameliorates intestinal inflammation in mice. Further insight into the functions of IL-36 ligands during intestinal inflammation may contribute to the development of novel therapeutic strategies for the treatment of human IBD.

OR.94. *Experimental Autoimmune Lacrimal Keratoconjunctivitis* Develops by Th-17 Cell Dependent on IL-12p35 and IL-12p40

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Desiccating stress is known to induce *experimental autoimmune lacrimal keratoconjunctivitis* (EALK) in mice model mimicking dry eye disease (DED). Evidence suggests that dry eye disease is a kind of ocular surface inflammatory disease combining lacrimal functional unit (LFU; cornea, conjunctiva, eyelid and lacrimal glands).In our study, development of *autoimmune lacrimal keratoconjunctivitis* by naïve T cell

transfer system. Inflammatory changes in the lacrimal functional unit (LFU) of adoptive transferred mice. Co-transfer of naïve T cell with regulatory $CD4^+$ T cells completely prevented the development of keratitis. It also showed that the production of IFN-gamma by $CD4^+$ T cell itself do not have major roles contributing to the expression of keratitis.However,IL-17A KO CD^+4 naïve T cell transferred mice showed reduced incidence of keratitis compared to the wild type $CD4^+$ naïve T cells transferred mice. When we adoptively transferred $CD4^+CD45RB^{hi}$ T cells from the spleen to C57BL/10 IL-12p35$^{-/-}$ RAG2$^{-/-}$ mice or IL-12/IL-23p40$^{-/-}$ RAG2$^{-/-}$ mice, there were no evidences of eye diseases in both mice. By the model of adoptive transfer of naïve $CD4^+$ $CD45RB^{hi}$ T cells to B10-RAG2 KO, we show the central role of $CD4^+$ cell in EALK as a model of DED and Sjögren's syndrome. This spontaneous disease model minimizing environmental damage demonstrates the disease process and involving mechanism precisely.

OR.95. Factors Mediating the Induction of IL-17$^+$ Regulatory T Cells in Inflamed Gut Mucosa

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We have recently shown that the unique inflammatory environment in Crohn's disease(CD) contributes to the generation of distinct IL-17$^+$Tregs in inflamed gut mucosa(Gastroenterology 2011,140:957).To further elucidate the factors coordinating Treg/Th17 axis,$CD4^+$LPLs from IBD patients and normal controls were incubated with supernatants from IBD and normal mucosa cultures.Supernatants from CD,but not UC or normal mucosa cultures, were able to induce IL-17$^+$Tregs in UC,but not normal $CD4^+$LPLs.Addition of α-TGF-β antibody abolished this induction.Importantly,the levels of bioactive TGF-β were significantly increased in CD gut mucosa.To assess whether the bacterial microbiota can influence the conversion of Treg to Th17,UC $CD4^+$LPLs were cultured with bacterial antigens(BAgs) derived from IBD patients and normal controls.Interestingly,BAgs derived from either source potently induced IL-17$^+$Tregs.The cognate T cell/BAg/MHC recognition was essential for this induction.Analysis of TCR repertoire revealed the predominant usage of Vβ5.1,Vβ2 and Vβ9 by IL-17$^+$Tregs induced by CD BAg,whereas IL-17$^+$Tregs induced by UC/normal BAgs shared Vβ2 usage.Altogether,given significantly elevated levels of bioactive TGF-β in CD mucosa as the ability of TGF-β to induce IL-17$^+$Tregs *in vitro*,our data suggest that TGF-β may be responsible,at least in part,for IL-17$^+$Treg development in CD.Our results also indicate a potential role of bacterial microbiota in modulation of IL-17 expression by LP $CD4^+$Tregs.

OR.96. TL1A, a Member of the TNF Superfamily, Modulates Differentiation of TH17 Cells and TH17 Cytokine Production by CCR6$^+$ Human Memory T Cells

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TL1A, a member of the TNF superfamily, mediates a strong co-stimulation of TH1 cells. It enhances the IFN-γ production by peripheral $CD4^+$ and mucosal $CCR9^+$ T cells. TL1A plays an important role in the development of chronic colitis, experimental autoimmune encephalomyelitis, and allergic lung inflammation by modulating TH1, TH17, and TH2 responses suggesting an important role in chronic inflammatory processes. Here, we demonstrate that TL1A, in combination with TGF-β and IL-6, promoted the differentiation of human TH17 cells from naive $CD4^+$ T cells. Additionally, TL1A in combination with TGF-β and IL-6 enhanced IL-17 production from $CD4^+$ $CD45RO^+$ memory T cells and induced IL-17/IFN-γ producing TH17 cells. In contrast, TL1A alone induced high levels of IL-22 in naïve and memory $CD4^+$ T cells. TL1A also enhanced IL-17 and IL-22 production by committed $CD45RO^+CCR6^+$ TH17 cells suggesting that TL1A is able to induce TH17 differentiation and enhances IL-17 and IL-22 secretion from committed TH17 cells. The mechanism leading to TL1A-induced secretion of TH17 cytokines is currently investigated. Thus, these observations establish an important role of TL1A in promoting human TH17 cell differentiation and function and may provide a potential target for therapeutic intervention in chronic inflammatory TH1 and TH17 driven diseases.

Poster Session: Wednesday, July 17

W.1. T Helper 17 Cells may not be a Major Factor in House Dust Mite Allergic Rhinitis

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Purpose: Interleukin-17 (IL-17) was reported to be related with symptom aggravation of *allergic rhinitis* (AR) and to be elevated in serum of patients of AR. However, the role of Th17cells was controversial in *allergic rhinitis*. In this study, we aimed to reveal the role of Th17 cell in patients with AR. Methods: Twenty patients with AR (sensitized with house dust mite (HDM)) and 10 controls with turbinate hypertrophy were included. Mononuclear cells were harvested from nasal turbinate and peripheral blood. ELIspot assay was performed to detect HDM allergen-specific peripheral blood T cells secreting Interleukin-4, interferon-gamma or IL-17. HDM specific secretion of IL-4, IL-5, IL-17 and interferon-gamma from nasal mononuclear cells were measured using cytometric bead array after stimulating with HDM(10ug/ml) for 96 hours. Additionally, IL-17-secreting potential of nasal mucosa-infiltrating T cells was assessed by intracellular cytokine staining after PMA/ionomycin stimulation. Results: While IL-4-secreting peripheral T cells were detected in only AR group, IL-17-secreting peripheral T cells were not detected in both AR and control group. After allergen stimulation of nasal mucosal-infiltrating T cells, IL-17 was not detected in both AR and control group, whereas Th2 cytokine, IL-5 was detected only in AR group. Moreover, flowcytometric analysis showed that the IL-17 secreting potential of mucosa-infiltrating T cells were significantly low in AR group ($p=0.03$). Conclusions: Th17 cells were not detected in peripheral blood and nasal mucosa of AR patients sensitized with HDM. This human study showed that Th17 cells may not be related with pathogenesis of AR sensitized with HDM.

W.2. Induction of Foxp3$^+$CD4$^+$ Regulatory T Cells as a Novel Mechanism Underlying the Therapeutic Action of Kakkonto, a Traditional Japanese Herbal Medicine, in a Murine Food Allergy Model

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Background & Aim: The number of food allergy (FA) patients has increased over the last several decades. We found that kakkonto, a traditional Japanese herbal medicine, suppressed the occurrence of allergic symptoms in an FA mouse model. Recently, intestinal mucosal immunity is considered to plays an important role in FA. The aim of this study is to examine whether kakkonto induce regulatory T cells in the FA mouse colon to ameliorate FA. Methods: BALB/c mice were systemically sensitized and then orally challenged with ovalbumin in the FA mouse model. Results: mRNA expression of IL-4 in the FA mouse colon and GATA-3 in CD4$^+$T cells from the FA mouse colon was significantly up-regulated, which was reduced by an oral administration of kakkonto. In contrast, the level of Foxp3 mRNA expression was increased in CD4$^+$T cells from the kakkonto-treated FA mouse colon. Furthermore, immunohistochemistrical analysis and flow cytometric analysis detected an increase in the number of Foxp3$^+$CD4$^+$T cells in the kakkonto-treated FA mouse colon. Conclusion: The present findings suggest that the immunosuppressive effects of kakkonto on FA mice are attributed to an increase in the number of Foxp3$^+$ CD4$^+$T cells in the intestinal lamina propria.

W.3. Dietary Intervention with Specific Non-Digestible Oligosaccharides and Bifidobacterium Breve M-16V Support the Development of Tolerogenic DC

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Dietary intervention with Bifidobacterium breve M-16V and specific non-digestible oligosaccharides (scGOS/lcFOS; GF/Bb) can prevent food allergic symptoms by inducing galectin-9 expression in intestinal epithelial cells. However, it is not known whether GF/Bb modulates DC and T cell responses in the intestine. Using an ovalbumin (OVA)-induced murine model for food allergy, we evaluated whether GF/Bb results in changes in DC and T cell subsets in the intestine. Upon OVA challenge, an increase in

$CD11c^+MHC\text{-}II^{mid}$ cells was observed in the small intestinal lamina propria (SI-LP). In allergic mice, $CD11c^+MHC\text{-}II^{mid}$ cells expressed lower levels of CD103. Furthermore, $CD11c^+$ cells in the SI-LP of allergic mice produce more IL-4, which was paralleled with an increase in $CD69^+GATA\text{-}3^+$ activated T_h2 cells and reduced $Foxp3^+$ T_{reg} cells in the SI-LP. These effects were at least in part abrogated by the GF/Bb diet. Supporting *in vitro* experiments show that galectin-9 secreted by IEC induces RALDH activity in human moDC. Galectin-9 conditioned moDC had increased capacity to induce functional $Foxp3^+$ Treg cells. In conclusion, dietary intervention using GF/Bb may support the generation of tolerogenic DC to reduce T_h2-associated cytokine production and enhance T_{reg} polarization during allergic sensitization.

W4. Unplugging Food Allergy: Understanding the Cellular Mechanisms Underlying Food Allergen Sensitization

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Previous mouse and clinical studies demonstrate a link between Th2 intestinal inflammation and the induction of the effector phase of food allergy. We have demonstrated that interleukin (IL-9) has an important role in this process. In addition, intestinal IL-9 overexpression (iFABPp IL-9 Tg) predisposes to oral antigen sensitization, which requires mast cells and increased intestinal permeability. We have extended these findings by investigating the role of the oral allergen-induced $CD4^+$ Th2 response in the perpetuation of intestinal allergic responses. Here, we seek to take these studies further by combining the iFABPp IL-9 Tg mouse with a newly generated IL-4 and IL-13 reporter mouse, resulting in a novel transgenic food allergy susceptible/Th2 cytokine reporter model that is able to reveal for the first time the earliest cellular and molecular events involved in allergic sensitization in the gut.

W.5. Circulatory Antigen Processing by Mucosal Dendritic Cells Controls $CD8^+$ T Cell Activation

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Circulatory antigens transit through the small intestine via the fenestrated capillaries in the lamina propria prior to entering into the draining lymphatics. But whether or how this process controls mucosal immune responses remains unknown. Here we demonstrate that dendritic cells (DCs) of the lamina propria can sample and process both circulatory and luminal antigens. Surprisingly, antigen crosspresentation by resident $CX3CR1^+$ DCs induced differentiation of precursor cells into $CD8^+$ T cells that expressed interleukin-10 (IL-10), IL-13 and IL-9 and could migrate into adjacent compartments. We conclude that lamina propria $CX3CR1^+$ DCs facilitate the surveillance of circulatory antigens and act as a conduit for the processing of self- and intestinally absorbed antigens, leading to the induction of $CD8^+$ T cells, that partake in the control of T cell activation during mucosal immune responses.

W.6. Improved Mucosal Delivery of Muc-Modified Peptides and Proteins

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Intense *in vivo* screening of various phage-display libraries lead to the discovery of mucosa-targeting peptides (Muc-peptides).These nine amino acid long cyclic peptides can target the mucosal surfaces of the gut and be taken up in the lymphatic system. Hence, Muc-peptides are attractive carrier candidates for the mucosal delivery of molecules. To investigate the potential of Muc-peptides to transport proteins, biotinylated Muc-peptide was coupled to fluorescently labeled streptavidin or antibody as model proteins. The constructs were injected into clamped intestinal loops of mice *in vivo* and the uptake was visualized

histologically. Muc-peptide coupled constructs, but not control constructs can be observed in a subset of intestinal epithelial cells, inside the villi and in $CD11c^+$ DCs in Peyer's Patches. Furthermore, Ovalbumin peptide (OVA) was used as a model antigen to analyze the mucosal delivery of antigenic peptides. Adoptive T cell transfer experiments have shown that mucosal treatment with Muc-coupled OVA-peptide leads to a highly increased proliferation of OVA-specific T cells in draining lymph nodes as well as in spleen. However, Muc-OVA-peptide treatment did not lead to an improved tolerogenic response. Our results suggest that Muc-peptide coupling can lead to intestinal uptake and therefore might be useful in the mucosal administration of biomolecules.

W.7. Limited Expression of T Cell-Independent Factors APRIL and TACI Prior to Infant Intestinal IgA Plasma Cell Development

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To identify mechanisms of intestinal IgA development early in human infancy, we characterized the kinetics and expression of colonic plasma cells by isotype, class switch recombination (CSR), and T cell-dependent (TD) and T cell-independent (TI) switch factors during the first 6 months of life. The density of intestinal IgA plasma cells, virtually absent in the first month, increased rapidly over 6 months, approaching but not attaining adult levels by 1 year. Activation-induced cytidine deaminase (AID), required for CSR, was primarily localized to isolated lymphoid follicles, not the lamina propria, and was expressed prior to the development of IgA plasma cells. Similarly, TD factors, including CD4, CD40L and CD40, were present before 1 month and neither increased over time nor correlated with IgA plasma cell density. In contrast, expression of TI factor APRIL and its receptor TACI was low in the first month of life and increased in association with IgA plasma cell densities. These findings suggest that TI CSR may play a pivotal role in intestinal IgA plasma cell development during early infancy, revealing potential targets for enhancing early infant IgA responses to enteric pathogens and vaccines.

W.8. Gut IgA Inductive Sites in GALT Differ with Regard to the Germinal Center Reaction

Rathan Komban, Anneli Stensson, Mats Bemark, Nils Lycke. Gothenburg University, Gothenburg, Sweden

A detailed study of the initiation of gut mucosal IgA B cell responses to oral immunizations has never been done. Therefore, we developed an adoptive transfer model with sorted high affinity B1-8hi NP-specific IgH knock-in GFP^+ B cells and NP-cholera toxin (CT) (NP; hapten nitrophenyl) that allowed us to visualize the IgA B cell response. Following oral immunization with NP-CT we monitored the development of germinal centers (GC) in GALT and identified cell proliferation using the Violet dye tracer. We observed a dramatic expansion of NP-specific B cells starting on day 4 in PP, followed by MLN, while only few B cells appeared in spleen. NP-specific B cells were found in multiple GC in PP while MLN exhibited fewer GC and spleen had no GC. Blocking lymphocyte-egress from GALT by FTY720 treatment, suggested that primed NP-cells migrated from PP to MLN and spleen, as the presence of GFP^+ cells dropped in MLN and spleen but not in PP. Hence, PP is critical for gut IgA B cell responses, while MLN exhibits both *de novo* B cell priming as well as cells primed in PP. The microenvironment for GC B cell expansion appears to be dramatically different in PP and MLN.

W.9. B-Lineage Cells and EBI2 Expression are Elevated in Nasal Polyps from Patients with Chronic Rhinosinusitis

Kathryn Hulse, James Norton, Lydia Suh, Qiu Zhong, Mahboobeh Mahdavinia, Patrick Simon, Robert Kern, David Conley, Rakesh Chandra, Bruce Tan, Anju Peters, Leslie Grammer, Kathleen Harris, Roderick Carter, Atsushi Kato, Robert Schleimer. Northwestern University, Chicago, IL

The mechanisms that drive maturation of B cells at mucosal sites in humans are not well characterized. Epstien Barr virus-induced protein 2 (EBI2) is critical for B cell and antibody responses in lymph nodes, but little is known about EBI2 at mucosal sites. Chronic rhinosinusitis (CRS) affects over 30 million Americans, and provides an opportunity to study mucosal inflammation in humans. Previous work suggests B-lineage cells play a role in CRS pathogenesis. We wanted to determine whether B cells were

expanded in nasal polyps (NP) from CRS patients, and whether EBI2 played a role. Flow cytometry analysis of cells from control uncinate tissue (UT) and NP revealed elevations of B cells, plasmablasts and plasma cells (PC) in NP compared to UT ($p<0.01$). NP-derived B cells had higher side scatter MFI (2.5 fold), suggesting they were activated *in vivo*. EBI2 gene and protein expression were elevated in NP extracts ($p<0.01$), and correlated with expression of PC markers. Most antibody isotypes were elevated in NP tissues and cultured supernatants (>5 fold), indicating local secretion *in vitro* and *in vivo*. These data suggest that B cells in NP are highly active, secrete antibodies, and that EBI2 may play a role in this process.

W.10. The J Chain of Sarcopterygian Fish: Evolutionary Implications in Mucosal Immunity
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Background: J chain is a 15KDa polypeptide that is involved in the transport of immunoglobulins across epithelial surfaces and is responsible for immunoglobulin polymerization. J chain has been identified in mammals, amphibians, reptiles, birds and shark. Sarcopterygian fishes (lungfish) are the closest ancestors to all tetrapods. Aim: to characterize the J chain in the African lungfish (*Protopterus dolloi*). Results: we have identified a J chain sequence in *P. dolloi*. Sequence analyses indicate that six out of the eight Cys found in mammalian J chain are conserved in *P. dolloi*. The deduced amino acid sequence of *P. dolloi* J chain is 44% similar to chicken, 40% to Xenopus, 37% to human and 31% to shark J chains. J chain is expressed in gut, spleen, lung, and kidney, the highest expression levels found in the gut and postpyloric spleen. Fluorescence *in situ* hybridization shows that gut epithelial cells and B cells express J chain. Laser capture microdissected gut epithelial cells express J chain. Conclusion: this is the first characterization of J chain in sarcopterygian fishes, the ancestor of all tetrapods, and it reveals the ancient role of J chain in mucosal immunity and a role for J chain in non-B cells such as enterocytes.

W.11. Crosstalk Between Complement 5a Anaphylatoxin Receptor and Toll-Like Receptor on M Cells is Utilized by Mucosal Pathogen for Its Infection
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The mucosa harbors many micro-organisms including commensal and pathogenic bacteria and it is frequently exploited by pathogens for their infection. Especially, a specialized epithelial cell, M cell, whose function is known to take up luminal antigens is exploited by many pathogens such as Salmonella, Listeria, and Yersinia. In this study, we suggest possible survival mechanism of Y. enterocolitica through crosstalk between the receptor for complement 5a anaphylatoxin (C5aR) and Toll-like receptor (TLR) during its M cell infection via outer membrane H, one of C5aR ligands. During the Y. enterocolitica infection, not only localization of C5aR and TLR on apical area of M cells but also regulation of cAMP- and /or autophagy-associated gene expression were identified. In addition, blocking C5aR using C5aR antagonist or knocking-out of C5aR reduced the survival rate of Y. enterocolitica. Collectively, we suggest that regulation of cAMP expression by interaction between Y. enterocolitica and both C5aR and TLR is closely associated with the bacterial survival in M cells. (This study was supported by the Korea Institute of Planning and Evaluation for Technology of Food, Agriculture, Forestry and Fisheries.)

W.12. Fetal and Adult Intestinal Epithelial Cells Display a Similar Response Pattern to Microbial Stimuli
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A major challenge in premature neonates is necrotizing enterocolitis. Bacterial colonization contributes to disease development, while breast milk is protective. Our aim was to investigate how fetal and adult intestinal epithelial cell lines (IECs) respond to different bacterial strains and how breast milk and baby formula modulate this response. Fetal and adult IECs of different gestational ages/degree of differentiation were stimulated with bacterial supernatants from strains of *Lactobacilli* and *Staphylococcus (S.) aureus* or *lipopolysaccharide* and peptidoglycan. There were 36 cytokines and chemokines analyzed

by cytokine array. All IECs produced a restricted and surprisingly similar pattern of factors upon stimulation, but only S. aureus induced production of the proinflammatory chemokines CXCL8/IL-8 and CXCL1/GRO-α. As verified with ELISA the fetal IECs had a higher basal and microbial induced production of CXCL8/IL-8 compared to adult IECs. In contrast to what we have seen in immune cells the simultaneous stimulation of IECs with *S. aureus* and *lactobacilli* did not result in a dampening of the inflammatory response. Breast milk increased the CXCL8/IL-8 response to bacterial stimuli in both fetal and adult cells while baby formula had less effect. In conclusion, fetal and adult IECs have a strikingly similar response pattern to microbial stimuli.

W.13. Influence of Vaginal Lactobacilli on Mobility of HIV Virions in Fresh Human Cervicovaginal Mucus

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To reach target cells underlying the vaginal epithelium, HIV must first penetrate cervicovaginal mucus (CVM) secretions. We previously found that native CVM from women with healthy, lactobacilli-dominated vaginal flora (pH ~3.5-4) effectively trapped HIV, but not when neutralized to pH ~6-7. Only one-third of women have lactobacilli-dominated vaginal microflora; the remaining either have bacterial vaginosis, an overgrowth of polymicrobes that deplete vaginal lactobacilli resulting in a loss of vaginal acidity, or intermediate microflora. Therefore, we investigated whether native CVM beyond those from women with lactobacilli-dominated microflora can provide an effective diffusional barrier against HIV. We measured the real-time mobility of hundreds of individual HIV pseudoviruses internally tagged with m-Cherry. In 10 of 14 CVM samples tested, which exhibited high levels of lactic acid, HIV was effectively trapped in native CVM, with ~100-fold reduced mobility compared to theoretical speeds in water. In contrast, CVM samples that failed to trap HIV exhibited substantially lower lactic acid content, suggesting lactic acid secreted by lactobacilli may directly influence HIV mobility in CVM. This may help explain why women with bacterial vaginosis and intermediate microflora, where vaginal lactobacilli are often depleted, are at markedly enhanced susceptibility to acquiring HIV and other sexually transmitted infections.

W.14. Ontogeny of Fut2 Expression is Sensitive to Microbiota and Glucocorticoids, and is Required for Intestinal Homeostasis

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Regulation of intestinal maturation by intrinsic (glucocorticoid) and extrinsic (microbiota) factors is central to adaptation from milk to solid food at weaning. The contribution of these factors toward maturation was measured as changes in α1,2-fucosyltransferase II (fut2) and sucrase-isomaltase (SI) gene expression in conventionally raised (CONV), germ-free (GF), and bacteria-depleted (BD) mice. In CONV pups, cortisone acetate (CA) precociously induced both Fut2 and SI gene expression up to 4 weeks of age, but not thereafter. In GF suckling mice, whose ontogeny of SI was delayed, CA precociously induced SI, but not fut2 expression. The presence of normal microbiome only affects fut2 induction, but not SI expression. In GF and BD mice, preweaning gut fut2 expression is not affected by differences in the microbiome. However, in microbiome deficient mice, fut2 expression remained at low suckling levels after 4 weeks; only after colonization with adult gut microbiota did these animals express adult levels. CONV mice were able to recover from DSS-induced mucosal injury, but not BD mice; restoration of fut2 expression in BD mice allowed full recovery from DSS injury. These findings link the high incidence of Fut2 polymorphisms in populations with chronic inflammatory disease (Crohn's and necrotizing enterocolitis) to inflammatory pathophysiology.

W.15. Goblet Cells Deliver Luminal Retinoids and Imprint CD103$^+$ Lamina Propria Dendritic Cells

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The intestinal *lamina propria* (LP) contains a large population of CD103$^+$ myeloid dendritic cells (DC). CD103$^+$ LP-DCs generate the vitamin A metabolite all-trans retinoic acid (ATRA) and promote intestinal

type responses including generating Foxp3$^+$ T regulatory cells, imprinting gut homing expression on lymphocytes, and facilitating IgA production. *In vivo* studies demonstrated that luminal retinoids from the diet or bile are required to condition intestinal LP-DCs to generate ATRA. How luminal retinoids confer this capacity is not known. Goblet cell associated antigen passages (GAPs) provide a portal for CD103$^+$ LP-DCs to acquire soluble luminal substances and goblet cell (GCs) proteins, suggesting a role for GCs and GAPs in imprinting CD103$^+$ LP-DCs. Using an *in vivo* two-photon (2P) imaging approach, we found that GAPs delivered luminal retinoids and the enzyme required to metabolize pro-vitamin A to LP-DCs. In the absence of GCs, LP-DCs are not imprinted with ALDH activity. CCR6 deficient LP-DCs, which fail to associate with the epithelium, do not acquire GC proteins or luminal antigens, do not become imprinted with ALDH activity, and are impaired at inducing ATRA dependent events. These findings demonstrate that GAPs deliver luminal retinoids and the enzymes required to metabolize these retinoids to CD103$^+$ LP-DCs.

W.16. Colonic Mucosal Dysbiosis in Chronic, Untreated HIV-1 Infection is Associated with Increased Mucosal T Cell and Dendritic Cell Activation

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HIV-1 infection disrupts the intestinal immune system, leading to microbial translocation and systemic immune activation. We investigated the impact of HIV infection on the intestinal microbiome and its association with mucosal T cell and dendritic cell (DC) frequency and activation. Bacterial 16S ribosomal DNA sequencing was performed on colon biopsies from 17 subjects with chronic, untreated HIV-1 infection (HIV$^+$) and 14 uninfected (HIVneg) control subjects. Colon T cell and DC frequency and activation state were determined by flow cytometry. HIV$^+$ subjects had increased abundance of Proteobacteria ($p=0.03$), largely comprised of Acinetobacter spp. ($p<0.05$), and decreased abundance of Firmicutes ($p=0.03$) relative to HIVneg donors. Despite similar abundance of Bacteroidetes phyla between the two cohorts, a significant increase in Prevotella ($p=0.01$) and decrease in Bacteroides ($p=0.04$) genera relative abundance was observed in HIV$^+$ subjects, suggesting an enterotype shift. This HIV-associated increase in Prevotella was not related to either BMI or dietary factors, but was associated with increased numbers of activated colonic CD4 ($r=0.67$, $p=0.003$) and CD8 ($r=0.65$, $p=0.004$) T cells and with activated myeloid DCs ($r=0.64$, $p=0.02$). These observations suggest that an important relationship may exist between mucosal bacterial enterotype and intestinal inflammation during chronic HIV infection.

W.17. hSPAG11B Alternative Splice Variants: Putative New Players on Intestinal Mucosa Homeostasis

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The human anti-microbial gene sperm-associated antigen 11 (hSPAG11) resides within a highly polymorphic β-defensin cluster in the chromosome 8p23, wherein a complex alternative splicing pattern results in 7 distinct transcripts (hSPAG11A-E, G and H). Aside from its antimicrobial activity, a yeast two-hybrid screening identified hSPAG11D as a competitive inhibitor of hTryptase-β1. Evidence is mounting that a relative deficiency on antimicrobial peptides and endogenous protease inhibitors are detrimental to intestinal mucosa homeostasis. In this way, the aim of the present study was to investigate the expression profile and regulation of hSPAG11B gene products in human colonic biopsies from inflammatory bowel disease patients. Preliminary RT-PCR experiments indicated the constitutive transcription of at least hSPAG11C, D and E in healthy individuals. Immunoreactivity for hSPAG11C, D/E was most evident in colonic epithelium, with a polarization towards the apical region of these cells. Notwithstanding, a deficiency on hSPAG11D/E expression was detected by immunofluorescence in either inflamed or uninflamed colonic biopsies from Crohn’s disease, but not from ulcerative colitis patients, in

comparison to healthy individuals. The functional relevance of these findings with regard to the pathophysiology of intestinal inflammatory diseases is under investigation.

W.18. Crosstalk Between Natural Killer and Monocyte-Derived Dendritic Cells

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Crosstalk between natural killer (NKs) and dendritic cells (DCs) during infection is well documented. Nevertheless, interaction between NKs and monocytes during monocyte-derived-dendritic cells (Mo-DCs) differentiation is less understood. During intra-peritoneal infection, NKs promote Mo-DCs differentiation through their IFN-γ production, however, it is not established if contact between cells is required. Here, we investigated if NK cells, through direct contact or by their products, affect Mo-DCs differentiation *in vitro*. $CD14^+$monocytes were purified from healthy donors' blood and co-cultured with isolated NK cells ($CD56^+$CD3-) in the presence of IL-4 and GM-CSF, in a transwell® system or in direct contact. At day 5, TNF-α was added and 48h later Mo-DCs were harvested, phenotyped and co-cultured with CFSE-labeled allogeneic T lymphocytes. After additional 5 days, lymphoproliferation was determined and cytokines in supernatants quantified by CBA (BD™). Mo-DCs differentiated in contact with NKs (NK-Mo-DCs) up-regulated the expression of HLA-DR, CD80, CD86, CD83, CCR7 and CD11c, were more potent stimulators of lymphocyte proliferation and induced a Th1-like cytokine profile (IL-2, IFN-γ and TNF-α) when compared to control and transwell groups. These results suggest that NK cells, through direct cell contact and not only by its products promote Mo-DCs differentiation and induce a polarized Th1-like profile.

W.19. Type I NKT Cells Promote Intestinal Tumor Development

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Type I NKT cells carry a semi-invariant T cell receptor that is restricted to the MHC class I-like molecule CD1d. They respond rapidly to activation and regulate diverse immune reactions. Murine Type I NKT cells have been shown to reduce tumor growth and prevent metastasis formation. Further, NKT cells were found to promote intestinal inflammation in a mouse model for colitis. $APCMin^+$ mice carry a deletion in the adenomatous polyposis coli (APC) gene, mutated in familial and sporadic human colorectal cancer. These mice develop small and large intestinal polyps, driven by the intestinal flora and inflammatory signals. While Type I NKT cells have a protective role in several tumor models, their ability to promote intestinal inflammation raised the question whether the cells would prevent or promote tumor formation in the intestine. Here we demonstrate that Type I NKT cells promote tumor formation in the APCMin mouse. We further show that treatment of mice with the Type I NKT cell ligand α-galactosylceramide reduced tumor growth, while treatment with altered ligands that induce a skewed cytokine profile could increase tumor growth. Our results reveal that type I NKT cells can naturally promote some forms of tumors, an effect enhanced by ligand stimulation.

W.21. Mucosal Imprinting of Vaccine Induced-$CD8^+$T Cells is Crucial to Inhibit the Growth of Mucosal Tumors

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Although many human cancers are located in mucosal sites, most cancer vaccines are tested against subcutaneous tumors in mice. We therefore wondered whether mucosa-specific homing instructions to the immune system might influence mucosal tumor outgrowth. We showed that the growth of orthotopic

head and neck or lung cancers was only inhibited, when a cancer vaccine was delivered by the intranasal (i.n) mucosal and not the intramuscular (i.m) route. This anti-tumor effect was dependent on $CD8^+$T cells. To explain this finding, we demonstrated that only i.n. vaccination elicited mucosal specific-$CD8^+$T cells expressing the mucosal integrin CD49a. Blockade of CD49a decreased intratumoral $CD8^+$T cell infiltration and the efficacy of cancer vaccine on mucosal tumor. We then showed that after intranasal vaccination, only dendritic cells from lung parenchyma, but not from spleen induced the expression of CD49a on co-cultured specific $CD8^+$T cells. Tumor-infiltrating lymphocytes from human mucosal cancer also expressed more CD49a than non-mucosal tumors supporting the relevance of these results in humans. We thus identified a link between the route of vaccination and the induction of a mucosal homing program on induced $CD8^+$T cells controlling their trafficking with a direct application on the efficacy of cancer vaccine to control mucosal tumors.

W.22. Tissue-Specific Knock Out Mouse Models Reveal a Crucial Role of Mitochondrial HSP60 in Intestinal Epithelial Cell Homeostasis

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Heat shock protein (HSP) 60, a mitochondrial (MT) unfolded protein response (UPR)-associated chaperone, is implicated in the pathogenesis of chronic intestinal inflammation. This study investigates the role of HSP60 in intestinal epithelial cell (IEC) homeostasis using novel tissue-specific knockout mouse models. Generation of epithelial-specific hsp60 knockout mice ($hsp60^{flox/flox}$XVillinCre) antagonized embryonic development upon day eleven after conception and induced embryolethality. Postnatal induction of an epithelial specific hsp60 knockout ($hsp60^{flox/flox}$XVillinCreER^{T2}) caused a severe phenotype with rapid weight loss and mortality. Histological evaluation of small intestinal gut sections revealed aberrations in villus-crypt architecture including focal crypt hyperproliferation originating from few HSP60-positive cells, which have escaped from the genetic knock out in the crypt. The morphological changes were accompanied by macrophage and dendritic cell infiltration. Colonic loss of HSP60 did not cause tissue pathology but induced hallmarks of the mtUPR in IEC, suggesting the presence of compensatory mechanisms upon loss of HSP60 at early phases of mtUPR. These include induction of mitochondrial chaperones like mtHSP70, proteases like the ATP dependent caseinolytic peptidase and transcription factors like C/EBP-homologous-protein. Tissue-specific deletion of hsp60 disrupts epithelial cell homeostasis both pre- and post-natally leading to crypt aberrations and compartmentalized pathologies in the small intestine.

W.23. GPR35: A Potential Colon Epithelial Cell Receptor for the Bacteroides Fragilis Toxin

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Bacteria are proposed as critical to the pathogenesis of gastrointestinal diseases such as colorectal cancer (CRC). *Enterotoxigenic Bacteroides fragilis* (ETBF) induces inflammatory diarrhea and promotes colon tumorigenesis in the multiple intestinal neoplasia mouse model ($Apcmin^+$). Binding and signaling of the *Bacteroides fragilis toxin* (BFT) through colon epithelial cells is pivotal for the carcinogenic potential of ETBF, resulting in cleavage of E-cadherin, β-catenin signaling and Th17-mediated tumorigenesis. Using a subtraction array in BFT responsive versus non-responsive cells and shRNA knock-down techniques, GPR35, a G-protein coupled receptor highly expressed in the colon, was identified as a candidate BFT-receptor. Both GPR35 knock-down and GPR35 antagonists reduced the HT29/C1 cell response to BFT including inhibition of E-cadherin cleavage and IL-8 secretion, a molecule involved in chemotaxis. Furthermore, Western blot and confocal microscopy revealed that BFT induces B -arrestin-2 cytoplasmic redistribution and nuclear translocation, similar to the GPR35 agonist compound 10. Our data strongly suggest that BFT acts through the colonic epithelial cell receptor GPR35 and therefore we hypothesize that GPR35 contributes to the pathogenesis of human CRC.

W.24. Caspase-1 Regulates Colorectal Cancer Metastasis to the Liver Through IL-18

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Colorectal cancer (CRC) often die because of primary tumor dissemination to secondary sites, primarily the liver. While inflammation has been implicated in modulating CRC metastasis, the role of the inflammasome in this process is unknown. In response to danger signals, the inflammasome is scaffolded by intracellular PRRs, such as the NLR, and recruit and activate Caspase 1. Caspase 1 cleaves pro-IL-1b and pro-IL-18 into their bioactive forms. To examine the role of the inflammasome in liver metastasis, MC38 cells were injected intrasplenically in mice. Twenty-one days later, Casp1$^{-/-}$/Casp11m mice had an increased metastatic burden compared to WT animals. The control of metastatic growth was dependent on IL-18 as Il18$^{-/-}$ and Il18r1$^{-/-}$ mice, but not Il1r1$^{-/-}$ mice, phenocopied the Casp1$^{-/-}$ mice. An important function of IL-18 is the stimulation of IFNg production. Consistently, Ifng$^{-/-}$ mice were also more susceptible to liver metastasis compared to WT animals. We show that tumor immune surveillance in this context might involve NK cells, as Rag1$^{-/-}$ mice, but not Jak3$^{-/-}$ mice had a comparable metastatic burden to that of WT mice. Our results thus indicate a pre-requisite role for Caspase 1 activation and subsequent IL-18 production in controlling metastatic growth.

W.25. Myosin Light Chain Kinase is Involved in the Development of an Animal Model of Colitis-Associated Tumor

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Background and Aim: Prolonged inflammatory bowel disease is associated with colitis-associated cancer (CAC). Myosin light chain kinase (MLCK) has been reported to be essential to the permeability of epithelial barrier in the setting of colitis, but its role in the development of CAC is still unknown and is the focus of our study. Methods and Results: Wild type C57BL/6 mice were treated three times with DSS to induce chronic colitis. Western blotting (WB) showed slight upregulation of MLCK in the colonic epithelia in association with up-regulated NF-κB. Next, the CAC model was accomplished by azoxymethane treatment before three cycles of DSS administration. NF-κB, and MLCK were further up-regulated in the tumor tissues compared to the non-tumor areas, as well as up-regulated tumor necrosis factor receptor (TNFR) 2 assessed by qPCR and WB. Immunohistochemistry (IHC) showed that the MLCK inhibitor, ML-7, treatment prevented tight junction disruption. IHC showed that anti-TNF mAb, MP6-XT22, treatment restored the disrupted tight junctions in tumors and suppressed tumor development. Conclusions: The permeability of epithelial layer in the CAC tissues is associated with MLCK up-regulation and susceptibility to proinflammatory cytokines that potentially promote CAC growth.

W.26. MUC1 and MUC13 Differentially Regulate Epithelial Inflammation in Response to Inflammatory and Infectious Stimuli

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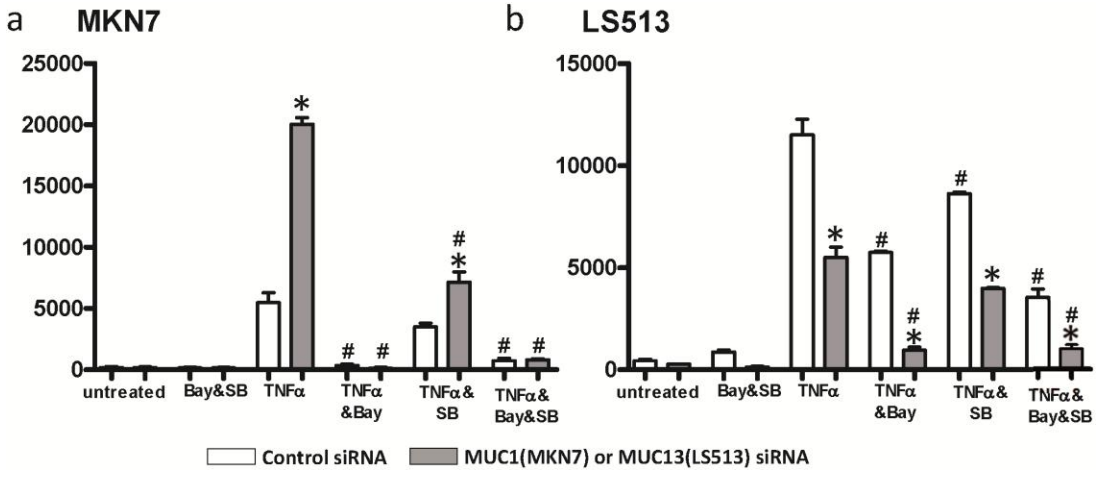

Background: MUC1 is the predominant gastric epithelial cell surface mucin, whereas MUC13 is a major intestinal cell surface mucin. Polymorphisms in MUC1 and MUC13 have been linked to IBD, and our aim was to characterize whether and how MUC1 and MUC13 modulate infectious and inflammatory signaling. Methods: We used gastrointestinal tissue from Muc1 or Muc13 deficient mice in *ex vivo* culture, MUC1 siRNA silencing in MKN7 gastric cells and MUC13 siRNA silencing in LS513 and LIM2463 intestinal cells. Results: 1) MUC1 inhibits whereas MUC13 enhances inflammatory responses of gastrointestinal epithelial cells to inflammatory and infectious stimuli. 2) MUC1 and MUC13 have actively opposing influences on inflammatory signaling in cells where both mucins were expressed. 3) The main mechanism by which MUC1 and MUC13 regulate chemokine secretion in gastrointestinal epithelial cells is through NF-κB dependent pathways, although MUC13 modulation can also involve p38 MAPK, but not via ErbB-1 or ErbB-2 signaling. Furthermore, MUC13 is also associated in complexes with p53, and this interaction is increased by apoptotic stimuli. Conclusion: Our data demonstrate that MUC1 and MUC13 have reciprocal inflammatory actions, and that disruption or inappropriate expression of either of these mucins could predispose to infectious and inflammatory disease and inflammation-induced cancer.

MUC1 and MUC13 differentially regulate IL-8 production by gastrointestinal cells in response to TNF-α, and the mechanism is through NF-κB dependent pathways, although MUC13 modulation can also involve p38 MAPK. cells were transfected with MUC1 (a) or MUC13 (b) or control siRNA for 48 h and pretreated with 20 μM of the NF-κB inhibitor Bay 11-7085 (Bay) or 20 μM of the p38 MAPK inhibitor SB 203580 (SB) or both for 1 h, then cells were stimulated with TNF-α (10 ng/ml) for 24 h, and IL-8 levels in culture media were measured by ELISA Statistics: mean ± SEM; n ≥ 4; Mann-Whitney U-test MUC13 siRNA vs control siRNA , * $P < 0.05$, control siRNA inhibitor and TNF-α treated cells vs control siRNA TNF-α treated cells and MUC13 siRNA inhibitor and TNF-α treated cells vs MUC13 siRNA TNF-α treated cells, # $P < 0.05$.

W.27. Characterization of Inflammatory Cytokine Response in Human Sporadic Colorectal Cancer
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Studies in experimental models of colon carcinogenesis have shown that T cell-derived cytokines have a dual role in the control of colorectal cancer (CRC) cell growth. However, the contribution that such cytokines play in the pathogenesis of human sporadic CRC is not fully understood. We here show that human sporadic CRC tissue is infiltrated with huge numbers of T cells and CRC-infiltrating T cell-derived supernatants stimulate the growth of cultured CRC cell lines. By flow-cytometry, we also show that the majority of T cells infiltrating either CRC tissue or normal adjacent colonic mucosa co-expresses T-β and RORγt even though CRC samples contained more T-β-/RORγt$^+$ T cells and less T-β$^+$/RORγt- T cells as compared to normal colonic samples. Consistently, transition from the normal colonic mucosa to the neoplastic area is marked by abundance of IL-17A-, IL-21- and IL-22-producing T cells and reduction of IFN-γ-producing T cell numbers. Moreover, a high percentage of IL-17A-producing Foxp3$^+$ cells co-expressing T-β and/or RORγt, but unable to produce IFN-γ is seen in CRC tissue. Data indicate that human sporadic CRC is associated with a predominant accumulation of IL-17A-producing RORγt$^+$ T cells, which could contribute to sustain neoplastic cell growth.

W.28. Down-Regulation of the Polymeric Immunoglobulin Receptor is a Common Feature of Human Ulcerative Colitis and Murine Models of Chronic Colitis and Colitis-Associated Cancer
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Secretory IgA (SIgA) antibodies promote intestinal homeostasis by limiting microbial access to immune cells in the lamina propria. SIgA is transported across intestinal epithelial cells (IEC) by the polymeric immunoglobulin receptor (pIgR). Analysis of pIgR mRNA levels in colonic biopsies revealed that pIgR was significantly down-regulated in patients with ulcerative colitis (UC) compared to healthy controls. We previously reported that pIgR is down-regulated in sporadic colon cancer. Because UC patients are at high risk of developing colitis-associated cancer (CAC), we hypothesized that down-regulation of pIgR may contribute to development of chronic colitis and CAC. To test this hypothesis, we used a murine

model of chronic colitis and CAC, involving multiple cycles of oral administration of the epithelial-disrupting agent DSS, with or without prior injection with the carcinogen azoxymethane. We found that down-regulation of pIgR mRNA was an early and sustained event in the development of chronic colitis, and that pIgR protein was substantially reduced in dysplastic and neoplastic colon tissues. Finally, we found that pIgR-deficient mice exhibited more severe colitis after multiple rounds of DSS than did wild-type mice. Our findings suggest that down-regulation of pIgR expression in IEC may contribute to the development of chronic colitis and CAC.

W.29. Early Maternal Separation in Mice Impairs Specific Immune Response Toward Luminal Content at Adulthood

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Early life stressful events contribute to development of functional gastrointestinal disorders such as irritable bowel syndrome (IBS). Using maternal separation (MS) as an experimental model mimicking IBS, we investigated whether early life stressful events in mice may trigger inappropriate immune responses toward luminal content and as a consequence induce IBS symptoms. MS leads to IBS-like symptoms (intestinal hyper-permeability and visceral sensitivity) and impairs immune system in adult male C_3H/HeN mice. Indeed, MS decreased by 20% fecal IgA concentration. Ig specificity against food antigens and commensal *E. coli lysate* was assessed by normalizing Ig concentrations between samples. MS significantly increased anti-*E. coli* IgG (Optical-Density 0.089±0.012 vs.0.033±0.005; $p<0.05$) but not IgA in plasma without affecting Ig response in Gut Fluid lavages (GFL). Furthermore, MS decreased levels of anti-food IgG in plasma (OD 1.277±0.160vs.0.785±0.125; $p<0.05$). *Ex vivo* stimulation of splenocytes with *E. coli lysate* induced higher IFNγ secretion (594±72 vs. 357±72pg/ml; $p<0.05$) and lower IL-10 (278±100vs.535±115pg/ml; $p<0.05$) and IL-17 (289±159vs.539±189pg/ml; $p<0.05$) secretion in MS mice compared to controls. These data show that early life stressful events trigger inappropriate immune response toward luminal content (food antigens and microbiota) that may contribute to IBS symptoms and highlight perinatal period as a critical window for immune system development.

W.30. Tumor Elicited Inflammation in Colorectal Cancer

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Chronic inflammation is associated with cancer development and often promotes tumor growth. Even initially non-inflammatory tumors inflammatory cells as a part of "tumor-elicited inflammation" (TEI). Various pro-inflammatory cytokines originating from tumor-infiltrating cells and acting on cancer cells, provide a possible link between inflammation and cancer. In colorectal cancer (CRC) tumor initiating oncogenic events linked to the epithelial activation of Wnt pathway lead to the local defects and permeability of epithelial barrier. Subsequent translocation of microbiota triggers tumor-specific activation of myeloid cells and production of tumor-promoting cytokines, such as Interleukin 23 (IL-23). Inactivation of IL-23 pathway results in decreased CRC tumorigenicity, and reduction in its downstream targets IL-6, IL-22 and IL-17 previously linked to intestinal homeostasis and tumorigenesis. We further define cytokines and endogenous factors that are essential for regulation of TEI and tumor promotion and progression.

W.31. Role of $CD8^+$T Cells in a Mouse Model of Enteropathy Induced by a Dietary Antigen

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A mouse model of enteropathy was obtained by chronic exposure of OTII mice (bearing OVA-specific TCR on $CD4^+$T cells) overexpressing human IL-15 in the intestine (OTII/IL-15Tge) to dietary ovalbumin (OVA). In contrast to OTII/IL-15Tge on control diet, OTII/IL-15Tge on OVA-containing diet displayed

decreased villous/crypt ratios and growth retardation. Specific Foxp3$^+$ Treg cells were functional, but *in vitro* studies revealed that IL-15 rendered effector T cells, notably CD8$^+$, resistant to Treg suppression. Pointing out the role of CD8$^+$T cells in the enteropathy, a dramatic upregulation of granzyme expression was observed in the CD8$^+$T cells that accumulated in the lamina propria (LP) of OTII/IL-15Tge mice on OVA diet. Dextramer (H2-Kb/SIINFEKL) staining failed to demonstrate CD8$^+$T lymphocytes specific for OVA. However, stimulation of LP cells from OTII/IL-15Tge but not from B6/IL-15Tge by OVA323-339 (CD4$^+$T cell specific) peptide in the presence of IL-15 led to the proliferation and activation of CD8$^+$T cells. Studies in progress aim at dissecting the mechanism of CD8$^+$T cell activation and the respective roles of CD4$^+$T cell help and IL-15 in this process. We expect that these data will shed light on the mechanisms that control the activation of CD8$^+$T in celiac patients exposed to dietary gluten.

W.32. Epithelial-Derived Thymic Stromal Lymphopoietin (TSLP) Negatively Regulates T Cell-Derived Cytokines in Celiac Disease (CD)

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Background and Aims: TSLP is required to protect the gut from inflammation. However, no information is available on TSLP in CD, a Th1-mediated enteropathy induced by the ingestion of gluten. We explored through *in vivo* and *ex vivo* experiments TSLP expression and function in CD duodenal mucosa. Methods: Transcripts and proteins of TSLP and TSLP receptor (TSLPR) were detected by qRT-PCR or immunoprecipitation/immunoblotting and confocal microscopy in duodenal biopsies from 14 CD patients before and after 12 months of gluten-free diet (GFD), and from 13 controls. IFN-γ and IL-17A production and phospho-STAT5 expression were evaluated in biopsies from 8 untreated CD patients cultured *ex vivo* with rhTSLP. Results: Transcript and protein levels of TSLP, but not TSLPR, were significantly reduced in untreated CD patients compared to CD patients after GFD and controls. By confocal microscopy, while TSLP was abundant in normal gut epithelium, it was virtually absent in untreated CD. rhTSLP markedly inhibited IFN-γ and IL-17A production by untreated CD biopsies grown *ex vivo*, and significantly enhanced STAT5 phosphorylation. Conclusions: Epithelial TSLP is decreased in untreated CD and normalizes after GFD. The *ex vivo* TSLP-induced down-regulation of T cell-derived cytokines, which are implicated in the generation of villous atrophy, suggests that removal of the negative regulation exerted by TSLP on activated T cells might have a pathogenic role in CD.

W.33. Production of CXCL10 in Intestinal Mucosa in Active Coeliac Disease

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Lymphocytic infiltration, mainly composed by CD4$^+$ Th1 cells and plasma cells, is a chararacteristic finding in intestinal mucosa in active Coeliac Disease (CD). Signals for specific recruitment of these cells have no been fully established. In previous work, we observed that the number of CXCR3$^+$ cells and CXCL10 expression are higher in lamina propria of untreated CD patients compared to healthy controls. The aim of this work was to extend the investigation on the role of CXCL10/CXCR3 axis in CD pathogenesis. Higher concentration of CXCL10 was found in serum samples from CD patients (n=26) compared to healthy controls (n=21)(p=0.0002). mRNA levels for CXCL10 and CXCL11, but not CXCL9, were significantly higher in duodenal biopsies from paediatric CD patients (n=20) compared to healthy controls (n=22) or treated patients (n=6)(p<0,01). We identified plasma cells, among other lamina propria CXCL10 producer cells. CXCL10 was also expressed by epithelial cells. *In vitro* assays showed that CXCL10 was induced in Caco-2 cells treated with IFNg and poly I:C. In conclusion, the massive CXCL10 production in the small intestine may be one of the main chemotactic pathways mediating T cell and plasma cell recruitment. CXCL10 production by enterocytes may be also a consequence of innate stimuli.

W.34. Intestinal Epithelial Cultures from Patients with Celiac Disease do not Differ from Controls

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The duodenum of patients with active celiac disease show distinctive features such as villus atrophy and crypt hyperplasia. These pathological features are known to result from activated T cells that recognize gliadin-peptides bound to HLA-DQ2 or -DQ8. It has been shown that gliadin or its derivative peptides can induce epithelial damage in biopsies from active celiac patients. However, as there are also immune cells present in biopsies, it is difficult to dissect the direct or indirect effects of gliadin on epithelial cells. Therefore, we generated epithelial organoid cultures from intestinal biopsies from celiac patients and healthy controls (n=3 per group), both with an HLA-DQ2.5 genotype. We cultured the organoids for 5 weeks and studied their properties. Preliminary data show that organoids from celiac patients and controls do not show clear differences in proliferation and apoptosis. We are currently investigating other epithelial properties, like differentiation and the direct effects of gliadin and gliadin peptides on epithelial cells.

W.36. Altered Expression, Desensitization and Recycling of CCR9 in the TNFΔARE Mouse Model of Crohn's Disease

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Background & Aims: CCL25 chemokine and its receptor CCR9 are critical for the homing of lymphocytes to the small intestine (SI) under physiologic conditions. Their role in homing under inflammatory conditions is less understood. Using a TNF-driven mouse model of Crohn's-like ileitis (i.e. TNFΔARE mice), we studied whether the expression, the dynamics of CCR9 desensitization and recycling in response to CCL25 were altered by the chronic inflammatory process. Results: CCR9 expression in ileal $CD8^+$ T cells from WT animals was lower (mean MFI 852±61, n=6) compared with cells from mesenteric lymph node (MLN; 1654±62, n=10) and spleen (1727±87, n=13), where CCL25 is not expressed. CCR9 internalizes *in vitro* upon ligation with CCL25 (69%±1 internalization, n=3) and recycles back to membrane in its absence (36%±0.8 recovery, n=3). We detected higher CCR9 density on the membrane of ileal $CD8^+$ T cells from TNFΔARE mice (mean MFI 1293±85, n=9) versus WT controls (852±61, n=6), despite higher levels of CCL25 in chronically inflamed terminal ilea. Conclusions: Although CCR9 behaves as a classic GPCR *in vitro* and *in vivo* in non-inflamed mice, the regulation of CCR9 expression and its response to CCL25 is multifactorial under conditions of chronic inflammation.

W.37. Enhanced Cancer Immunotherapy Using Nanoparticle-Based Modulation of SOCS1 Genes in Dendritic Cells

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Although dendritic cells have important roles in cancer immunotherapy, their therapeutic efficiencies were limited due to the presence of immunosuppressive factors. Here, we report the fabrication and use of polymer nanoparticles for the simultaneous delivery of antigens and small interference RNA (siRNA) of immune suppressor genes into dendritic cells. The polymer nanoparticles containing ovalbumin (OVA, as a model antigen) and siRNA of suppressor of cytokine signaling 1 (SOCS1) were fabricated by double emulsion solvent evaporation method. The encapsulation of siRNA was about 57.6% when OVA was present during the preparation, while that was only 2 % without the OVA. The polymer nanoparticles containing OVA and siRNA of SOCS1 were efficiently taken up by the dendritic cells and showed no detectable toxicity. The knockdown of SOCS1 genes in dendritic cells induced pro-inflammatory and anti-inflammatory cytokine expression. The polymer nanoparticles are expected to be potent nanotechnology platform for targeted delivery of siRNA and antigens into dendritic cells, and use as $CD8^+$ T cells-based cancer immunotherapy.

W.38. Multifunctional Hybrid Nanoconjugates for Efficient *in vivo* Delivery of Immunomodulating Biomolecules and Enhanced Anti-tumor Immunity

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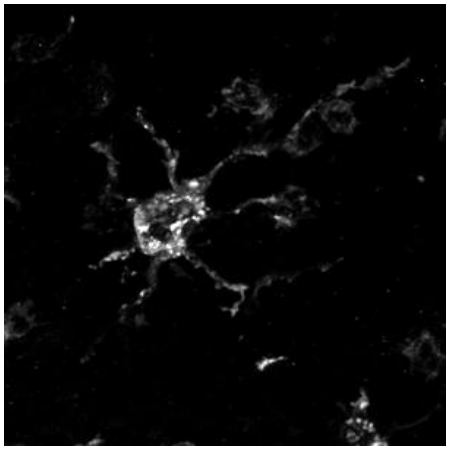
Immunotherapy is regarded as a method of attractive treatment for cancer. Especially, dendritic cells (DCs) represent important targets for cell-mediated immunotherapy in cancer because they can capture tumor antigens that are released from tumor cells and migrate to the tumor-draining lymph nodes, where they present the antigens to T cells and secrete the pro-inflammatory cytokines that enhance T cell activation. However, immunosuppressive factors, such as signal transducer and activator of transcription-3 (STAT3), represent a major limitation for DCs-based cancer therapies. In this study, we have designed and synthesized an immunomodulatory hybrid nanoconjugates (HNC) system based on polymer nanocomposites containing quantum dots (QDs; as imaging tracers) that are decorated with CpG ODNs (as a TLR9 ligand) and STAT3 siRNAs (as an immunosuppressive gene silencer) for the efficient immunotherapeutic cancer therapy. These HNC efficiently targeted immune cells, induced TLR activation, and silenced immunosuppressive genes. Simultaneous *in vivo* delivery of STAT3 siRNA and CpG ODN to DCs in the tumor microenvironment induced both the inhibition of STAT3 and activation of DCs by CpG ODNs, and synergistically elicited anti-tumor effects. By using NIR-emitting QDs, the migration of *in vivo* DCs to lymph nodes was also tracked by real-time NIR fluorescence imaging. In the future, these studies are expected to facilitate the development of immune cell-based cancer therapy.



W.39. Origin of Peyer's Patch Dendritic Cells

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The mammal small intestine possesses specific sentinel sites, named Peyer's patches (PPs), which are composed of clustered B cell follicles. Above each follicle the subepithelial dome contains dendritic cells (DC) among which a myeloid DC subset, called LysoDC, highly expresses the bactericidal agent lysozyme. Here, we defined a gating strategy to isolate each PPDC subset to generate a gene-expression database. LysoDC expressed genes typically related to the monocyte-derived pathway of differentiation namely CSFR-1, MafB, C-Maf and CCR2 whereas all other PPDC subsets expressed genes of the common DC precursor pathway of differentiation such as Id2, BatF3 and Flt3. Since monocyte egress from the bone marrow is dependent on CCR2 we investigated whether LysoDC distribution was altered in $CCR2^{-/-}$ mice. PP lysozyme-expressing cells could be split in 2 subsets based on their MHC-II and CD11b expression, their level of autofluorescence (AF) and their CCR2 dependency: the $MHCII^{Hi}CD11b^{Hi}AF^{lo}$ subset was almost completely depleted in $CCR2^{-/-}$ mice while the $MHCII^{Lo}CD11bintAF^{Hi}$ subset was not altered. However, in bone marrow chimeric mice, both subsets derived only from the wild-type bone marrow while conventional PPDC derived equally from wild type and $CCR2^{-/-}$ bone marrows. In addition, we observed that the $MHCII^{Hi}CD11b^{Hi}AFlo$ subset had a much faster turnover than the $MHCII^{Lo}CD11bintAF^{Hi}$ subset. Our results indicate that PP lysozyme-expressing cells are monocyte-derived while others PPDC belongs to the conventional DC. Moreover, the former are composed of two subsets: the LysoDC subset which is related to the monocyte-derived DC ($MHCII^{Hi}CD11b^{Hi}AF^{lo}$) while the other one is related to the monocyte-derived macrophages ($MHCII^{Lo}CD11bintAF^{Hi}$).

W.40. Regulation of Integrin αvβ8 Expression on Dendritic Cells Modulates TGF-β-Dependent Immune Responses

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Induction of regulatory T cells (Tregs) by the pleiotropic cytokine TGF-β is necessary in the gut to suppress immune responses to commensal bacteria and innocuous antigen. In the mouse gut, expression of integrin αvβ8 on $CD103^{+}$ dendritic cells is important for TGF-β activation, Treg induction,

and protection from colitis. Modulation of integrin αvβ8 expression might therefore have therapeutic potential in human inflammatory bowel disease. However, the pathways that control integrin αvβ8 expression and function remain to be discovered. To investigate how αvβ8 expression is regulated, we treated human monocyte-derived dendritic cells (MoDC) with a panel of immunomodulatory stimuli and analysed the expression levels of αvβ8 and maturation markers using flow cytometry. We found that treatment of MoDC with a range of bacterial species caused upregulation of integrin αvβ8 expression. Increased expression was also observed when the cells were treated with purified toll-like receptor ligands, indicating that pathogen-associated molecular patterns trigger upregulation of the αvβ8 integrin in MoDC. Our current work is aimed at understanding the functional consequences of regulated αvβ8 expression, focusing on how alteration of the αvβ8-TGF-β pathway modifies downstream T cell responses.

W.41. Dendritic Cells Use IgE for Regulating Th2 Immunity at Mucosal Surfaces

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Immunoglobulin-E (IgE) is well recognized for its role in inducing allergic reactions in response to antigen via activation of mast cells and basophils. Comparable to these allergic effector cells, human dendritic cells (DCs) also constitutively express Fc-epsilon-RI, the high-affinity IgE receptor, and use this Fc receptor to carry IgE on their cell surface. Since murine DCs do not constitutively express Fc-epsilon-RI, the physiological consequences of IgE-mediated antigen recognition by DCs are poorly defined. Therefore, we used 'Fc-epsilon-RI-humanized' transgenic mice, which express this receptor on DCs like humans. At steady state slightly elevated levels of IL-4 and IL-13, but no signs of an inflammatory response were detectable in these animals, suggesting that IgE contributes to maintaining immune homeostasis. Using a food allergy and an allergic airway inflammation model, we demonstrate that inflammatory Th2-associated immune response at mucosal surfaces can be dampened by Fc-epsilon-RI expressing DCs. At the cellular level, we found that IgE is an extremely efficient mechanism for antigen uptake and induction of T cell responses. Importantly, IgE-mediated antigen recognition inhibits NF-kappa B signaling and the production of inflammatory cytokines by DCs. Collectively, our data suggest a novel immunoregulatory function of IgE and DCs in orchestrating Th2 immunity.

W.42. Antigen-Targeting to DEC-205 Regulates T Cell Immunity and Abrogates Intestinal Inflammation

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Crohn's Disease and Ulcerative Colitis are the two major types of inflammatory bowel disease (IBD) in humans. The loss of tolerance towards commensal gut bacteria and/or food antigens seems to be an initial event for the manifestation of disease. Therefore, a promising approach for the prevention of IBD seems to be the induction of tolerance in the gut. Antigen-targeting to DEC-205 expressed by dendritic cells was reported to induce tolerance in different experimental disease settings. However, whether this approach is sufficient to also induce tolerance in the intestine is currently unknown. In the present study, we demonstrate that antigen-targeting to DEC-205 under steady state conditions leads to a significant abrogation of inflammation in a newly established, antigen-specific $CD4^+$ T cell-mediated mouse model for intestinal inflammation. This is represented by the reduction of weight loss, histopathology and secretion of pro-inflammatory cytokines like IFN-γ and IL1-β in the colon. Importantly, in this experimental setting antigen delivery to dendritic cells via DEC-205 leads to the reduction of inflammation via the silencing of Th1 cells. In further studies the potential of antigen-targeting to DEC-205 for the induction of tolerance will be tested during active inflammation and remission.

W.43. CD1a-/CD207-/$CD11c^+$ Human Dendritic Cells Bind βv1 Allergen Within the Oral Mucosal Tissue

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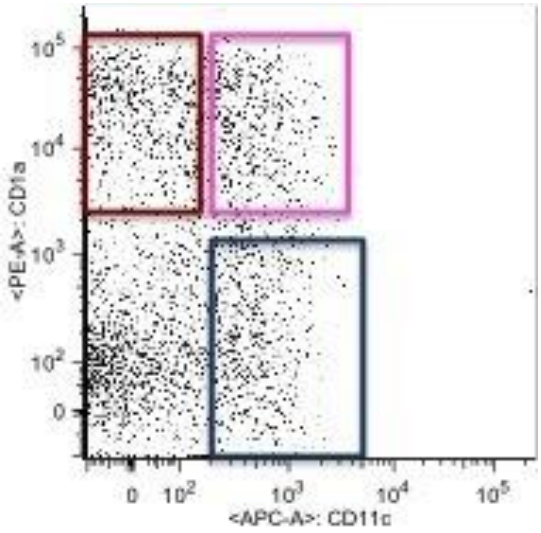
Background: Sub-lingual allergen-specific immunotherapy has been shown to be an effective treatment

for allergic rhinitis. Most likely, dendritic cells (DCs) play a role. In human oral mucosal tissue (hOMT) CD1a$^+$ Langerhans cells represent the major DC population, whereas in mice CD11b$^+$ and CD11c$^+$ DCs predominate. Little is known about CD11c$^+$/CD11b$^+$ DCs in human OMT. Thus, DC subpopulations in human OMT and their role in allergen uptake were analyzed. Material and Methods: DCs of hOMT were analyzed by HLA-DR and other DCs markers by flow cytometry (FACS) after trypsinization. hOMT was incubated with FITC-coupled βv1 solution prior to culture in RPMI. DCs migrated out of hOMT were investigated for βv1 binding by FACS. Results: DCs subsets within the HLA-DR$^+$ cell population expressed CD1a$^+$/CD207$^+$/CD11c-, CD1a$^+$/CD207$^+$/CD11c$^+$ and CD1a-/CD207-/CD11c$^+$. All subpopulations expressed CD1c and CD11b but no CD163 or CD68. Moreover stimulation of OMT with βv1 solution led to its binding mostly to CD1a$^+$/CD207$^+$/CD11c- and CD1a$^+$/CD207$^+$/CD11c$^+$ DCs but also to a lower proportion to CD1a-/CD207-/CD11c$^+$ DCs. Conclusion: CD1a-/CD207-/CD11c$^+$ DCs appear to represent a separate DCs subpopulation in hOMT. Although CD1a$^+$/CD207$^+$ oLC appear to be the predominant βv1 binding subpopulation, CD1a-/CD207-/CD11c$^+$ also bind βv1.



W.44. Differences in the Phenotype and Function of Lamina Propria Dendritic Cells from the Human Ascending and Descending Colon

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Background: Dendritic cells (DC) are the most potent antigen presenting cells determining the nature and type of immune responses. Ascending and descending colon have different embryological origins, blood supply, enzymatic activities and gene expression profiles suggesting that they may be different immunological entities. Methods: Paired colonic samples from ascending and descending colon of healthy controls were obtained. After collagenase digestion DC were identified (HLA-DR$^+$CD3-CD14-CD16-CD19-CD34-) and characterized by flow cytometry. Paired t-test was applied on at least 10 independent comparisons. Results: Larger numbers of DC were found in the ascending colon ($p<0.001$). Myeloid DC were increased in the descending colon ($p<0.001$). Innate immunity receptors TLR2 ($p<0.05$) and TLR4 ($p<0.05$) and intestinal homing markers β7 ($p<0.01$) and CCR9 ($p<0.01$) were decreased on ascending colon DC. CD103 was only expressed on β7$^+$ DC and was also decreased on ascending colon ($p<0.001$). Ascending colon DC were more mature as determined by increased CD40 ($p<0.05$) and CD80 ($p<0.05$) expression, decreased ILT3 ($p<0.05$) and phagocytic capacity ($p<0.05$), and increased stimulatory capacity ($p<0.05$) on allogeneic T cells. Conclusions: DC from human ascending and descending colon differ in their phenotype and function. Future studies should identify factors controlling DC compartmentalization through the length of the intestine.

W.45. Shaping the Autoimmune Response in the Gut: The Role of Intestinal Immunity in the Pathogenesis of Type 1 Diabetes

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Environmental factors acting at the intestinal level such as diet, infections and microbiota modulate the pathogenesis of autoimmune Type 1 Diabetes (T1D). Our recent finding that T1D patients show a defective Foxp3$^+$ Treg cell differentiation in the gut mucosa due to an impairment of DC tolerogenic function (Badami E. et al. Diabetes 2011) suggests that those environmental factors modulate

autoimmune T1D by shaping gut immunity and immune regulation. To test this hypothesis, we asked whether a dietary regimen that protects NOD mice from autoimmune diabetes (gluten-free diet) prevents autoimmunity by restoring a normal Th17/Treg cell ratio in the gut and pancreatic tissues. We found that NOD mice fed with a standard diet carry a significantly reduced number of tolerogenic DCs and have an increased Th17/Treg cell ratio in the gut mucosa compared to control mice. Importantly, removing gluten from the diet of NOD mice protected from diabetes by rescuing the number and tolerogenic function of intestinal DCs and restoring a normal Th17/Treg cell ratio in the gut mucosa and pancreatic lymph nodes. Our data indicate that environmental factors that act at the intestinal level such as diet modifications affect autoimmune T1D by modulating gut immunity.

W.46. Mucosal Education of Dendritic Cells by Intestinal Epithelial Cells and Luminal Contents
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Dendritic cells (DCs) are the principal initiators as antigen-presenting cells (APCs) to drive a series of immune responses. Depending on the microenvironments around them, DCs are educated to acquire the unique functional property to properly control immune responses. In particular, the functions of DCs in the lamina propria are known to be regulated by intestinal epithelial cells (IECs) themselves and IECs that have been stimulated by various luminal contents. Here we examine the effects of IECs and luminal contents on the functional education of DCs in mucosal tissues of the gastrointestinal tract. To analyze the communication between IECs and DCs, we constructed a IECs-DCs contact culture using a transwell system in which murine epithelial cells CMT-93 are attached at the reverse side of the polycarbonate membrane to form a monolayer and immature bone marrow-derived DCs (BMDCs) are placed inside the insert. The contact to CMT-93 monolayer led BMDCs to induce the increase in the expression level of CD103 surface antigen. The expression level of mRNA for aldehyde dehydrogenase (aldh1a2) and TGF-β significantly increased when the soluble extract derived from Bifidobacterium longum was added to CMT-93 monolayer, suggesting a regulatory role of Bifidobacterium longum in mucosal immune response.

W.47. Disconnect Between LPS-Induced Cytokine mRNAs and Secreted Proteins in Dendritic Cells Tolerised by Intestinal Epithelia
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In the gut, dendritic cells (DCs) interact with epithelial cells (ECs) resulting in DCs that are hypo-responsive to Toll-like receptor (TLR) activation. However, molecular mechanisms underpinning this "tolerisation" remain ill-defined. Our aim was to investigate the DC phenotype mediated by epithelia contact. Human monocyte-derived DCs were co-cultured with Caco-2 (ECs) and stimulated with LPS for six hours. Tolerised DC (DC-EC) phenotypes were confirmed by flow cytometry and ELISA. Genome-wide mRNA expression analysis was performed on the non-tolerised and tolerised $DCs^{+/-}$ LPS. Gene Ontology enrichment analysis identified differing immune response characteristics following co-culture. EC co-culture increased CCR2 and CCL24 with a reduction in CCL2 and IL-1b mRNA expression in DCs. Stimulation of the DC-EC cells with LPS resulted in differential cytokine production, with increased CXCL9 and reduced EGR1 expression. In response to LPS, there is a disconnect between induced mRNA and protein levels. DC-EC had reduced cytokine production (TNFa, IL-1b and IL-6) following LPS stimulation; however the corresponding mRNA transcripts was unchanged. These results demonstrate that tolerised DCs still respond to TLR stimuli, such as LPS - with induction of cytokine mRNAs, but the subsequent production of secreted cytokines is altered due to factors in the epithelia microenvironment.

W.48. Intestinal CD103- $CX3CR1^{+}$ Dendritic Cells Constitutively Migrate in Lymph and Prime Effector T Cells
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Dendritic cells (DCs) are crucial for the initiation of adaptive intestinal immune responses, as they migrate through lymphatics from the periphery to the mesenteric lymph nodes (MLNs) and prime naïve T cells.

Much recent research has focused on a specialised population of migrating intestinal DCs expressing CD103. Here we unequivocally demonstrate the existence of two populations of migratory CD103- lymph DCs (LDCs). These represent previously-unidentified subsets of intestinal DCs, which share Flt3-dependent ontogeny with $CD103^+$ DCs. Both CD103- and $CD103^+$ LDCs efficiently induce proliferation of naïve T cells and induce the expression of CCR9, a gut-homing chemokine receptor. Surprisingly, CD103- $CD11b^+$ LDCs express CX3CR1, albeit at lower levels than intestinal macrophages. Importantly, this subset of LDCs is able to induce the differentiation of IFN-γ and IL-17-producing effector T cells, both *in vitro* and *in vivo*, without additional stimulation. Priming by CD103- $CD11b^+$ DCs therefore represents a novel mechanism for rapid generation of effector T cell responses in the gut. The identification of CD103- $CD11b^+$ LDCs provides important new insights into the mechanisms for initiation of inflammatory responses in the gut, and these cells may prove valuable targets for the development of effective oral vaccines.

W.49. Direct Interaction Between Cholera Toxin and Dendritic Cells is Required for Oral Adjuvant Activity

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New effective vaccines against mucosal pathogens are needed, but the number of mucosal adjuvants is very limited. Cholera toxin (CT) is a very potent mucosal adjuvant in rodents but the mechanisms underlying the supreme activity are not known. In this study we explore the cellular interactions required for oral adjuvant activity by the use of mice deficient in GM2/GD2 synthase and hence lack all complex gangliosides, including GM1, the only known receptor for CT. Chimeric mice that lack GM1 on non-hematopoietic cells still mounted sufficient antibody responses to OVA fed in the presence of CT. In contrast, mice deficient in GM1-expression on hematopoietic cells generated no intestinal anti-OVA IgA and reduced serum titers of IgG specific for OVA. In addition, feeding OVA and CT to these chimeric mice resulted in reduced proliferation and impaired expression of PD-1 and CXCR5 by adoptively transferred OVA-specific $CD4^+$ T cells (OT-II) indicating impaired T follicular helper cell development. To determine whether CT was acting directly on dendritic cells (DCs), we used mixed bone marrow chimeras in which the presence or absence of GM1-expressing $CD11c^+$ cells was controlled by injection of diphtheria toxin. In these mice the expansion of OT-II T cells was decreased, indicating a dependence of DC expressed GM1. In summary our results show a complete dependence on direct stimulation of hematopoietic cells for adjuvant activity of CT. These results have important implications for the generation of novel oral adjuvants.

W.50. Vaginal Dendritic Cells are Functionally Distinct in their Ability to Induce IL-17 Production from $CD4^+$ T Cells, Compared to Lung and Intestinal Antigen Presenting Cells

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The mucosal immune system is functionally distinct from the systemic immune system, yet it is unknown whether resident antigen presenting cells (APCs) can prime distinct immune responses among different mucosa. In this study, we examined the phenotype and function of APCs from the respiratory, genital and intestinal mucosa. Tissue-resident and draining lymph node APCs from the lungs, vagina and intestine were isolated and analyzed. To examine functional differences, APCs were pulsed with ovalbumin peptide and co-cultured with OT-II $CD4^+$ T cells. Flow cytometry and multiplex assays were used to examine $CD4^+$ T cell proliferative and cytokine responses, respectively. APCs from all tissues induced $CD4^+$ T cell proliferation. However, vaginal APCs induced remarkably higher levels of IL-17 and IL-6 in co-cultures, compared to lung or intestinal APCs. Flow sorting demonstrated that $CD11c^+$ dendritic cells, and to a lesser extent, $CD11b^+$ $F4/80^+$ macrophages, were the principal vaginal APCs that induced IL-17 in co-cultures. Strikingly, the lymph nodes draining these tissues did not show any distinct characteristics and induced similar cytokine responses. This IL-17 polarization by vaginal APCs appears to be IL-23 and TGF-β independent, but possibly IL-6 or IL-1β dependent. These results indicate that discrete mucosal immune microenvironments may induce distinct immune responses.

W.51. Human Dendritic Cells Model in Testing of the Effect of Edible Plants' Extracts
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Human dendritic cells derived from monocytes (moDCs) were exposed to extracts of Nettle, Dill, Kale, Persimmon, Pomegranate, and Sideritis at a concentration 2 mg/ml for 6 and 24 hours. Plant extracts are inducing a shift in expression of CD1a and CD80 molecules of naïve matured, activated and test extract exposed moDCs in a time-dependent and plant-specific manner. Nettle significantly effects expression of pro-inflammatory $CD1a^+$ and the total amount of $CD1a^+CD80^+$ ($p< 0.01$) compared to control. Dill and Kale demonstrated similar but smaller influence, while Sideritis is statistically lowering the expression of $CD1a^+$ in 6-, but not in 24-hour incubations. Dill (6 and 24 hours) and Nettle (6 hours) induced IFN-g secretion. IL-4 production of exposed moDCs was initiated by Nettle (6 and 24 hours) and Kale (6 hours), a less significant but more stable effect is induced by Sideritis, while Pomegranate is not affecting IL-4. TNF-α dramatically increased after DCs exposure to Kale, Dill and Nettle (6 h) while the level induced by Persimmon and Pomegranate was less increased but detectable in 24-hour incubations. IL-10, IL-12, IL-17 and IL-1b were not changed compared to the control.

W.52. Comparative Transcriptomics in Mice and Men Reveal an Evolutionary Conserved Role for the Bcl6/Blimp-1 Axis in Intestinal Dendritic Cell Specification
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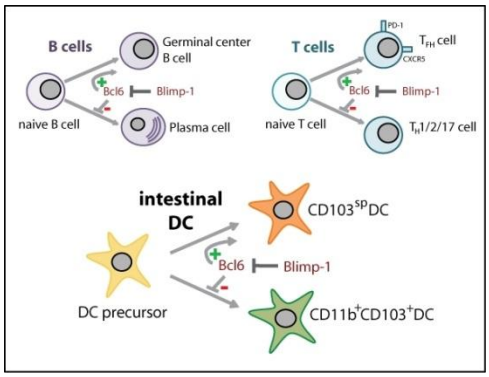

Dendritic cells (DCs) maintain immune homeostasis in the gut wall. Intestinal DC subsets with specialized tolerogenic and immunogenic properties act in both mice and men, but due to the lack of usage of conserved markers direct comparison across species remains difficult. We used comparative transcriptional analysis to evaluate regulatory programs in DC subsets. A few transcription factors, including Bcl6 and Blimp-1, were differentially expressed in functionally defined DC subsets in both species. Bcl6 and Blimp-1 are antagonistic transcriptional repressors that control fate decisions in B and T cells. Based on conservation of differential expression in DC subsets, we hypothesized that these transcription factors influence DC specialization. Using Bcl6 and Blimp-1 mutant mice, we showed that the Bcl6/Blimp-1 axis regulates the development of murine $CD103^+CD11b^+$ and $CD103^+CD11b$- intestinal DCs, subsets that differ in their ability to recognize pathogens, to cross-present, and to direct T helper cells. DC-specific Bcl6-deficient mice lack $CD103^+CD11b$- DCs, whereas DC-specific Blimp-1 deficiency impacts $CD11b^+CD103^+$ DCs. In human intestine, $CD103^+$Sirpα- DCs are equivalent to mouse $CD103^+CD11b$- DCs. Critically, these DCs express high levels of Bcl6 protein. In summary, we identified Bcl6 and Blimp-1 as evolutionary conserved regulators that drive specification of phenotypically and functionally related intestinal DC subsets.

W.53. PTX-Related Inhibition of Plasmacytoid Dendritic Cell Functions, and Imprinting of Mucosal Associated Trafficking Receptors on Th17 Cells During *Bordetella Pertussis* Infection in Mice
Victoria Wu, Tuan Nguyen, Ryan Ferguson, Christopher Sequeria, Shahryar Niknam, Judy Wan, Marlene Taylor, Tzvia Abramson. San Jose State University, San Jose, CA

Whooping cough, caused by *Bordetella pertussis* (Bp), is a highly contagious respiratory disease. Pertussis toxin (PTX) is largely responsible for the prolonged disease. We previously showed PTX-associated reduced imprinting of mucosal associated trafficking receptors (MATR) and impairment of

effector memory CD4$^+$ cells lung migration. Others deemed Th17 cells responsible for microbial clearance. Plasmacytoid dendritic cells (pDC) are activated during lung inflammation in response to pathogens, but their role during Bp infection is understudied. Using murine infection models of Bp and BpΔPTX (Bp strain devoid of PTX), we tested the hypothesis that PTX inhibits pDC function leading to reduced imprinting of MATR on Th17. Lung pDCs during infections emerged 4- to 7-fold five days post infection (p.i.). Reduced Bp-derived pDCs expressed costimulatory molecules, CD40, CD86, and MHC class II, which restored 10 days p.i. Fewer Bp-derived pDCs expressed CCR7, which persisted 10 days p.i., perhaps explaining lung pDC accumulation. Circulating and lung Th17 cells increased significantly in Bp-infected mice, peaking 15 days p.i. However, fewer expressed MATR compared to BpΔPTX-infected cells. Temporal lung pDC accumulation and increased Th17 responses suggests a role for pDCs in Th17 differentiation, possibly driven by opsonized Bordetella antigens. Altogether, these observations may explain a prolonged disease.

W.54. The Aryl Hydrocarbon Receptor and Mucosal Dendritic Cells: Sentinels for Maintaining Intestinal Homeostasis

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The aryl hydrocarbon receptor (AhR) is a ligand induced transcription factor expressed in lymphocytes and myeloid cells. Interestingly, microbiota mediated metabolism of tryptophan has been shown to generate planar indoles which can act as ligands for the AhR. This suggests that AhR activation in the intestine may be conditionally regulated by the microbiota postnatally and hence may potentially modulate microbe-immune interaction. One of the key cell types known to communicate with microbiota are the mucosal dendritic cells (mDCs) and the understanding of how AhR signaling can impact mDC function is not known. Here, we demonstrated that the absence of AhR activation in DCs resulted in irregular intestinal DC homeostasis where conventional DC surface makers were down-regulated in all three major mDC subsets present in the lamina propria, concomitant with elevation of maturation markers such as CD86, CD80 and MHC II molecules. In addition, we observed an elevation of anti-microbial peptides expression in the colons of DC AHR KO mice. Furthermore, while we noted an increased susceptibility to DSS induced colitis in DC AHR KO mice, no differences were observed in T cell specific AhR KO mice. Our data support a role for AhR signaling in mDC function.

W.55. Epithelial Notch-1 Signaling Bridges Innate and Adaptive Immune Responses

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Introduction: Lamina propria (LP) lymphocytes promote barrier function in the RAG-transfer model putatively due to the Notch-1 pathway. Notch-1 activation is increased in Crohn's Disease epithelia compared to normal or ulcerative colitis. AIM: To determine the role of epithelial Notch-1 in colitis onset. Methods: WT mice were treated with Notch-1 or scrambled siRNA with or without a 3% DSS regimen. Notch-1, Hes-1, CDX-2 and claudin-5 mRNA expression was quantified. Permeability was assessed by Ussing chamber. Colonic tissues were stained for claudin-5 and Foxp3. Foxp3$^+$ cells and cytokine secretion were assessed in MLNs. Cytokines from TNFa stimulated Notch-1 knocked-down (KD) Caco-2 cells and T cell subsets from Notch-1 KD Caco-2 cells supernatant stimulated PBMCs were assessed. Results: Notch-1, Hes-1, CDX-2 and claudin-5 mRNA expression and staining were decreased in siRNA treated animals, and permeability was increased. These mice were more susceptible to DSS colitis, showed decreased LP and MLN Foxp3$^+$ cells, and decreased effector T cell cytokine. Notch-1 KD Caco-2 cells had lower basal cytokines and dampened response to TNFα, their supernatant induced a 45% decrease in Foxp3$^+$ CD4$^+$ T cells from stimulated PBMCs. Conclusion: Epithelial Notch-1 signaling shapes the underlying immune responses at steady state and during injury.

W.56. Bacterial Outer Membrane Vesicles are Processed into Antigen Presenting Exosomes by Epithelial Cells, Which Induce the Proliferation of Human T Cells

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We previously reported that outer membrane vesicles (OMVs) from Gram-negative bacteria are capable of entering epithelial cells and initiating immune responses *in vivo*. Precisely how OMVs gain entry to non-phagocytic cells and the mechanisms whereby these bacterial-specific adaptive immune responses are generated remain unknown. In this study we demonstrated that OMVs enter epithelial cells via macropinocytosis-, clathrin- and caveolin-mediated endocytosis, and that their size dictates the route of entry. Once internalised, OMVs up-regulated the expression of HLA Class II molecules on the cell surface. Furthermore, stimulation of polarised epithelial cells with OMVs resulted in basolaterally-produced exosomes containing OMV proteins, which could constitute a link between luminal antigens and the local immune system. Proteomic analysis revealed indeed that these exosomes contained several known immunogenic OMV proteins. Finally, these exosomes had immunostimulatory abilities, as they induced antigen-specific proliferation of human T cells. Collectively, our findings suggest that upon entry into epithelial cells, OMVs are processed and their contents packaged into exosomes, which facilitate the generation of antigen specific T cell responses. We propose that these exosomes are important for presenting antigen to immune cells, thereby providing a link between the generation of innate and adaptive immune responses at the mucosal epithelium.

W.57. Use of CEACAM5 Synthetic Peptide Combinatorial Library to Characterize Binding of CEACAM5 to CD8α

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$CD8^+$ regulatory T cells can be activated by intestinal epithelial cells (IECs) through the complex formed by CD1d and gp180 both expressed on IECs. mAb B9 (anti-gp180) blocks this induction. We demonstrated sequence and properties homology between gp180 and CEACAM5. The aim of this study is to characterize CEACAM5-CD8 binding. CEACAM5 and CEACAM5 mutants were purified from supernatants of PIPLC treated transfected cells. A deletion of the N42 to I46 (ΔNI), two conservative point mutations K35A and N42D, and a N70, 81A mutation which prevents N domain glycosylation were selected for Biacore analysis. An overlapping peptide library of N domain was tested in its ability to phosphorylate CD8 associated p56Lck kinase. CEACAM5 binds to CD8α, not CD4, with an affinity comparable to MHC Class 1. The loss of glycosylation of the N domain resulted in the greatest loss of affinity for CD8α and in impaired phosphorylation of LcK. B9 blocks CEACAM5/CD8 binding. The collection of peptides induced phosphorylation of Lck. Pool 3>2 (pool 2: G30-T55; pool 3: P66-Y107) activates $CD8^+$ T cells. Our data suggest the role of the N domain sugar bridge site in CEACAM5 binding to CD8α and in the activation of $CD8^+$ Tregs.

W.58. High Fat Diet Aggravates Crohn's Disease-Like Ileitis Independently of Obesity

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Background: Recent studies indicate that diets rich in fat impact the pathogenesis of colitis, but evidence for effects on pathology of the ileum is scarce. We therefore challenged the $TNF^{\Delta ARE/WT}$ mouse model of Crohn's Disease-like ileitis using a high-fat diet (HFD) to elucidate the interrelations between diet-induced obesity, intestinal microbiota, barrier function and inflammatory processes in the intestine. Methods and Results: HFD accelerated ileitis in $TNF^{\Delta ARE/WT}$ mice independently of obesity and obesity-associated metabolic parameters. Ileal occludin expression was markedly reduced by HFD and endotoxin translocation was increased. Epithelial cells showed enhanced expression of markers of immune activation. Consistent with enhanced recruitment mechansism, for example increased expression of the chemokine CCL20, recruitment of dendritic cells into the lamina propria was increased under HFD. *In vitro* experiments revealed higher chemotactic potential of epithelial cells towards bone-marrow-derived

dendritic cells when stimulated with cecal lysates from HFD vs. control mice. Cecal content analysis revealed HFD-induced alterations in bile acid profiles as well as microbial composition and diversity. Conclusion: Dietary fat aggravated ileal inflammation through changes in epithelial cell homeostasis independently of obesity.

W.59. MUC2 Mucin Regulates the Anti-microbial Activity of β-Defensin 2 in Colonic Mucus
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In this study we investigated the interactions between MUC2 mucin and the antimicrobial peptides β-defensin. Of the colonic cells tested, β-defensin 2 expressions was highest in MUC2 producing human LS 174T and the lowest in HT-29 and Caco-2 cells. Exogenous MUC2 mucin and IL-1β upregulated β-defensin mRNA expression and protein production in HT-29 as compared to IL-1β only. Purified MUC2 mucin impaired the antimicrobial activity as β-defensin-susceptible Escherichia coli survived when both components were incubated together. In $Muc2^{-/-}$ mice, β-defensin 4 (orthologous to human β-defensin 2) was down regulated in the colon and harbored significantly less commensal Bacteroides and Firmicutes spp as compared to Wt controls. Mechanistically, we found that bacteria bound MUC2 via N- and O-linked oligosaccharides to resist β-defensin 2 antimicrobial activities. These studies summarize that MUC2 mucin can regulate the bioactivity of antimicrobial peptides in the colonic mucosa enhancing epithelial induction of β-defensin but at the same time, protecting the enteric microbiota by inhibiting β-defensin antimicrobial activity. A compromised mucin barrier may allow β-defensin resistant bacteria to colonize and cause disease and impair adequate β-defensin stimulation common in inflammatory bowel diseases.

W.60. Immunomodulating Ability of Pectin is Toll-Like Receptor Dependent
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Pectin is a plant polymer of predominantly galacturonic acid, constituting a portion of our daily diet through consumption of fruits, grains, and food additives. Health benefits are recognized for pectin, but the mechanisms remain unknown. Commercial pectins are heterogeneous molecules having varying degrees of methyl esterification (DM). It is not known how this influences bioactivity as only heterogeneous mixtures have been tested. We hypothesize that pectin interacts with gut immune cells through Toll-like Receptors (TLR). TLR responsive reporter gene assays were applied to test activating and inhibitory effects of pectin differing in DM. Our studies show that pectin can inhibit TLR. The strongest inhibition was observed for TLR9 followed by TLR2, TLR4 and TLR5. TLR9 is inhibited by all DM pectins but in different degrees. TLR2 inhibition was observed more with low DM (0, 7, 22 and 45) pectin than with high DM pectin (65 and 70). TLR4 and TLR5 could be inhibited to a comparable extent by all DM levels of pectin. Thus, these results give an insight into anti-inflammatory effects of pectin with reference to its methyl esterification. These new insights will contribute to the design of science-based pectin-containing immunomodulating foods.

W.61. Mucosal Immunity Mediated by Antibody-Mucin Crosslinking
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IgG is the predominant immunoglobulin in cervicovaginal mucus (CVM) secretions, yet the mechanisms by which IgG in mucus protects against infections are not fully understood. IgG diffuses rapidly through cervical mucus, slowed only slightly by transient adhesive interactions with the mucus gel. We hypothesize that this almost unhindered diffusion allows IgG to accumulate rapidly on pathogen surfaces, forming weak yet polyvalent adhesive crosslinks that effectively trap (immobilize) the pathogens in mucus

and prevent them from initiating infections. Here, we report that herpes simplex virus Serotype 1 (HSV-1) readily penetrated fresh, pH-neutralized *ex vivo* samples of CVM with low or no detectable levels of anti-HSV-1 IgG, but was effectively trapped in samples with modest levels of anti-HSV-1 IgG. Moreover, addition of anti-HSV-1 IgG affinity-purified from intravenous immunoglobulin potently trapped virions at concentrations below those needed for neutralization. Deglycosylating the purified anti-HSV-1 IgG, or removing its Fc component, markedly reduced trapping potency. Finally, a non-neutralizing monoclonal IgG against HSV-gG significantly protected mice against vaginal infection, and removing mouse vaginal mucus by gentle lavage abolished protection. These observations suggest the Fc component of IgG has a glycan-dependent "muco-trapping" effector function that provides an exceptionally potent protective mechanism at mucosal surfaces.

W.63. Dual Oxidase 2 (DUOX2) is Responsible for Hyperoxia-Induced Lung Injury in Mice
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Acute lung injury (ALI) and its more severe form, the acute respiratory distress syndrome (ARDS), are syndromes of respiratory dysfunction that result from acute pulmonary edema, pulmonary contusions and inflammation. In the lungs of patients with ALI-ARDS, increased reactive oxygen species (ROS) were observed. ROS are chemically very reactive oxygen-containing molecules. Modest levels of ROS participate in signal transduction and host defense but it is harmful with high levels causing cell damage. To mimic human ALI-ARDS, investigators generally have used animal models of hyperoxia. In laboratory mice, a prolonged exposure of oxygen caused symptoms similar to ALI-ARDS such as pulmonary edema and respiratory failure. Several studies have shown that hyperoxia induce high levels of ROS and they lead to apoptosis and necrosis. The major source of ROS is NADPH oxidses. However, which kind of NADPH oxidase plays a critical role to induce apoptosis in alveolar type II cells is not well known. Our study showed that DUOX2 is highly expressed in alveolar epithelial cells, and responsible for hyperoxia-induced lung injury.

W.64. Double-Edged Sword of Early Growth Response 1 in Epithelial Inflammatory Response Under Mucosal Ribosomal Insults
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Gut epithelia strictly regulate NF-κB expression and activity which prevent overstimulation of pro-inflammatory response following exposure to commensal bacteria. Previous studies from our group have suggested that ribosomal insults induce pro-inflammatory chemokines via early growth response gene 1 product (EGR-1) in gut epithelial cells. Based on these fondings, roles of EGR-1 in responses to ribosomal insults were further assessed in cells exposed to bacterial endotoxin. Although lipopolysaccharide (LPS) enhanced NF-κB activation, chemokine expression was marginally affected. In contrast, simultaneous exposure to LPS and ribosomal insults attenuated NF-κB activation while chemokine expression was more elevated. Similar to the previous reports, EGR-1 expression was up-regulated by ribosomal insults and positively modulated chemokine gene expression. However, EGR-1 suppression caused super-induction of chemokines by simultaneous treatment, indicating EGR-1 can be a negative modulator of chemokine gene expression. Especially, mucosal ribosomal insult-triggered EGR-1 mediated PPARγ induction, which blocked NF-κB activation by LPS. It can be thus concluded that EGR-1 regulates NF-κB activation by LPS via PPARγ although EGR-1 is a positive mediator of chemokine expression following ribosomal insult in gut epithelia (This work was supported by the Basic Science Research Program through the National Research Foundation of Korea, funded by Ministry of Education, Science, and Technology Grant 2012R1A1A2005837).

W.65. M**icroRNA-Mediated Regulation of TLRs by Immunosuppressive Agent Increases Susceptibility to Bacterial Infections**
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Urinary tract infections are among the most common infectious diseases responsible for 7 to 10 million cases reported each year in USA and are mostly caused by Uropathogenic Escherichia coli (UPECs).

Acute pyelonephritis (APN) is frequently encounters after renal transplantation and represents an independent risk factor associated with graft survival. Bacterial attachment to mucosal epithelial cells represents the initial step in the pathogenicity and Toll-like receptor (TLR)-mediated recognition of UPECs by epithelial cells elicits potent inflammatory response for host defense. However, the consequences of long-term immunosuppressive drugs on TLR expression and function in renal transplanted patients exhibiting frequent APN have not yet been investigated. We showed that treatment with cyclosporine A (CsA), widely used for the prevention of graft rejection, increases UPEC infection. Incubating cells with CsA induced a dose dependent inhibition of TLR expression concomitantly with the increase of specific microRNAs. The inflammatory response, as well as TLR expression, was restored by using anti-microRNA. Thus, CsA-induced microRNAs downregulate TLR expression, which could explain the greater sensitivity of patients to APN after transplantation and highlight the possible use of microRNAs as therapeutic tool to prevent progressive alteration of the graft.

W.66. Dissecting the Mechanisms of M Cell Differentiation

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Microfold cells (M cells) are specialized intestinal epithelial cells in the follicle associated epithelium overlying the Peyer's Patches. M cells deliver luminal antigens to immune cells and thereby form a necessary link between the gut lumen and the mucosal immune system. *In vivo* studies have shown that M cell differentiation can be induced upon binding of Rank ligand (RankL) to its receptor Rank. Recently, we used an intestinal organoid culture system to further investigate M cell differentiation. We have shown that upon incubation with RankL, small intestinal organoids developed into Annexin V and GP2 expressing M cells. These cells were able to take up beads, confirming M cell function. Furthermore, we have shown that RankL-induced M cell development was dependent on the transcription factor SpiB. Organoids established from SpiB-deficient mice did not develop into functional M cells upon incubation with RankL despite expression of Rank. These data showed that Rank-RankL interaction results in upregulation of SpiB, which is crucial for M cell development. However, we now show that forced overexpression of SpiB in intestinal organoids did not induce M cell development, suggesting that SpiB is not operating autonomously. Therefore, we are currently looking into the involvement of another transcription factor in the induction of the M cell developmental program.

W.67. cFLIP is Essential for Intestinal Epithelial Cell Survival and Immune Homeostasis by Controlling the Activation Level of Caspase-8

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We have previously demonstrated the importance of cell death regulation by Caspase 8 (Casp8) in the intestinal epithelium, the important barrier in the gut protecting the organism from gut contents. We now investigated the role of Casp8 regulator cFlip in intestinal epithelial cells (IECs) in gut homeostasis. Therefore, we intended to generate mice with IEC-specific deletion of cFlip but cFlipΔIEC embryos were lethal. We then created mice with an inducible conditional deletion of cFlip. The deletion of cFlip in IECs in adult animals caused continuous weight loss and ultimately led to death. We observed general tissue destruction of the small and large intestine, as visualized by endoscopy and HE-staining of gut cross-sections. The histological analysis further revealed abundant cell shedding into the gut lumen and inclusions of dead IECs. To examine cFlip induced cell death in detail, organoids were grown and cFlip deficiency was induced. cFlip deficient organoids did not die on their own but after addition of the cell death ligands TNFα and CD95L. Our data show that cell death in the gut is constitutively triggered by cell death ligands such as TNFα requiring the presence of the Casp8 regulator cFlip.

W.68. Surviving Influences the Homeostasis of the Intestinal Stem Cell Niche

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Intestinal stem cells and transient-amplifying cells at the base of the crypts give rise to all terminally differentiated cells in the gut. The balance of the replacement of dead cells and the production of new cells has to be tightly regulated. Survivin is a unique member of the inhibitor of apoptosis (IAP) family with bifunctional properties involved in controlling apoptosis and cell division, respectively. Therefore, we intended to generate mice with a specific deletion of survivin in intestinal epithelial cells (IECs) but unfortunately these mice were embryonic lethal. For this reason, we created mice with an inducible deletion of the survivin gene in IECs. Control and knockout mice where histologically analysed by immunohistochemistry, revealing a localisation of survivin in transient amplifying cells and stem cells. The specific deletion of survivin in IECs leads to a fatal destruction of the intestinal epithelium and remaining IECs showed abnormally enlarged nuclei, suggesting a defect in cell cycle progression. Western-blot analysis and qPCR indicated increased apoptosis and reduced gene expression levels of stem cell genes in survivin depleted IECs. Our results demonstrated the important contribution of survivin for the intestinal stem cell niche and the preservation of epithelial cells in the gut.

W.69. Morphine Compromises Intestinal Barrier Function in a TLR-dependent Manner
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As one of the most prescribed drugs for pain management, opiate use or abuse results in significant gut bacterial translocation and increases risk of serious infections with gut origin. The mechanism underlying this defect is still illusive. In the present study, we observed significant bacterial translocation to mesenteric lymph node (MLN) and liver following morphine treatment in wild-type (WT) animals that was dramatically attenuated in Toll-like receptor (TLR2 and 4) knockout mice. We further observed significant disruption of tight junction protein organization and up-regulation of pro-inflammatory cytokines such as IL-17, which is shown to be involved in tight junction modulation in endothelial and epithelial cells and pathogenesis of inflammatory bowel disease. This study demonstrates that morphine induced gut epithelial barrier dysfunction and pro-inflammatory cytokine up-regulation are mediated by TLR signaling and thus TLRs can be exploited as potential therapeutic targets for alleviating infections in morphine-using or abusing populations. In addition, it may also provide novel personalized therapeutic strategies to control pain and inflammation in IBD patients.

W.70. Adult Stem Cells in the Small Intestine are Intrinsically Programmed with their Location-Specific Differentiation Fate
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In mammals, the small intestinal epithelium is highly specialized along the cephalocaudal axis with different absorptive and digestive functions in duodenum, jejunum and ileum. Several transcription factors, such as GATA4 and CDX2, have been described to regulate location-specific gene expression in the mouse small intestine. However, it is not known whether the intestinal environment, such as the mesenchyme, luminal content or the microbiota is necessary to maintain the location-specific functional properties of epithelial cells. By using the organoid culturing technique, we cultured pure epithelial cells derived from location-specific crypts of mice and human to exclude the effect of extrinsic factors. We determined expression of location-specific genes, such as GATA4, disaccharidases and bile acid transporters. We show that the *ex vivo* expression signatures remained stable in location-specific mouse organoids that were expanded for up to 12 weeks. Furthermore, human duodenal and ileal organoid cultures that were expanded for seven weeks and were then induced to differentiate, also maintained their functional identity corresponding with their original location. These data show that within the small intestine, location-specificity is intrinsically programmed in the adult stem cells and is independent of extracellular signals from either mesenchyme or luminal content.

W.71. Cytochrome P450 Epoxygenases Regulate Macrophage Phagocytosis and Lung Bacterial Clearance of Streptococcus Pneumoniae
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Recent reports have demonstrated that CYP-derived eicosanoids are also involved in regulating the inflammatory response. The role of CYP-derived eicosanoids in regulating macrophage function remains unknown. In this study, we used soluble epoxide hydrolase (sEH) deficient mice to examine the role of CYP-derived eicosanoids in the regulation of macrophage maturation, activation and phagocytosis. Flow cytometry results show that expression of CD80, CD86 and MHC class II molecules were significantly decreased in $sEH^{-/-}$ bone marrow-derived macrophage compared to wild-type cells when they were stimulated with different pathogen-associated molecular patterns including bacterial lipopolysaccharide (LPS), lipoteichoic acid (LTA), peptidoglycan (PGN), mannan (Man) and zymsome (Zym). However, we did not found the defects of this molecular expression on peritoneal macrophages. Consistent with this observation, we observed significantly reduced phagocytosis by $sEH^{-/-}$ macrophages. Examination of potential signaling mechanisms showed that $sEH^{-/-}$ macrophages had reduced TLR2 and Pgylrp1 expression. Moreover, quantitative RT-PCR analysis revealed that expression of TNF-α, IL-6 and IL-1 mRNAs were also markedly downregulated in $sEH^{-/-}$ macrophage stimulated with PGN. More importantly, there was significantly impaired of lung bacterial clearance to Streptococcus pneumoniae in $sEH^{-/-}$ mice. Together, these results demonstrate that CYP-derived eicosanoids regulate macrophage function and inflammation.

W.72. Oncostatin M Decreases Barrier Function in Human Airway Epithelial Cells

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Dysfunctional sinonasal epithelial barrier is thought to play a role in chronic rhinosinusitis (CRS). Activators of STAT3 such as Oncostatin M (OSM) are known to modulate epithelial function. Analysis of CRS samples showed elevations of OSM mRNA (6.5 fold, $p<.05$, $n=6$) and protein (4.4 fold, $p<.05$, $n=12$-19) in nasal polyps compared to control uncinate tissue by microarray and luminex assay. To study the impact of OSM on epithelial barrier function, normal human bronchial epithelial (NHBE) cells, and nasal epithelial cell (NEC) samples from CRS patients were cultured at air-liquid interface (ALI) conditions, grown until fully differentiated at day 21, and then were left unstimulated or stimulated with OSM at 100ng/mL for 48 hours. OSM stimulation reduced barrier function measured by transepithelial electrical resistance (TEER) in NHBE cells (63% reduction, $p<.005$, $n=4$) and NEC cultures (35% reduction, $p<.05$, $n=4$). This decrease in barrier function was not a result of decreased mRNA expression of tight junction proteins, claudin 1, E-cadherin, occludin, and zonula-occludens 1. Elevated levels of OSM found in nasal tissue from patients with CRS may play a role in the known loss of barrier function in this important disease.

W.73. Oral and Vaginal Epithelial Cells are Activated and Transmitted by HIV-1 to Permissive Cells Without Productive Infection

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In contrast to vaginal mucosa, the question of whether oral mucosa can act as a portal of entry for HIV-1 infection remains a controversial issue. To address potential differences with regard to the fate of HIV-1 after exposure to oral and vaginal epithelium, we utilized two oral (TR146 and FaDu) and one vaginal (A431) epithelial cell (EC) lines to assess interactions of oral and vaginal mucosa with HIV-1. We demonstrate that although lacking CD4, CXCR4 or CCR5, these cells can capture X4 and R5 virus before transferring infectious virus to permissive cells (TZM-bl) either directly via cell-cell contact or indirectly via transmigration. However, we found no evidence of proviral integration or *de novo* viral synthesis post-infection. Notably, X4 and R5 binding activates different intracellular signaling pathways in oral and vaginal ECs, although neither pathway activation results in cytokine production including IL-6, IL-1α, G-CSF or GM-CSF. These data demonstrate that under normal conditions, whilst ECs can capture and respond to infective HIV-1, productive infection is unlikely. However, whilst oral and vaginal ECs can act

as immune sensors, they can also be mediators of systemic viral dissemination through attachment and transfer of HIV-1 to permissive cells.

W.74. Host Discrimination of Commensal versus Pathogenic Bacteria in the Human Vaginal Epithelium

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The vaginal mucosa of the female reproductive tract is the first line of defense against invading pathogens and also the residence for nonpathogenic commensal microflora. The vaginal epithelium represents a dimorphic innate immune environment that must protect against invading pathogens without compromising resident commensal bacteria. We demonstrate a unique innate immune signature that differs between commensal *Lactobacillus* and bacterial vaginosis-associated bacteria (BVAB). For this purpose we utilized a human 3-D vaginal epithelial cell (3-D EC) model, shown to recapitulate relevant barrier properties including mucus secretion. Infection of 3-D ECs with BVAB strains *Atopobium vaginae* and *Prevotella bivia* resulted in a significant increase in several mucins, however colonization of 3-D ECs with *Lactobacillus spp.* did not alter mucin expression. Furthermore, BVAB infection significantly increased expression of IRAK and IL-1 family members in 3-D ECs compared to uninfected and *Lactobacillus* colonized 3-D ECs. *A. vaginae* is significantly associated with BV symptoms and elicited a robust increase in IL-6 and IL-8. These data demonstrate a unique innate response signature elicited by pathogens that is absent with commensal bacteria. These findings highlight a role for vaginal epithelial cells in distinguishing pathogenic versus beneficial bacteria to maintain mucosal homeostasis, while remaining responsive to pathogen insult.

W.75. SLPI is Predominantly Expressed by the Distal Colonic Goblet Cells and its Deficiency Protects Distal Colon During Acute Murine Colitis Through Reduced IL-1β

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Seceretory leukocyte protease inhibitor (SLPI) is a mucosal anti-microbial and immune regulator. We investigated the role of SLPI during acute and chronic colitis and also in intestinal mucosal wound healing. Wild-type BL/6 and SLPI deficient mice were employed in the study. DSS colitis model was used for acute colitis and wound healing while Winnie spontaneous model of colitis was employed for chronic colitis. Relative contribution of epithelium and leukocytes was studied. Our studies show that SLPI is predominantly expressed by the goblet cells, in the distal colon during acute colitis. SLPI deficient mice were protected from acute colitis only in the distal colon region ($p>0.001$) through the down regulation of IL-1β. Further, SLPI deficiency did not impair colonic wound healing post DSS colitis contrary to recent reports. Winnie mice expressed high levels of SLPI (13 fold more than controls) in the goblet cells of distal colon. Winnie mice with SLPI deficiency also showed mild protection in the distal colon. SLPI expression in non-haematopoietic cells was sufficient for the increased severity. Our studies demonstrate that SLPI is mainly expressed in the distal colon and SLPI overexpression in an inflammatory setting aggravates colitis but doesn't play a role in wound healing.

W.76. Early Maternal Separation In Mice Triggers a Specific Immune Response Toward Luminal Content at Adulthood

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Early life stressful events contribute to development of functional gastrointestinal disorders such as irritable bowel syndrome (IBS). Using maternal separation (MS) as an experimental model mimicking IBS, we investigated whether early life stressful events in mice may trigger inappropriate immune responses toward luminal content and as a consequence induce IBS symptoms. MS leads to IBS-like symptoms (intestinal hyper-permeability and visceral sensitivity) in adult male C3H/HeN mice. Furthermore, total plasmatic IgG concentrations were increased by 20% whereas fecal IgA were decreased by 20% in MS

mice. Thereof, Ig specificity against soluble food antigens and commensal *E. coli lysate* was assessed by normalizing Ig concentrations between samples. Plasmatic IgG specificity against food antigens (optical density 0.68±0.08 vs 0.47±0.07, P<0.05) and *E. coli lysate* (0.04±0.005 vs 0.28±0.15) was increased in MS mice. *Ex vivo* stimulation of splenocytes with *E. coli lysate* induced higher IFNγ secretion (594±72 vs 357±72pg/ml; p<0.05) and lower IL-10 (535±115 vs 278±100pg/ml; p<0.05) and IL-17 (539±189 vs 289±159pg/ml; p<0.05) secretion in MS mice. These data show that early life stressful events trigger inappropriate immune response toward luminal content (food antigens and microbiota) that may contribute to IBS symptoms and highlight perinatal period as a critical window for immune system development.

W.77. Secretory Leukocyte Protease Inhibitor (SLPI) is a Crucial Mediator for the Acquisition and Maintenance of Hyporesponsive Mucosal Epithelium

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The aim of this study was to investigate how primary mucosal epithelial cells (EC) become hyporesponsive to TLR stimulation and what mechanisms are involved. Using primary buccal EC we established that at birth neonatal EC spontaneously produced CXCL-8 and were highly responsive to microbial stimuli. Within the first weeks of life buccal EC became hyporesponsive, having sustained levels of IκBα while activated neonatal EC displayed IκB degradation. Microbially-induced hyporesponsiveness of a buccal EC line, TR146, elicited expression of the negative NF-κB regulator secretory leukocyte protease inhibitor (SLPI). SLPI constitutively maintained TR146 epithelial hyporesponsiveness as knockdown of SLPI expression resulted in increased spontaneous production of CXCL-8. In agreement, hyporesponsive adult primary buccal EC expressed SLPI mRNA and had nuclear SLPI protein whereas activated neonatal buccal EC did not. In the intestine SLPI also contributed to acquisition of homeostasis as its expression was upregulated upon long term colonization of adult germ free mice with commensal microbiota (conventionalization) and was inversely related to expression of the murine CXCL-8 homolog CXCL-2 in colonic epithelium. Conversely, in normally colonized SLPI deficient mice CXCL-2 expression was constitutively increased in colonic epithelium. In conclusion, SLPI mediates hyporesponsiveness of mucosal epithelium in the first weeks after microbial colonization.

W.78. Role of Glucocorticoid Receptor in Regulation of Pulmonary Dendritic Cells Gene Expression

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Respiratory dendritic cells are continuously challenged with inhaled antigens but under homeostatic conditions no inflammatory reaction is induced. This tolerogenic milieu is partially due to anti-inflammatory factors secreted by lung epithelial cells. We recently demonstrated that the treatment of bone-marrow-derived dendritic cells with lung-epithelial cell conditioned medium (ECCM) lead to a decrease in LPS induced MHC class II and CD86 surface presentation. Performing now a whole genome expression array we observed that 67 genes were additionally modulated by ECCM treatment. Interestingly, the most up-regulated genes within DCs were Ms4a8a and Ym1, marker genes of alternatively activated macrophages (M2). Bioinformatics analysis showed an overrepresentation of hormone-nuclear receptors, among them was the glucocorticoid receptor (GR). In line with this, pharmacological blockade or genetic manipulation of the glucocorticoid receptor inhibited Ms4a8a and Ym1 expression as well as MHC class II or CD86 down-regulation. Surprisingly, we were unable to detect any glucocorticoids to be present in ECCM. Further experiments proved that the factor is an organic lipophilic epithelial cell derived compound. Therefore we speculate that an unknown lipophilic factor is involved in the GR dependent regulation of the immune system of the lung.

W.79. Aging Impairs the Functional Maturation of M Cells and their Ability to Sample Lumenal Antigens

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Microfold cells (M cells), present in the follicle-associated epithelium (FAE) of Peyer's patches, are specialised enterocytes which transcytose particulate lumenal antigens, facilitating the induction of immune responses towards these. Aging is known to affect systemic immunity; however, the effect on M cells was undetermined. In aged mice, the density of M cells in the FAE was significantly reduced resulting in an impaired ability of Peyer's patches to transcytose lumenal antigens. Expression of Spi-B, a transcription factor critical for the functional maturation of M cells, was reduced in aged mice; however, similar densities of immature M cells were present. Aging did not affect expression of RANK or RANKL, both critical for M cell differentiation, however, expression of the chemokine CCL20 in the FAE was reduced, resulting in a reduction in the number of B cells found within the FAE. The results of this study suggest that aging may impair mucosal immunity by reducing the ability of the immune system to sample lumenal antigens via M cells.

W.80. *Salmonella Typhimurium* Favours Its Dissemination Modulating Wnt/β-Catenin Pathway and Inducing Intestinal Barrier Breakdown

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In order to protect itself from a wide range of potentially harmful environmental agents, the intestine has developed a number of barrier mechanisms. Indeed, it has to constantly deal with pathogenic microorganisms but also with innocuous food antigens and an enormous number of commensal microbes with which the intestine has to maintain symbiotic host-microorganism relationships avoiding pathologies such as bacteremia and chronic inflammation. Our mucosal immune system induces systemic tolerance towards food antigens and local tolerance towards the microbiota. How this is established is still unknown. Here we describe the existence of a new barrier that controls the dissemination of antigens to systemic districts. We found that *S. typhimurium* is able to modify this structure and, therefore, also the function of the intestinal barrier. Indeed, we have shown that the infection is paralleled by an increased permeability of molecules to the systemic circulation. Moreover, *S. typhimurium* changing the intestinal barrier properties is able to spread in the early phases of the infection to lymphoid organs, and at later stages to distal ones, such as liver and spleen. The capability of *S. typhimurium* to change the permeability of the intestinal barrier and to colonize distal organs is mediated by the modulation of the Wnt/β-catenin signaling pathway.

W.81. Decreased Chemokine Levels in Supernatants from Primary Murine Gut Epithelial Cells Stimulated with Soluble Factors From *Lactobacillus Reuteri*, Compared With that of Staphylococcus Aureus

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The gut microbiota is thought to affect gut immune-responses and some bacterial strains may even regulate inflammatory responses within the tissue. A direct interaction between the gut microbiota and epithelial cells is likely to be invovled in these effects. To further elucidate the molecular mechanisms by which bacteria may affect gut epithelial responses, we cultured primary murine colonic epithelial cells and stimulated them with diverse stimuli including supernatants from the non-pathogenic *Lactobacillus reuteri (L. reuteri*) and the pathogenic *Staphylococcus aureus (S. aureus*). Cytokine/chemokine production was determined in cell culture supernatants using a mouse cytokine profiler array and quantified with an ELISA. Stimulating gut epithelial cells with S. aureus elicited secretion of cytokines, chemokines and

growth factors including TNF, IL-6, IP-10, RANTES, KC, MCP-1, and GM-CSF. A similar profile was seen after stimulation with supernatants from *L. reuteri* but the concentration was generally lower than that of cells stimulated with *S. aureus*. Furthermore, the pro-inflammatory chemokines IP-10 and RANTES were not detected after stimulation with *L. reuteri*. The decreased chemokine levels observed by *L. reuteri*-stimulated epithelial cells may lead to a reduced influx of inflammatory cells or an alteration in the cell types attracted to the tissue.

W.83. Anti-Inflammatory Treatments can Protect Female Genital Tract Mucosal Epithelial Barrier from Disruption by HIV-1

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We previously showed that direct interaction between HIV-1 and the female genital mucosa leads to induction of inflammatory factors including TNF-α from genital epithelial cells (GECs) resulting in mucosal barrier impairment and microbial translocation. Here, we examined if treatment with IL-22, an epithelial reparative cytokine or curcumin (diferuloylmethane), a well-characterized natural wide-spectrum anti-inflammatory agent or TGF-β, an immunoregulatory cytokine present in semen, could block induction of inflammatory responses in GECs and therefore protect the mucosal barrier. Primary GECs isolated from endometrial and cervical tissues were pre-treated with IL-22 (10ng/ml), curcumin (5uM) or TGF-β (5ng/ml) prior to HIV-1 exposure. GEC monolayers were protected from HIV-mediated barrier disruption following pre-treatment with IL-22, curcumin or TGF-β as seen by high transepithelial barrier measurements and intact tight junction immune-staining compared to HIV-treated monolayers. To define the mechanism of protection, we examined pro-inflammatory cytokine production by GECs in response to HIV exposure, the key mechanism involved in barrier disruption. Anti-inflammatory treatments completely suppressed TNF-α production by GECs, indicating that protection is mediated by direct or indirect blocking of TNF-α pathway. Treatment with anti-inflammatory and immunoregulatory factors can strengthen and protect the genital tract mucosal barrier from HIV, forming the basis for novel future prophylactic strategies.

W.84. Gut Barrier Defense Systems are Compromised Following Infection with EcoHIV and Exacerbated with Morphine: A Novel Murine Model of HIV and Drug Abuse

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Compromised gut barrier function, which is exacerbated by opiates, is believed to be integral for early pathogenesis of HIV; however no mouse infection model currently exists to study epithelial and immune functional changes in the gut. EcoHIV was developed to simulate HIV pathogenesis by genetically altering HIV to infect mouse cells by substituting gp80 from Murine Leukemia Virus for gp120 of HIV. We show that chronic morphine in EcoHIV treated mice additively enhances bacterial translocation from the gut to systemic tissues beyond what is seen in either treatment alone; this replicates human studies which implicate LPS in serum as a marker for translocation. Epithelial cells are a major physical barrier against bacteria and we observed that tight junctions are modulated severely in combined morphine and EcoHIV treated animals. Interestingly, we have also seen decreased IgA secretion and alterations in goblet cells. Cumulatively, these results imply a severe deficiency in the first line defenses that typically keeps bacteria outside the normally sterile body cavity and is a potential mechanism for how bacterial translocation is exacerbated in human HIV patients who abuse opiate drugs.

W.85. Not all Quiet on the Mucosal Front: Innate Mucosal Immunity in Resistance to HIV Infection in Kenyan Commercial Sex Workers

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Studies of female commercial sex workers (CSWs) in Kenya were among the first to identify highly HIV-1-

exposed seronegative (HESN) individuals. Since natural resistance is usually mediated by innate immune mechanisms, we focused on determining whether expression and function of innate signaling pathways were altered locally in the genital mucosa of HESN CSWs. Cervical mononuclear cells (CMCs), cervical epithelial cells (CECs) and cervicovaginal lavage (CVL) from HESN, HIV-positive (HIV-P), and new HIV-negative (HIV-N) CSWs were investigated. Our results demonstrated that pattern recognition receptors (PRRs) were significantly reduced in expression in CMCs from HESN compared to HIV-N and HIV-P groups. Although baseline levels of secreted TNFα and IL-10 were reduced in CMCs of HESN, these cytokines were highly stimulated following exposure to ssRNA40 *in vitro*. CECs from HESN also expressed significantly reduced TLRs, but importantly, expression of TLR3 and TLR7 were significantly enhanced in these cells. NFκB and AP-1 were highly expressed and activated in CECs from HESN. Lastly, CVL of HESN had reduced levels of inflammatory cytokines. Our data reveals a local mucosal microenvironment of resistance consisting of basal immune quiescence with a focused, potent innate anti-viral response that plays a critical role in prevention of sexual transmission of HIV-1.

W.86. Human Stromal Cell Function in Chronic Intestinal Inflammation
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Chronic inflammatory disorders of the intestine, such as Crohn's Disease, arise as a result of a complex interplay of genetic and environmental factors. Although several mechanisms involving hematopoietic immune cells are known to contribute to disease, the role of non-hematopoietic stromal cells (SCs) in the initiation and/or perpetuation of chronic intestinal inflammation is not fully described. Here we examined the impact of chronic inflammation on the human intestinal stromal compartment. $CD90^+$ colonic SCs isolated from inflamed mucosa displayed a persistently activated phenotype, characterized by exaggerated and sustained expression of adhesion molecules and inflammatory cytokines. Furthermore, SCs from inflamed tissue differentially regulated the functional responses of primary myeloid cells when assessed using a novel 3D organotypic co-culture model of the human mucosa. We are currently; 1) extending the characterization of SCs during chronic inflammation; 2) examining mechanisms underlying the persistent inflammatory stromal phenotype, including transcriptional and epigenetic profiling by RNA-seq and ChIP-seq; and 3) assessing the impact of chronic inflammation on multiple SC-leukocyte interaction pathways in 3D co-culture. Our data indicates that persistent alterations in SC activation accompany chronic intestinal inflammation in humans and suggests that SCs may contribute to pathology by providing multiple inflammatory signals in the inflamed tissue microenvironment.

W.87. Dendritic Cell-Specific Roles for Nod2 Activation *in vivo*
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Background: Nod2 is a cytosolic pattern recognition receptor that detects muramyl dipeptide (MDP), a component of bacterial peptidoglycan. This protein is known to play a critical role in the defense against infection, and may promote the generation of an immunologically tolerant state in bacteria-heavy environments such as the intestine. This is highlighted by the fact that single-nucleotide polymorphisms in the gene encoding for Nod2 are associated with an increased risk of developing inflammatory bowel disease (IBD). However, the relative contributions of specific tissues to the Nod2-mediated maintenance of homeostasis remain unexplored. Methods: Mice that specifically lack NOD2 expression in dendritic cells (DC) were generated using the Cre-lox expression system. These mice, along with wild-type controls, were systemically exposed to MDP via intraperitoneal injection. Serum was collected 4 hours post-injection, and circulating levels of KC and IL-6 assessed by ELISA. Splenocytes were isolated at 24 hours post-injection, and evidence of dendritic cell maturation assessed by flow cytometric analysis of surface marker expression. Results: RT-PCR analysis revealed that Nod2 mRNA expression was selectively knocked out in CD11c-expressing splenocytes. Systemic administration of MDP resulted in an elevation of serum levels of KC and IL-6 in both wild-type and Nod2-deficient DC-bearing mice at 4 hours post-injection. Similarly, administration of MDP led to the upregulation of the maturation markers MHC class II and CD40 on splenic DCs collected 24 hours post-injection. Once again, this was independent of DC Nod2 expression. Conclusions: DC-derived Nod2 is not required for systemic immune responses to MDP, including the maturation of splenic DCs, which must therefore be due to a mediator produced by a

non-DC cell type.

W.88. Air Pollution Induces NLRP3 Inflammasome-Independent, Uricase Sensitive, Allergic Sensitization and Airway Inflammation

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Our overarching objective is to characterize the inflammatory responses mediated by the airway epithelium to various environmental stimuli. We hypothesized that the airway epithelium expressed a NLRP3 inflammasome that mediates immune responses to urban particulate matter (PM) facilitating sensitization to allergens. *In vitro* studies were performed by exposing human airway epithelial cells to PM in the presence of silencing RNA for NLRP3, and assessing IL-1β, GM-CSF, CCL-20 protein. *In vivo* exposure of NLPR3$^{-/-}$ and wild-type control mice to ovalbumin, PM, or ovalbumin/PM was performed with outcome measurements of lung inflammation, goblet cell metaplasia, serum immunoglobulins, and lung gene expression. Uricase intervention studies were also performed. *In vitro* and *in vivo* PM exposure resulted in NLRP3 inflammasome sensitive production of IL-1β, CCL-20 and GM-CSF. *In vivo* ovalbumin/PM exposure resulted in development of a Th2 skewed immune response that was present in both wild-type and NLRP3$^{-/-}$ mice. Uricase intervention during ovalbumin/PM exposure resulted in complete inhibition of allergic sensitization and airway inflammation. Conclusions: PM exposure activates the NLRP3 inflammasome in airway epithelium to induce IL-1β, GM-CSF, and CCL-20. *In vivo*, NLRP3 is not required for PM-facilitated sensitization and Th2 skewed immunity to ovalbumin - a process that is uricase sensitive.

W.90. Intestinal Epithelial Cells Deficient in ATG16L1 Favours the Persistence of Adherent-Invasive *Escherichia Coli* Resulting in Increased AKT Activity and Reduced Autophagosome Formation and Barrier Integrity

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Adherent and invasive *Escherichia coli* (AIEC) are commonly found in lesions of Crohn's Disease (CD) patients. AIEC can survive, grow and induce pro-inflammatory cytokines in macrophages suggesting AIEC may play a role in the immunopathology of CD. ATG16L1 is a member of the ATG5-ATG12 autophagy complex and a well-known CD susceptibility gene. In this study, we sought to examine the biological response of intestinal epithelial cells deficient in ATG16L1 expression to infection with the colonic AIEC strain HM605. Polarized cells lacking ATG16L1 expression (ATG16L1KD), due to lentiviral transfection, and infected with HM605 displayed higher bacteria invasion and IL-8 secretion accompanied by a reduced autophagy response and trans-epithelial resistance in comparison to Non Target- (NT)-infected cells. Infected-ATG16L1KD and NT cells displayed similar responses in ERK 1/2, p38 MAPK, JNK-signalling pathways. In contrast, a delayed PI3K-independent AKT-activation starting 7hrs post infection was found in infected-ATG16L1KD compared to NT-infected cells. This data suggests that the AIEC-induced PI3K-independent AKT-activation in ATG16L1-deficient epithelial cells contributes to the impaired bacterial handling, autophagosome formation and barrier function. Thus, we provide a model of how a dysfunction in ATG16L1 can contribute to a persistence AIEC-infection in epithelial cells supporting the role of AIEC in Crohn's disease pathogenesis.

W.91. Epithelial-Specific A2BAR Protects the Intestinal Mucosa During Acute Colitis

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Central to IBD pathogenesis is uncontrolled intestinal inflammation due to epithelial barrier dysfunction and leukocyte infiltration. Emerging evidence indicates that adenosine is a mucosal protective signaling molecule. The A2B adenosine receptor (A2BAR) is of particular interest in intestinal inflammation as it is expressed to a high level in the epithelium and studies illustrate a barrier protective and anti-inflammatory role for A2BAR during acute mucosal inflammation. We hypothesize that A2BAR plays a protective role in the crosstalk between epithelial dysfunction and leukocyte-mediated inflammation central to IBD.

Enhanced severity of acute colitis was observed in A2BAR deficient mice (A2BAR$^{-/-}$), associated with increased colonic permeability, bacterial translocation and dramatic colonic granulocyte infiltration compared to controls. Bone marrow chimeras revealed that A2BAR expression on bone marrow derived cells did not significantly contribute to colitis severity in A2BAR$^{-/-}$. Intriguingly, specific deletion of A2BAR in the intestinal epithelium (A2BARfl$^{+/+}$/VillinCre^{+}) pointed to a prominent protective role for A2BAR expression on the epithelium in experimental colitis, characterized by increased weight loss, colonic shortening and tissue permeability in A2BARfl$^{+/+}$/VillinCre^{+} compared to VillinCre^{+}. Critically, A2BAR agonist treatment attenuated these parameters. We conclude that A2BAR intestinal epithelial signaling is a protective mechanism that may be pharmacologically exploited to attenuate experimental colitis.

W.94. Role of TNFα in Female Sex Hormone Related Immunopathology in a Mouse Model of Genital HSV-2 Infection

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Female sex hormones are known to regulate susceptibility to sexually transmitted infections in experimental models. Previously, we reported that estradiol- or progesterone-treated mice immunized with attenuated TK-HSV-2 are protected against subsequent WT HSV-2 challenge. While estradiol-treated mice do not develop pathology, progesterone-treated mice develop significant genital pathology. We hypothesized that increased levels of inflammatory mediators, such as TNFα, may lead to increased pathology in progesterone-treated mice. While excess TNFα induces immunopathology following Mtb infections, its role in the development of immunopathology following HSV-2 infections is not known. To examine the role of TNFα in hormone-induced immunopathology following HSV-2 infections, TNFα-/- mice were treated with estradiol, progesterone or saline and immunized intravaginally with TK-HSV-2. Following intravaginal WT HSV-2 challenge, progesterone-treated TNFα-/- mice showed protection, with no genital pathology. However, surprisingly, estradiol-treated TNFα-/- mice showed significant immunopathology and significant number succumbed to infection. Estradiol-treated mice had very high levels of vaginal IFNγ compared to other experimental groups. Thus, while the absence of TNFα in progesterone-treated mice improves immunopathology, estradiol-treated mice have uncontrolled IFNγ responses, which may lead to increased immunopathology. This relationship between sex hormones, TNFα and IFNγ is being further examined to gain better insight into their role in HSV-2 immunopathology.

W.95. Examining the Impact of HSV-2 Infection on HIV Susceptibility in the Female Genital Tract

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Herpes simplex type 2 (HSV-2) infection is associated with a 3-fold increased risk of HIV acquisition. We previously found that HSV-2 seropositive women had increased numbers of CCR5^{+} CD4 T cells in the cervical mucosa. The current study aimed to further elucidate HSV-2-associated immune alterations in the cervical mucosa of African/Caribbean women from Toronto, Canada. Cervical mononuclear cells isolated from two cytobrushes were stained with two panels of monoclonal antibodies that included T cell markers (CD3, CD4, CCR5, CD69, CD38, HLA-DR, CD25 and CD39), and dendritic cell markers (CD1a, CD14, CD11c, DC-SIGN, langerin and mannose receptor). We enrolled 42 HIV-uninfected women; 23 were HSV-2 seropositive (without clinical or virological reactivation), and 19 were HSV-2 uninfected. Overall, HSV-2 positive women had increased absolute numbers of cervical CD4 T cells (P=0.011), and an increased absolute number and proportion of cervical CD4 T cells expressing the following: CCR5^{+}, CD38^{+}HLA-DR^{+} and CD25^{+}CD39^{+}. In addition, mannose receptor (CD206) expression was increased in the CD11c^{+} (mDC) and CD14^{+} (monocyte) subsets (P=0.011 and P=0.010, respectively). HSV-2 seropositive women had increased HIV target cells in the endocervical mucosa even in the absence of herpes reactivation. This suggests that HSV-2 induces a persistent state of increased mucosal HIV susceptibility.

W.96. Persistence of Mucosal T Cell Responses to HSV-2 in the Female Genital Tract

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Relatively little is known about the T cell response to HSV-2 in the female genital tract, a major site of heterosexual HSV-2 acquisition, transmission and reactivation. In order to understand the role of local mucosal immunity in HSV-2 infection, T cell lines were expanded from serial cervical cytobrush samples from 30 HSV-2 infected women and examined for reactivity to HSV-2. T cells expanded from 48 of the 60 total cytobrush samples obtained from the HSV-2$^+$ women and of these, 31 contained T cells that proliferated in response to HSV-2; none of the T cell lines expanded from the 2 HSV-seronegative subjects proliferated in response to HSV-2. T cell lines were tested for antigenic reactivity to pools of overlapping HSV-2 peptides representing 16 HSV-2 proteins including immediate-early and virion (glycoproteins, capsid, tegument) proteins. The HSV-2 proteins most frequently recognized by cervical T cell lines (in descending order) were ICP4 (72% of subjects), UL39 (72%), UL49 (50%), ICP0 (44%), UL46 (44%), gB-2 (39%), gD-2 (39%). Importantly, T cells directed at the same HSV-2 peptide were often detected in serial cytobrush samples suggesting that HSV-specific T cells persist in the cervix. Greater antigenic diversity was observed in the cervical T cell lines compared to *ex vivo* PBMC although T cells directed at the same HSV-2 peptide pool were often measured in both compartments. Thus, broad and persistent T cell responses to HSV-2 were frequently detected in cervical T cell lines from HSV-2$^+$ women and were enriched in the cervix compared to the blood. Understanding the role of these T cells at this most biologically relevant site of virus exposure will be central to the elucidation of adaptive immune mechanisms involved in controlling asymptomatic and symptomatic HSV-2 disease.

W.97. *Chlamydia* Infection Causes Destruction of Male Germ Cells and Sertoli Cells That is Partly Prevented by Immune CD4 T Cells

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Chlamydia trachomatis infections are increasingly prevalent worldwide. Male chlamydial infections are associated with urethritis, epididymitis and orchitis, however the role of *Chlamydia* in prostatitis and male factor infertility remains controversial. Using a mouse model of *C. muridarum* infection in male C57BL/6 mice we investigated the effects of chlamydial infection on spermatogenesis and determined the potential of immune T cells to prevent infection-induced outcomes. Infection disrupted seminiferous tubules causing loss of germ cells at four and eight weeks post infection, with the most severely affected tubules containing only Sertoli cells. Increased mitotic proliferation, DNA repair and apoptosis in spermatogonial cells and damaged germ cells was evident in atrophic tubules. Caspase 3 staining revealed increased (6-fold) numbers of Sertoli cells with abnormal morphology that were Casp3 positive in tubules of infected mice indicating significant levels of apoptosis. Sperm count and motility were both decreased in infected mice and there was a significant decrease in morphologically normal spermatozoa. Interestingly, adoptive transfer of immune CD4 cells prior to infection prevented these effects on spermatogenesis and Sertoli cells. These data suggest that chlamydial infection adversely affects spermatogenesis and male fertility and that vaccination can potentially prevent the spread of infection and these adverse outcomes.

W.98. Crosstalk Between Respiratory and Reproductive Immunity by Effector T Cells Generated by Intranasal Immunization with Herpes Simplex Virus Type 2

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Genital herpes is one of the most common sexually transmitted diseases (STDs), causes primary infection in the genital epithelium and establishes lifelong latency in the sacral ganglia. In attempts to elicit protective immunity within genital tract, a number of vaccine types have been administered to humans and experimental animals by systemic and mucosal immunization routes. However, a licensed vaccine for genital herpes has not been established although these vaccines induce antigen (Ag)-specific antibody

responses in the host. The induction of Ag-specific effector T cells at genital mucosa has been reported to be a key to develop the protective immunity against genital herpes infection. In this study, we show that intranasal (inl) immunization with live attenuated thymidine kinase-defective HSV-2 strain (HSV-2 TK-) successfully induces HSV-2 specific Th1 cells in the female genital tract, which is critical for the protective immunity against intravaginal (ivag) challenge with wild type HSV-2. Ag-presenting dendritic cells (DCs) and Ag-specific Th1 cells but not HSV-2 genomic DNA were detected in the cervical lymph node (cLN) of immunized mice, suggesting that migrant nasal DCs harboring the HSV-2 peptide but not cLN regident DCs present the Ags to naïve CD4 T cells in cLNs. Moreover, we showed that cLN effector CD4 T cells had the ability to migrate into iliac lymph nodes and vaginal mucosa and provide against secondary challenge with ivag WT HSV-2 infection. Thus, we established the mouse model to examine the reproductive imprinting system, which will lead to the development of effective inl vaccine against STDs.

W.99. The Protective Efficacy of Antibodies Targeting Intracellular and Extracellular Chlamydial Antigens is Dependent on Isotype and Transcytosis

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Immunoglobulin-mediated protection is believed to involve antibody binding to extracellular chlamydial antigens. However, both IgG and IgA can be internalized by their respective transport receptors the neonatal Fc receptor (FcRn) and the polyimmunoglobulin receptor (PIgR), both expressed throughout the reproductive tracts. Because of this, antibody directed at antigens expressed by intracellular *Chlamydia* could protect by interfering with replication, whilst antibody against surface antigens may actually enhance infection; particularly IgG. *In vitro* studies showed that IgG targeting the extracellular major outer membrane protein (MOMP) significantly enhanced infection by 74% ($p < 0.01$) whist IgG targeting an intracellular inclusion membrane protein IncA significantly reduced infection by 25% ($p < 0.05$). The function of both IgG-MOMP and IgG-IncA was pH-dependent and required FcRn-mediated transcytosis. Furthermore, infection of female mice with IgG-opsonized EBs resulted in prolonged infection and enhanced upper reproductive tract pathology in wild-type (WT) but not in B2M-/- mice lacking a functional FcRn. Interestingly, IgA specific for both MOMP and IncA neutralized *in vitro* and *in vivo* chlamydial infection in a PIgR-dependent manner. These data show that the subclass and antigen specificity are important considerations for vaccine development and that FcRn and PIgR-mediated antibody transport can either enhance or protect against chlamydial infection.

W.100. Chronic Cigarette Smoke Exposure Alters the Murine Gut Microbiome

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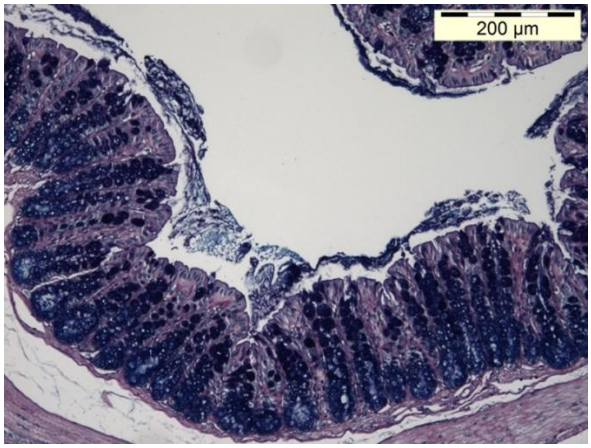

This study aims to investigate the influence of cigarette smoke on the microbiome, in particular the mucosa-adherent microbiota, and how this is linked to changes in mucin production. Analysis of the microbiome in smoke- and air-exposed C57BL/6 mice, using denaturing gradient gel electrophoresis (DGGE) and 454 pyrosequencing, revealed that microbial community structures changed significantly after cigarette smoke exposure (CSE) for 24 weeks in all parts of the gut. In addition, the abundance of specific species, in particular *Bifidobacterium sp.* and *Clostridium sp*., tended to decrease in response to CSE in both ileum and colon. Furthermore, analysis of mucin expression, using the Alcian Blue (AB)/Periodic Acid Schiff (PAS) and High Iron Diamine (HID)/AB staining methods, could not

demonstrate an altered expression of acidic and neutral mucins, nor changes in sulphated and sialylated mucins after CSE. However, in the ileum, mRNA expression of MUC2 and MUC3 significantly increased after CSE. In contrast, colonic expression of MUC2 and MUC3 was unaltered, but an increased expression of MUC4 was observed. Interestingly, additional analyses demonstrated that the CSE-induced increase of MUC4 significantly correlates with the CSE-induced shift in the microbiome. Intestinal mucins play an important role in the gut barrier, but also in the colonization efficiency of specific gut microbiota. These findings may point to a role for altered interactions between the microbiome and intestinal mucosa contributing to the effect of smoking on intestinal homeostasis. Protein expression of mucins in distal colon after smoke exposure. Acidic mucins are stained in blue, neutral mucins are stained in purple-pink.

W.101. Comparison of Mucosal Portals of Entry for *Salmonell*a in Poultry

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Salmonella enterica, one of the most prevalent species causing food borne illness in humans, has an established oral-fecal route of horizontal transmission. *Salmonella* invade mucosa, translocate and cause a systemic infection and disease through survival in macrophages. We hypothesized, the respiratory tract, bearing a similar mucosal barrier, can also be a viable portal of entry for *Salmonella* in chickens. In a series of *in vivo* trials, we evaluated *Salmonella* recovery in ceca and cecal tonsils, in broiler chickens challenged intratracheally (IT) with *S. enteritidis* (SE), *S. typhimurium* (ST) and *S. senftenberg* (SS). Recovery of SE from ceca (mean±SEM of $\log_{10}$CFU's/gram) in week old broiler chickens challenged intratracheally were: 1.1±0.4 for 10^4; 3.2±0.17 for 10^6 and, 5.1±0.5 for 10^8, as compared to 1.8±0.4, 1.9±0.4 and 5.9±0.5, respectively for orally challenged groups. Recovery in liver-spleen was also significantly higher with 50%, 83.3% and 91.7% samples positive as compared to 0%, 16.7% and 9.1% recovery for respective routes of challenge. An *in vivo* trial involving mucus coated SS failed to support the hypothesis that *Salmonella* given intratracheally are cleared by the mucociliary escalator and directed back to the oral route. Another hypothesis, supposing *Salmonella* invasion of blood directly from the parabronchial region without macrophages and spread systemically, was also evaluated by intravenous challenge of chickens with SE and SS. The hypothesis was questioned by careful comparison with initial SE trials; further supporting macrophage mediated systemic transportation of *Salmonella*. Overall, we advocate respiratory route as a viable portal of entry involving *Salmonella* pathogenesis, suggesting future research.

W.102. Impact of Dietary Protein on Caecal Microbiota: A Comparison Between Normal and Interleukin-10 Gene-Deficient Mice

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Gastrointestinal homeostasis is maintained by interactions between host, microbiota and diet; however, defects in the immune system lead to microbiota perturbation and inflammation. We investigated the effect of dietary protein on microbiota profiles, in normal and Interleukin-10 gene-deficient ($Il10^{-/-}$) mice. Five-week-old C57BL/6J (n=8/group) and $Il10^{-/-}$ (n=15/group) SPF male mice were assigned to three treatment groups randomized by weight, and inoculated with endogenous intestinal bacteria. Groups were fed diets (modified AIN-76A) containing 20% protein from soy, cow milk or goat milk for six weeks with body weight and condition monitored. Mice were then euthanized and caecal microbiota composition analysed by pyrosequencing of PCR amplicons of the bacterial 16S rRNA gene. $Il10^{-/-}$ mice fed soy-protein gained weight similar to C57BL/6J mice. In contrast, $Il10^{-/-}$ groups fed milk-protein lost weight and developed diarrhoea. Evaluation of caecal microbiota showed clear distinctions between $Il10^{-/-}$ and C57BL/6J mice, with greater diversity in C57BL/6J mice. Comparison between diets showed caecal microbiota of C57BL/6J groups were similar, whilst $Il10^{-/-}$ mice fed soy-protein were different compared with $Il10^{-/-}$ groups fed milk-protein. These differences may in part account for disease amelioration in $Il10^{-/-}$ mice fed soy compared with those fed milk-protein.

W.103. Antibiotic-Induced Alteration of the Gut Microbiota Protects TNFΔARE Mice from Crohn`s Disease-Like Ileitis

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Background: The functional role of intestinal bacteria in initiating and progressing ileal Crohn`s disease is unclear. We used the TNFΔARE Crohn`s Disease-Like Ileitis model to assess the role of antibiotic treatment on inflammation and microbial composition. Methods and Results: Inflammation was scored by microscopic observation of distal-ileal sections. Antibiotics (vancomycin (0.25 g/l) and metronidazole (1 g/l) for 4 weeks) substantially reduced ileitis in TNFΔARE mice (histopathology of 2.2 ± 1.6 vs. 4.9 ± 0.8 in the antibiotic and control group, respectively; n = 6; $p < 0.001$). Four weeks after antibiotic treatment, recurrence of inflammation was observed (score of 4.1 ± 1.5). High-throughput 16SrRNA sequencing showed marked changes in bacterial diversity and composition in the caecal lumen and the ileal mucosa. Total bacterial counts were not affected. In control mice, *Firmicutes* and *Bacteroidetes* were the dominant phyla (>82% total sequences). Antibiotics increased e.g. the abundance of *Lactobacilli* (19.6 ± 27.6% vs. 76.3 ± 19.8%), whereas the proportion of *Bacteroidetes* was reduced (7.2 ± 10% vs. 15.0 ± 6.0%). Metabolite profiling by FT/ICR-MS indicated changes in the bile-acids and carnitine metabolism. Conclusion: Our findings indicate an essential role of intestinal bacteria in the development of Crohn's Disease-Like Ileitis.

W.104. Immune Response and Invasiveness Ability of *Adherent-Invasive Escherichia Coli*: Invasiveness in Eukaryotic Cells

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Adherent-invasive Escherichia coli (AIEC) strains have been associated to Crohn's Disease (CD) pathogenesis. The aim of this study is to evaluate the genetic difference and invasiveness *in vitro* of AIEC strains isolated from Chilean patients and to determine the induction of immune response in macrophages. AIEC strains from intestinal biopsies from eight CD patients, two UC and one control were evaluated. The strains were characterized for genetic heterogeneity by PCR and pulsed-field gel electrophoresis (PFGE), respectively. Invasive and replicative ability was assessed by infections of epithelial and macrophages cell lines *in vitro*, respectively. The secretion of proinflammatory cytokines in macrophages exposed to bacterial isolates was determined by ELISA. Strains from different patients are heterogeneous, but those from the same patient are closely related (over 95%). The ability to invade epithelial cells and survive is variable in different strains, but in most cases is higher than commensal *E. coli*. AIEC strains are able to induce the secretion of cytokines such as TNF-α, IL-6 and IL-1β in macrophages. AIEC strains studied have different virulence genes that give the ability to invade and survive in eukaryotic cells and induce an innate immune response.

W.105. Role of Crohn's Disease Risk Loci (FUT2) on the Functional State of the Normal Colonic Mucosal Microbiota

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Fucosyltransferase 2 (FUT2) gene is responsible for the presence of the ABO histo-blood group antigens in body fluids and on the intestinal mucosa. Non-secretors (Se-), who are homozygous for the loss-of-function alleles of FUT2 gene, are associated with increased susceptibility of Crohn's Disease. We collected 75 endoscopic lavage samples from the cecum and sigmoid of 39 healthy subjects (27 Se^{+}, 12 Se-). The 16S rRNA gene of the microbiota was sequenced on an Illumina HiSeq 2000. The gene content of 1,119 KEGG reference genomes was used to infer the approximate gene content of the detected

phylotypes. Compared with non-secretors, secretors exhibited higher phylogenetic diversity. The PCoA plot of weighted UniFrac distance showed that the samples clustered by secretor status (Adonis test P = 0.034). The functional enrichment in secretors highlighted the amino acids metabolism related pathways. In non-secretors, the glutathione metabolism pathway and xenobiotic metabolism pathways were enriched. In conclusion, the colonic microbiota of non-secretors was significantly different from that of secretors at systematic and individual phylotypic level. The changes of metagenomic functions in non-secretors indicated the adaptation of gut microbiome to the presence of oxidative stress, which might be the potential link to the susceptibility of Crohn's Disease.

W.106. Altered Migratory and Functional Properties of Dendritic and T cells Predispose to Wound Failure in Crohn's Disease

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Introduction: Crohn's Disease (CD) patients are predisposed to post-surgical wound failure. Dendritic cells (DC) dictate type of T cell immunity,T cell homing profiles, and play a central role in CD pathogenesis;however wound DC have yet to be identified and characterized. Hypothesis: Changes in immune cell function contributes to wound failure in CD. Methods: DC and T cells were identified from wound tissue and blood samples from CD and non-CD (control) patients by flow cytometry. DC were co-cultured with T cells in a five-day mixed leucocyte reaction. Results: DC were successfully identified from wound tissue; wound DC in all cases expressed gut-homing marker (β7). Expression of skin-homing molecule (CLA) was reduced on wound DC in CD compared with controls(48.3±8.2vs.69.9±4.7,p=0.04). Wound DC stimulated dose-dependent allogeneic T cell proliferation; both wound and blood DC from CD patients were significantly less stimulatory than their control DC counterparts. Furthermore, DC from CD patients generated T cells with enhanced expression of CLA compared to T cells stimulated by control DC in wound tissue (16.7±1.1vs.7.1±1.5,p=0.006) and blood(28.3±3.2vs.12.5±3.7,p=0.03). Conclusions: Aberrant expression of skin-homing marker CLA on DC and T cells that they stimulate may contribute to alterations in immune cell migration in CD. Taken with the restricted stimulatory capacity of DC in CD wounds, it is likely that a loss of DC function occurs contributing to wound failure.

W.107. Lactobacilli Dampen IL-17 and IFN-γ T Cell Responses Against *Staphylococcus Aureus in vitro*

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Altered exposure to micro-organisms is linked to inflammatory disease like allergy. We have previously shown that early colonization with lactobacilli reduces the risk for allergy development later in life, irrespective of allergic heredity, while early *Staphylococcus (S.) aureus* colonization was associated with an increased risk. In retrospect, we investigated how these microbes influence the immune system. We stimulated the intestinal epithelial cell lines (IEC) with bacteria culture supernatants (-sn) from seven Lactobacillus strains and three *Staphylococcus aureus* strains. *S. aureus*-sn induced CXCL8/IL-8 production by IEC, which was partially regulated by MyD88. Further, peripheral blood mononuclear cells (PBMC) from healthy donors were stimulated directly by bacteria or indirectly with bacteria conditioned IEC-sn. In both conditions, Lactobacillus and *S. aureus* strains were able to induce a wide range of cytokines, but only *S. aureus* induced the T cell associated cytokines IL-2, IL-17 and IFN-γ. Intracellular staining of T cells further revealed that *S. aureus* induced IFN-γ and IL-17 production was down regulated by the simultaneous presence of lactobacilli. This was not due to the low pH of lactic acid or histamine release from lactobacilli as suggested previously. This study reveals a possible role of lactobacilli in modulating immune cells response toward *S. aureus*.

W.108. Recovery of Neutrophil Phagocytic Capacity in Patients with Cirrhosis and Hyperammonemia Through Intestinal Microbiota Modifications Induced by Blue Agave Inulin
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Intestinal bacterial overgrowth and endotoxemia play an important role on the cirrhosis complications. The increased susceptibility to infection of cirrhotic patients is by peripheral blood neutrophil deranged phagocytosis and increased proinflammatory cytokine production. The aim of this study was to stablish the blue agave inulin effect on cirrhotic and hyperammonemia patients through reducing serum levels of ammonium, TNF-α, restoration of the phagocytic capacity of neutrophils and increasing colonization resistance against invading pathogens by gut microbiota modifications. The study included 16 cirrhotic patients with hyperammonemia/Minimal hepatic encephalophaty. Two groups of eight patients randomized: the first was treated with inulin and the second with lactulose. Ammonium levels was measured, phagocytic index and phagocytic rate, microbiota was assessed by RT-PCR and TNFα by ELISA. Inulin or lactulose did not decrease ammonium arterial levels, same as TNFα (P=0.268 and P=0.422). However, inulin increased phagocytic index and phagocytic rate (P=0.016 and 0.031), and significantly increased the counts of acid-lactic bacteria (P=0.020), while lactulose did not (P=0.473). The Inulin effect of five days of treatment did not decrease arterial ammonium but restore neutrophil phagocytic capacity. Additionally, inulin effect induces an increase in the number of lactic-acid bacteria but not lactulose. Finally, patients refer best health-related quality of life after inulin ingestion.

W.109. A Novel Probiotic Function for the Activation of Intestinal Epithelial Autophagy
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Background: The effects of probiotics on the maintenance of intestinal homeostasis, as well as the modulation of immunity, are well known. Autophagy is also known to be associated with intestinal inflammation, and patients with genetic variations of molecules in the autophagy pathway are susceptible to inflammatory bowel diseases. This study proposes that bioactive molecules derived from the probiotic Bifidobacterium breve (BB) enhance the intestinal autophagy. Methods: Intestinal epithelial cells (Caco2BBE, IEC18) were treated with the conditioned media (CM) of BB. Activations of MAP kinases and LC3 conjugation were examined by a Western blotting. Bioactive molecules derived from the BB-CM were characterized using a molecular weight column and tests of protease-sensitivity, heat stability and pH dependency. Results: BB-CM induced autophagy in a time- and concentration-dependent manner. BB-CM also activated p38 MAPK and JNK within 30 minutes, and induced LC3II conjugation. Pretreatment with either a p38MAPK or JNK inhibitor blocked the LC3II conjugation by BB-CM. The bioactive molecules derived from BB-CM are regarded to be heat stable, low pH-resistant small peptides. Conclusions: This study demonstrated a novel function of the probiotic BB on the enhancement of autophagy through the activation of MAPKs, which was mediated by bioactive molecules secreted from the probiotics.

W.110. Selection of Treg-Inducing Bacteria from the Human Microbiota
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The mammalian intestine harbors a large and complex microbial community, known as the gut microbiota that is essential for the development of the immune system. The microbiota establishes a mutually beneficial relationship with their host. Environmental factors and host genetics may trigger changes in diversity and composition of the gut microbiota. An altered microbiota can disrupt this relationship and lead to inflammatory and autoimmune disease. However, because microbiota contains hundreds of different bacterial species, how the specific gut bacteria influence the host immune system is not yet fully understood. We previously reported that strains from the murine microbiota can induce regulatory T (Treg) cells. Here we demonstrate that Treg-inducing strains rationally isolated from the human indigenous microbiota. Starting from a healthy human fecal sample, a sequence of selection steps were applied to obtain gnotobiotic mice colonized with a human microbiota enriched in Treg-inducing strains. We cultured and selected 17 strains of bacteria mainly belonging to the genus Clostridium. When the

mixture of 17 strains was inoculated into germ-free mice, we observed a strong accumulation of Treg cells in the intestine. Furthermore, oral administration of 17 strains attenuated disease in adult mouse models of colitis and allergic diarrhea.

W.111. The Influence of Intestinal Microbiota on Tumor Growth and Metastases to the Lung
Alissa Cait, Michael Hughes, Kim Synder, Kelly McNagny, William Mohn. University of British Columbia, Vancouver, BC, Canada

The mammalian gastrointestinal tract harbors a complex community of micro-organisms and is one of the densest microbial habitats. These symbiotic microbes have significant influence on the development of the immune system, with consequences relevant to many human diseases. For example, specific microbes and microbial products exert a profound effect on the process of T helper (TH) cell differentiation. Appropriate balance and development of various TH cell subsets influences the way the body responds to various disturbances of homeostasis. Skewing of this balance may contribute to the development of intestinal inflammation or disease in distant tissues including the lung. Mice reared on antibiotics have previously been shown to be highly susceptible to allergic asthma and are predominately TH2-skewed compared to their conventionally raised littermates. We aim to uncover the particular taxa and molecular mechanisms that allow intestinal flora to influence disease in the lung through the skewing of peripheral and intestinal immune responses. Using a mouse model of lung metastases we are characterizing the changes that occur in the microbiota with antibiotic treatment and correlating specific changes in community structure to changes in the seeding and growth of melanoma in the lung.

W.112. *Bifidobacterium breve* NCC2950 Regulates Intestinal Immunity and Inflammation by Inducing the Expression of Antimicrobial RegIII in Colonic Epithelial Cells Through MyD88-Ticam Pathway
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Antimicrobial RegIII proteins are induced by intestinal bacteria, and play a role in spatially segregating bacteria, preventing a potentially harmful immune response, and protecting the host from infection. It is unknown whether specific commensal bacteria and probiotics differentially regulate RegIII proteins and the underlying mechanism. We showed that the probiotic *Bifidobacterium breve* NCC2950 stimulates intestinal epithelial innate defense through up-regulation of inducible RegIII proteins. Germ-free mice have low levels of RegIII-γ mRNA in their colon. Monocolonization with either *B. breve* or non-probiotic *Escherichia coli* JM83 up-regulated the expression of RegIII. However, *B. breve* induced a greater *in-vivo* expression of RegIII-γ than *E. coli*. Induction of RegIII-γ by *B. breve* was abrogated in mice lacking MyD88 and Ticam signaling. *In vitro*, both live and heat-killed *B. breve*, but not Spent Culture Media (SCM) from *B. breve*, induced the expression of RegIII-α (human counterpart of RegIII-γ) in human colonic epithelial Caco-2 cells compared to *E. coli*. Administration of live, but not heat-killed or SCM *B. breve*, to mice before dextran-sulfate sodium decreased intestinal inflammation. Taken together, these results suggest that induction of RegIII at the intestinal epithelial cell is mediated by MyD88-Ticam pathway. Specific induction of RegIII by *B. breve* NCC2950 has pathogenetic implications and may prevent intestinal injury.

W.113. Enterococcal Polysaccharide Antigen (Epa) Contributes to Virulence of Pathobionts in Infection and Chronic Inflammation Models
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Introduction: *Enterococcus faecalis*, a commensal of the human intestinal microbiota, harbors several putative virulence factors that mediate its bacterial pathogenicity and make it an exemplary model to analyze structure-function relationships for microbe-host interactions. The enterococcal polysaccharide antigen (Epa) was previously described to affect *E. faecalis* virulence. Methods and Results: In this study, we characterized enterococcal virulence using a newly generated epaB deletion mutant (TX5692). A

deletion of epaB resulted in diminished biofilm generation including attenuated formation of micro-colonies and impaired adhesion to epithelial cells *in vitro*. To further analyze microbe-host interaction, germ-free Manduca sexta larvae, a natural host to *E. faecalis*, were mono-associated with *E. faecalis* mutant and wild-type strains. Compared to wild-type OG1RF, the ΔepaB mutant showed altered adhesion to larval intestinal epithelium resulting in reduced expression levels of peptidoglycan-recognition protein. In a recent mono-association experiment, IL-10$^{-/-}$ mice associated with ΔepaB mutant strain showed diminished levels of chronic inflammation markers compared to OG1RF-associated mice. Conclusion: Our experiments in a colitis mouse model suggest an impact of epaB on intestinal pathogenesis. These studies indicate to mechanisms related to altered adhesion to intestinal mucosal surfaces and may unravel a track of how epaB-mediated adhesion contributes to *E. faecalis*-host interaction.

W.114. Mucosal DN T Cells Response Differently to the Systemic Cytokine/Chemokine Milieu

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The activation of the mucosal immune system has an important influence on the susceptibility to HIV infection. The CD3$^+$ double negative (DN) T cells have been associated immune activation. However, little is known about their presence at the genital tract and how the milieu influences their activation. In this study, we analyzed the influence the systemic and mucosal milieu on cervico mononuclear cells (CMC) T and DNT T cells. CVL and plasma from 120 commercial sex worker HIV- were analyzed for the presence of 22 chemokines by bead array. CMC were analyzed for cellular activation marker by flow cytometry. By analyzing the ratio of systemic/mucosal cytokine-chemokine on CMCs activation, we observed that the systemic milieu did not influence the activation of DNT cells (CD3$^+$CD4-CD8-CD69$^+$; CD3$^+$CD4-CD8-HLA-DR$^+$) as it did for the CD3$^+$CD4$^+$HLA-DR$^+$ (p=0.04) and CD3$^+$CD8$^+$HLA-DR$^+$ (p=0.002). However, the ratio of systemic/mucosal of Fractalkine, IFN-a2, MIP-1b, IFN-g and IL-6 negatively influence the frequency of mucosal DN T cells. Surprisingly, the mucosal milieu seems to have less impact of the activation and frequency of the DN T cells than does the systemic milieu. This study showed that DN T cells do not react the same way as CD4$^+$ and CD8$^+$ T cells.

W.115. Unexpected Differences in the Cervical and Cervico-Vaginal Mucus of HIV Positive and Negative Women

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Hindering transport of virions within the female reproductive tract is an attractive mechanism for transmission prevention. Here we expand on previous studies to determine the effect of HIV infection, menstrual cycle and immune responses to HIV on viral transport. We have established a cohort of 150 HIV $^+$ve and –ve women to study longitudinally for correlates of modulated transport phenotypes. Cervical and cervico-vaginal mucus samples (CM and CVM) systemic and mucosal immune monitoring samples are collected. Viral transport and capture assays employ a panel of viruses. Unexpectedly, mucus of HIV infected individuals is more permissive to viral transport. Hormone profile demarcating menstrual cycle phase correlates strongly with diffusivity. Further analysis revealed that CVM is particularly permissive to virus during the luteal phase of HIV-ve women and restricts in HIV $^+$ve women; bead mobility is unaffected. Mucosal antibodies isolation shows cross clade binding specificity amongst infected individuals. Correlates of diffusivity are not restricted to HIV-specific responses but also derived from mucus. The effect of infection within the reproductive tract resembles that of a “runny nose” response to pathogen clearance. This study reveals the significance of immunity and hormones on HIV-1 transmission mechanisms and viral transport in the female reproductive tract.

W.116. *Lactococcus Lactis Subsp. Cremoris* FC Triggers IFN-γ Production from NK and T Cells via IL-12 and IL-18

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Lactic acid bacteria (LAB) benefit health as probiotics in a strain-dependent way. In this study, we investigated the immunomodulatory effects *of Lactococcus lactis subsp. cremoris* FC (LcFC) on dendritic cells (DCs), natural killer (NK) cells and T cells. LcFC induced the production of cytokines such as IL-12, IL-6 and TNF-α from murine bone marrow DCs (BMDCs) via MyD88-dependent pathway. IL-10 production was partially dependent on c-type lectin pathway also. LcFC induced particularly high production of IL-12 while induction of IL-6 was moderate. Consequently, LcFC triggered IFN-γ production in splenic NK, $CD8^+$, and $CD4^+$ cells. Most prominent effect of LcFC on IFN-γ production was observed in NK cells, followed by $CD8^+$ cells, which was completely inhibited by combination of neutralizing anti-IL-12 and anti-IL-18 mAbs. Moreover, oral administration of LcFC enhanced the production of IFN-γ and IL-10 from splenocytes of treated mice. These findings suggest that this LAB strain is an efficient activator of protective cellular immunity via stimulation of myeloid cells including DCs.

W.117. α4β7 Expression on Blood T Cells is Increased in Herpes Simplex Type 2 Infection and Correlates with Markers of HIV Susceptibility in the Female Genital Tract.

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T cell immune activation in the cervical mucosa correlates with increased HIV susceptibility, but genital sampling is impractical in many contexts. The integrin $\alpha_4\beta_7$ homes T cells to mucosal surfaces. Therefore, we assessed the activation state of $\alpha_4\beta_7$-expressing T cells in blood, and their association with cervical T cell populations and with infection by herpes simplex virus type 2 (HSV2), which is known to enhance HIV susceptibility. Cervical and blood mononuclear cells and blood were isolated from 43 HIV-uninfected African/Caribbean women from Toronto and assessed by multi-parameter flow cytometry. In blood, α4β7+ CD4+ T cells expressed higher levels of the HIV co-receptor CCR5 ($p=0.005$) and the immune activation markers CD38/HLA-DR ($p=0.014$). Furthermore, blood α4β7 expression strongly correlated with an increased number of CCR5+ ($p=0.005$) and CD38/HLA-DR+ ($p=0.004$) CD4+ T cells in the cervix. HSV2 infection (22/43 participants; 51%) was associated with both systemic and genital immune activation, and with a dramatic increase in α4β7 expression on blood CD4+ T cells (19.60 vs. 8.76; $p<0.001$). We conclude that α4β7 expression on blood T cells was increased in HSV2 infection and was associated with increased cervical T cell immune activation and CCR5 expression.

Poster Session: Thursday, July 18

T.1. *Helicobacter Pylori* Infection Alters Homing Receptor Expression on Human Peripheral Blood Regulatory T cells

Katherine Cook, Darren Letley, Richard Ingram, James Crooks, Khiyam Hussain, John Atherton, Bruno Gran, Karen Robinson. University of Nottingham, Nottingham, United Kingdom

Helicobacter pylori (Hp), the main cause of peptic ulceration and gastric cancer, persistently colonises the gastric mucosa and stimulates inflammation with increased mucosal and blood regulatory T cells (Tregs). Recently, Hp has been linked to protection against immune-mediated conditions, including asthma and multiple sclerosis (MS). We investigated whether the infection modifies homing receptor profiles on Tregs. Blood and gastric biopsy samples were obtained from 27 infected and 38 uninfected patients undergoing an upper gastro-intestinal tract endoscopy. Cells were isolated, stained for Treg markers CD4, CD25 and Foxp3, and homing receptors CD62L, CD103, CCR4, CCR6, CCR7, CCR10, CXCR1, CXCR2, and integrin β7, before flow cytometry analysis. A higher proportion of blood Tregs from infected patients expressed CCR6 ($p=0.03$), CXCR1 ($p=0.04$) and CXCR2 ($p=0.04$) compared to uninfected patients. There were no differences amongst the other homing receptors. CCR6 was expressed by almost all gastric Tregs. Its ligand, CCL20, was expressed at 10-fold higher concentrations in Hp^+ biopsies ($p<0.001$), indicating its importance in recruiting these suppressive cells to the inflamed mucosa. In conclusion, Hp-infection modifies the homing potential of peripheral blood Tregs. This could permit their

entry to other CCL20-expressing tissues (such as the central nervous system in MS), to suppress pathogenic T cell activity.

T.2. Role of H. Pylori γ-glutamyl Transpeptidase During Early and Chronic Infection
Stefanie Wüstner, Florian Anderl, Markus Gerhard. Technische Universität München, Munich, Germany

Helicobacter pylori colonizes the stomach of half of the world's population. The infection persists despite a strong Th1/Th17 driven immune response that is characterized by an active gastritis. The virulence factor γ-glutamyl transpeptidase (gGT), a secreted enzyme of *H. pylori*, impairs the T cell response and is essential for persistent colonization. *In vitro* studies have shown that *H. pylori* gGT (HpgGT) depletes anti-oxidative substances and inhibits proliferation of T lymphocytes. This might display an effective mechanism by which the pathogen dampens the adaptive immune response. To elucidate this aspect, we infected mice with virulent *H. pylori* PMSS1 strain and gGT deficient mutant. We analyzed the colonization level and the inflammatory profile in the stomach and in associated secondary lymphoid organs. Hereby, we observed that *H. pylori* ΔgGT colonized at lower levels and with greater variability during the early infection compared to the wild type. This implies a general *in vivo* growth defect. In the chronic phase, *H. pylori* ΔgGT were cleared in a lymphocyte dependent manner as $Rag^{-/-}$ mice (T/B cell deficient) were still stably infected at high levels. Thus, we propose a dual role of HpgGT in metabolism and immune evasion.

T.3. Cellular Source of *Helicobacter Pylori*-Induced Gastric IL-17 and its Role in Human Disease
Emily Staples, Richard Ingram, Khiyam Hussain, Abed Zaitoun, John Atherton, Karen Robinson. University of Nottingham, Nottingham, United Kingdom

Helicobacter pylori (Hp) is a major cause of peptic ulcer disease (PUD). Gastric IL-17 levels are increased in Hp infection but its cellular source and role in human disease are unclear. Gastric biopsies were collected from 79 Hp^+ and 56 Hp- patients undergoing routine endoscopy. IL-17 and RORC2 mRNA was quantified by real-time PCR. IL-17, CCL20 and IL-8 protein concentrations were measured by Luminex. Frequencies of IL-17-secreting $CD4^+$ and $CD8^+$ T cells were assessed by flow cytometry. Inflammation and neutrophil infiltration (activity) were scored from H&E stained histological sections. IL-17 expression was increased in Hp^+ gastric biopsies (mRNA: 42.6-fold, $p<0.0001$; protein: 3.5-fold, $p<0.0001$). IL-17 concentrations correlated with concentrations of IL-17F ($\rho=0.80$, $p<0.0001$) and chemokines CCL20 ($\rho=0.59$, $p<0.0001$) and IL-8 ($\rho=0.49$, $p=0.0004$). High IL-17 concentrations were associated with increased inflammation (2.4-fold, $p=0.024$) and activity (2.4-fold, $p=0.031$). There was no association between IL-17 and PUD. The majority of IL-17 was Th17-derived with increased $\%CD3^+CD4^+IL\text{-}17^+$ (3.0-fold, $p=0.003$) and increased RORC2 expression (2.7-fold, $p<0.0001$) in Hp^+ biopsies. Low numbers of $CD3^+CD8^+$ and $CD3^+$CD4-CD8- IL-17-secreting cells were also present, and at higher frequencies in Hp^+ biopsies (3.2-fold, $p=0.02$ and 2.9-fold $p=0.03$ respectively). In conclusion Th17-derived IL-17 is important in Hp-induced gastritis, but not associated with PUD.

T.4. The Mucin Muc1 Suppresses *Helicobacter Pylori*-Induced Gastritis by Regulating the NLRP3 Inflammasome
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Background: Muc1 is an important cell surface mucin, expressed by epithelial cells lining the gastric mucosa and immune cells. Previously, we demonstrated Muc1 as a key regulator of gastritis induced by the mucosal pathogen *Helicobacter pylori*, the main cause of gastric cancer. Results: The critical importance of Muc1 was demonstrated in long-term experiments. $Muc1^{-/-}$ mice began to die six months post-*H. pylori* infection with >50% mortality by nine months, while wild-type mice remained asymptomatic. Stomachs from these $Muc1^{-/-}$ mice had severe pathology, dysplasia and contained precancerous epigenetic changes absent in infected wildtype mice. Using bone marrow chimaeras, we found Muc1 regulation of *H. pylori*-induced gastritis is mediated by haematopoietic and not epithelial cells. Analyses of cytokines within the *H. pylori*-infected gastric mucosa revealed an association between Muc1 deficiency

and IL-1β levels. *In vitro* studies showed Muc1$^{-/-}$ macrophages produce elevated IL-1β levels upon stimulation, compared to wildtype cells. By the use of specific activators, we demonstrated that Muc1 selectively regulates activation of the NLRP3 inflammasome. LPS-activated Muc1$^{-/-}$ cells and the gastric mucosa of *H. pylori* infected Muc1$^{-/-}$ mice both express elevated levels of Nlrp3. Conclusion: Muc1 critically regulates inflammation in the gastric mucosa by suppressing activation of the NLRP3 inflammasome.

T.5. Insufficient Apoptotic Cell Clearance may Contribute to Chronic Inflammation in Human *H. Pylori* Infection

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Increased apoptotic death of gastric epithelial cells is a hallmark of *H. pylori* infection, and altered epithelial turnover is an important contributor to gastric carcinogenesis. To address the fate of apoptotic gastric epithelial cells and their role in *H. pylori* mucosal disease, we investigated phagocyte clearance of apoptotic gastric epithelial cells in *H. pylori* infection. Using immunofluorescence analysis of human gastric tissue specimens, we show that mucosal HLA-DR^{+} mononuclear phagocytes contain cytokeratin-positive and TUNEL-positive apoptotic epithelial cell material, indicating that gastric phagocytes participate in apoptotic epithelial cell clearance. We further show that *H. pylori* both increased apoptosis in primary gastric epithelial cells and decreased phagocytosis of apoptotic epithelial cells by autologous monocyte-derived macrophages. Reduced macrophage clearance of apoptotic cells was mediated in part by *H. pylori*-induced TNF-α, as neutralization of TNF-α in *H. pylori*-stimulated macrophage cultures reversed the inhibitory effect of *H. pylori* on macrophage phagocytosis. Notably, TNF-α was expressed at higher levels in *H. pylori*-infected, compared to uninfected, gastric mucosa. Such insufficient phagocyte clearance of apoptotic epithelial cells may contribute to the chronic inflammatory status of the *H. pylori*-infected gastric mucosa, consistent with the pathogenic role of impaired apoptotic cell clearance in other autoimmune and chronic inflammatory diseases.

T.6. Peyer's Patches Harbor 'Resident' CD4^{+} T Cells that Recognize Antigens from the Intestine

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Lymphocytes circulate through secondary lymphoid organs (SLOs) in search for their cognate antigens. Although the mechanisms regulating the entry of lymphocytes into SLOs have been studied extensively; study of lymphocyte egress separately from entry has been particularly difficult due to continuous entry and exit of lymphocyte. Using a photoconvertible protein, Dendra2, we established an illumination-based in situ cell labeling system to study lymphocyte egress from Peyer's Patches (PPs) *in vivo*. We identified an effector/memory population of CD4^{+} T cells which stays inside PPs for at least 30 days without proliferation, making approximately 30% of the total CD4^{+} T cell pool in a PP. This 'resident' helper T cell population is a heterogenous population reflecting the overall composition of CD4^{+} T cells in a PP, including follicular helper T cells and regulatory T cells. These cells require TCR signaling to stay inside PPs as ovalbumin specific OT-II cells do not form this population unless the antigen is provided. We speculate that these 'resident' T cells recieve TCR signaling from microbiota and food antigens to stabilize the homeostatic immune interactions.

T.7. Postnatal Maturation Dynamics of the Enteric Mucosal Immune System

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The complex cellular architecture of the small intestinal immune system is required to allow discrimination between harmless food antigens, beneficial commensal microbiota members and pathogenic microorganisms and facilitate mucosal homeostasis. After birth the intestine undergoes a dramatic transition from a sterile to an increasingly colonized environment accompanied by significant changes in the cellular composition of the mucosal immune system. Here we evaluated the kinetics of the major immune cell populations (B and T lymphocytes, myeloid cells) during the postnatal period. Total small intestinal immune cells were isolated at different time points after birth and subjected to FACS analysis. Additionally, the impact of external and internal factors such as microbiota, mode of delivery, breast

feeding or innate immune mediators on the neonatal immune cell composition was investigated. Our results demonstrate the highly dynamic process of the cellular maturation during the immediate postnatal period. T and B cell populations appear in waves starting at birth with $TCRab^{+}CD4^{+}$ cells, followed by $CD19^{+}$ cells that peak during the second week of life and $CD8^{+}$ T cells that become the predominant fraction after weaning. Alterations in the immune maturation may contribute to the establishment of the microbiota and confer susceptibility to enteric infections.

T.8. Increased Gut Homing (β7) on Dendritic Cells in Fistulating Perianal Crohn's Disease

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Background: The aetiology of fistulating perianal Crohn's Disease (CD) remains unclear and the disease a challenge to treat due to poor healing and high recurrence. Dendritic cells (DC) play major roles in the pathogenesis of CD and express tissue-specific homing markers. Data on the characterisation of DC from CD fistulae are sparse. We aimed to determine alterations in homing marker expression on DC from Crohn's and idiopathic perianal fistulae. Methods: Biopsies were taken from anal fistula tracts of 17 Crohn's and 16 idiopathic patients. DC were characterised by flow cytometry and expression of skin-homing marker CLA, and gut-homing marker β7 was determined. Results: Percentage expression of β7 on DC was significantly higher in Crohn's compared with idiopathic perianal fistulae ($p<0.03$). There was no significant difference in CLA expression, which was low in both groups. When comparing the proportion of DC that are double positive for both β7 and CLA between the Crohn's and idiopathic groups, we found that these cells are significantly lower in Crohn's ($p<0.002$). Conclusions: Increased expression of β7 on DC of Crohn's perianal fistulae may lead to increased gut DC infiltrates contributing to impairment in healing. Anti-β7 therapies may aid healing when applied to perianal Crohn's fistulae.

T.9. Wiskott-Aldrich Syndrome Protein (WASp) Deficiency Results in Increased Stool IgA Associated with B Cell Sensitivity to TGFβ

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Background: WASp is a hematopoetic protein that activates actin polymerization in response to surface receptor signals. Mutations in WASp result in an immunodeficiency characterized by recurrent infections and autoimmunity. WASp-deficient humans and mice may develop colitis that is associated with defects in the number and function of Foxp3+ regulatory T cells (Tregs). Increased serum IgA has been reported in patients and mice with WASp-deficiency. In this study we explore the role of WASp in regulating mucosal IgA secretion. Methods/Results: Stool from WASp-deficient mice had marked increase in IgA measured by ELISA (WT=42 mcg/g $^{+/-}$ 14 versus WASp-deficient =1169 mcg/g $^{+/-}$ 697, $p=.02$). Tregs have been shown to be a major helper subset for IgA production. However, there were fewer FoxP3+ Tregs among CD4s measured by flow cytometry in the lamina propria of precolitic WASp-deficient mice (WT= $19\%^{+/-}4$, KO= $13\%^{+/-}1$, $p=.03$). This suggested a Treg-independent mechanism for increased IgA in these mice. Since TGFβ is a critical cytokine involved in IgA class switching, we investigated the response of splenic B cells to stimulation with 2 ng/mL of TGFβ. Flow cytometric analysis demonstrated increased levels of phosphorylated SMAD2/3 both at baseline and 20 minutes post-stimulation in WASp-deficient B cells compared to WT. Conclusions: Increased sensitivity to TGFβ in WASp-deficient B cells may drive IgA class switching and high levels of intestinal IgA in pre-colitic animals. These findings mirror increased intestinal and serum IgA reported in patients with inflammatory bowel disease (IBD) and raise the possibility that dysregulated IgA secretion contributes to the pathogenesis of IBD.

T.10. A Plant-Produced Cholera Toxin B Subunit Prevents Acute Colitis in a Mouse Model

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Background: The Cholera Toxin B subunit (CTB) has shown great utility in its ability to exhibit a strong mucosal immunomodulatory effect. We have produced CTB in Nicotiana benthamiana plants (pCTB),

which has significantly higher scalability than bacterial culture systems and thereby facilitates the protein's availability for broad pharmaceutical applications. Besides strong mucosal humoral immunogenicity, CTB has shown anti-inflammatory effects in several diseases. This led us to investigate if it could also protect against acute colitis induced by dextran sulfate sodium (DSS). Methods: C57BL/6J mice were orally administered 30 µg pCTB 2 weeks before and the day of initiation of DSS exposure. Mice were exposed to 4% DSS water ad libitum for eight days and allowed to recover for six days before sacrifice. Results: Body weights decreased in mice exposed to 4% DSS in drinking water. This weight loss was significantly blunted by pCTB pretreatment. Additionally, inflammation, histologically scored, in the colon was significantly decreased. Massons' Trichrome staining revealed decreased fibrosis in the pCTB-treated mice. Immunohistochemical analysis suggested a decreased T cell population in the inflamed tissue by pCTB pretreatment. Conclusion: Pretreatment with pCTB protected against DSS-induced acute colitis by preventing inflammation and aberrant tissue regeneration.

T.11. IL-23R^{+} Innate Lymhoid Cells Induce Colitis via an Interleukin-22 Dependent Mechanism

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Polymorphisms of IL-23R and signaling components are associated with several autoimmune diseases including inflammatory bowel diseases (IBD). Similar to Th17 lineage, Type 3 innate lymphoid cells (ILC) express RORγt and IL-23R, hence produce Th17 type cytokines, and are implicated in IBD; however, how IL-23R signaling in RORγt $^{+}$ILCs contributes to pathogenesis is unknown. IL-22, produced in copious amounts by Type 3 ILCs, was reported to have both beneficial and pathogenic effects in adaptive, yet only a pathogenic role in innate colitis models. Herein, by employing chronic CD45RBhigh CD4 T cell-transfer and anti-CD40 antibody-induced acute innate colitis models in Rag1$^{-/-}$ mice, we demonstrated opposite roles for IL-23R in colitogenesis: in the former a protective, and the latter a pathogenic role . Furthermore, we show that IL-23R signaling promotes innate colitis via IL-22 since neutralization of IL-22 protected mice from colitis and adding back of IL-22 to the IL-23R deficient animals restored the disease. Collectively, our results reveal that similar to its controversial role during chronic or adaptive colitis, IL-22 may also play opposite roles in innate colitis pathogenesis in a context and insult dependent manner.

T.12. Investigation of Anti-Inflammatory Effects of Carbon Monoxide (CO) Liberated from a Novel Water-Soluble CO-Releasing Molecule (CORM-3) on Trinitrobenzene Sulfonic Acid (TNBS)-Induced Colitis in Mice

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Background and Aims: Carbon monoxide (CO) has been shown to confer anti-inflammatory effects. However, the precise effects of CO on the function of CD4^{+} T cells remain unclear. Therefore, we assessed the effects of a novel water-soluble CO-releasing molecule (CORM-3) on trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice and measured the cytokine expressions in CD4^{+} T^{+}cells and differentiation of T helper cells. Materials and Methods: TNBS-induced colitis was produced in C57BL/6 male mice and CORM-3 was intraperitoneally administered. We measured colonic damage scores, tissue-associated myeloperoxidase (MPO) activity, and TNF-α, IFN-γ and IL-17A expressions in the colonic mucosa. CD4^{+} T cells isolated from the spleen were stimulated with/without CORM-3 and the production of TNF-α, IFN-γ and IL-17A was measured. Results: Mucosal damage scores were significantly inhibited in TNBS-induced mice treated with CORM-3. The increases of MPO activity and expressions of TNF-α, IFN-γ and IL-17A in TNBS-induced mice were significantly inhibited in CORM-3-treated mice. TNF-α, IFN-γ and IL-17A productions in CD4^{+} T cells were significantly decreased by CORM-3. Furthermore, the differentiation of Th1 and Th17 cells in naïve CD4^{+} T^{+}cells was inhibited in CORM-3-treated mice. Conclusion: These findings indicate that CO might be a new therapeutic molecule for inflammatory bowel disease.

T.13. The Effects of Change in the Intestinal Microbiota Through Antibiotics Administration on the Manifestion of DSS Colitis

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The role of intestinal microbiota in maintaining mucosal homeostasis cannot be more emphasized. IL-17A have been shown to have both pathogenic and protective roles in animal models of colitis. We investigated the effects of intestinal microbiota change through antibiotics administration on dextran sodium sulfate (DSS) colitis in IL-17A$^{-/-}$ mice. C57BL/6 wild-type (WT) and IL-17A$^{-/-}$ mice were assigned to different three groups: control group without treatment, antibiotics (vancomycin, ampicillin, neomycin and metronidazole) plus subsequent 1.7% DSS treated group and DSS treated group. Clinical activities including weight loss and histologic findings of colonic segments were examined. Proinflammatory cytokine levels were measured by ELISA in the supernatants of colonic tissue explants. To characterize the change of intestinal microbiota, high throughput sequencing for sequential feces is under way. Antibiotics treatment induced a transient weight loss in both WT and IL-17A$^{-/-}$ mice. WT mice developed more severe DSS colitis than IL-17A$^{-/-}$ mice. After antibiotics treatment, both mice displayed significantly enlarged ceca similar to germ-free mice. Antibiotics treatment attenuated DSS-induced increase of histologic inflammation and proinflammatory cytokine levels, especially in WT mice. IL-17A ablation decreases severity of colitis in DSS murine model. The change of intestinal microbiota through antibiotics administration affects the susceptibility of DSS colitis.

T.14. Tissue-Specific Overexpression of UPR-Related C/EBP Homologous Protein Impairs Epithelial Cell Restitution in Response to Experimental Colitis and Mechanical Injury

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Endoplasmic reticulum (ER) unfolded protein responses (UPR) in intestinal epithelial cells (IEC) have been implicated in chronic intestinal inflammation. In this study, we characterized the consequence of epithelial cell-specific UPR-related C/EBP homologous protein (CHOP) overexpression on intestinal homeostasis in response to bacterial-driven and Dextran Sodium Sulphate (DSS)-induced colitis, as well as mechanical injury. Chop$^{IEC\ Tg/Tg}$ mice show no spontaneous inflammatory phenotype, while expression profiling of primary colonic IEC identifies changes in the inflammatory gene expression program. Under conditions of acute colonic inflammation, transgenic mice are not affected by Citrobacter rodentium infection, but reveal increased susceptibility to DSS-induced colitis associated with delayed tissue regeneration. Although Chop is transcriptionally induced on mRNA level, CHOP protein is protectively down-regulated in wildtype mice three days after DSS administration. In contrast, Chop$^{IEC\ Tg/Tg}$ mice continue to express transgenic CHOP protein, which seems to be transcriptionally activated by MAPK P-p38-mediated phosphorylation that is induced in transgenic mice and controls, thus leading to a severe inflammatory phenotype. To further examine the impact of CHOP on epithelial cell restitution, Chop$^{IEC\ Tg/Tg}$ mice were mechanically injured demonstrating impaired wound healing. In conclusion, tissue-specific overexpression of CHOP protein aggravates experimental but not infectious inflammation associated with impaired epithelial cell restitution.

T.15. The Preventive Role of a Water-Soluble Extract from Ganoderma Lucidum Fungus Mycelia (Designated as MAK) on Indomethacin-Induced Small Intestinal Injury in Mice

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Introduction: We have previously reported that a water-soluble extract from the cultured medium of Ganoderma lucidum fungus mycelia (designated as MAK) has anti-inflammatory effects in murine colitis induced by trinitrobenzene sulphonic acid. The present study was designed to investigate the preventing effects of MAK on the indomethacin-induced ileitis. Methods: To assess the preventive role of dietary

MAK, the mice were sacrificed after 24 hours of indomethacin treatment and the intestinal inflammation was evaluated. Peritoneal macrophages (PMs) were taken from C57BL/6 mice and stimulated *in vitro* with MAK for 12 hours. The PMs (1×107/mouse) were adoptively transferred intra-peritoneally to other mice, which were then given indomethacin orally. After 24 hours, the mice were sacrificed and evaluated for small intestinal inflammation. Results: The number of ulcers induced by indomethacin was decreased by feeding with MAK in a dose-dependent manner. The injury was also significantly prevented in the transfer of PMs that had been stimulated *in vitro* with MAK. The transferred PMs were detected in mesenteric lymph node and the lamina propria of the small intestine, but not spleen. Conclusion: PMs stimulated by MAK accumulated into the small intestine, and may contribute to the anti-inflammatory response.

T.16. Molecular Analysis of the Gut Microbiota Following Therapeutic Treatment With a Monoclonal Anti-IL-12/23p40 Antibody in Experimental Colitis

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Molecular analyses have utility in discriminating between gut microbiota of altered compositions. This has been demonstrated in studies involving both experimental animals and humans with bowel inflammation. Although alterations in the composition of the microbiota can be demonstrated, future studies need to provide links between the microbiota composition and clinical signs of disease. In this study, we have evaluated the microbiota composition, following therapeutic treatment with a monoclonal anti-IL-12/23p40 antibody in a T cell transfer colitis model. The microbiota compositions in stool, ceceum and colon samples were evaluated by PCR followed by denaturing gradient gel electrophoresis. Colon and stool samples were found to correlate with central disease parameters (e.g. colon histopathology, CD3 density in colon, endoscopy score and colon W:L ratio). Moreover, a significant alteration in microbiota composition could be observed in colon samples when comparing mice treated with anti-IL-12/23p40 with mice treated with either isotype control or NaCl. Additionally, anti-IL-12/23p40 treated mice did not revert to the same microbiota composition observed in healthy control mice, although clinical disease parameters seemed to be normalized. These results suggest that microbiota composition, in both stool and colon could be used to evaluate clinical disease and efficacy of novel drug candidates.

T.17. Tauro-Ursodeoxycholic Acid Inhibits NF-κB Signaling in Intestinal Epithelial Cells, and Ameliorates Experimental Colitis and Colitis-Associated Colon Cancer in Mice

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Background: Tauro-ursodeoxycholic acid (TUDCA) exhibited anti-inflammatory and anti-cancer activity. However, little information is available on intestinal inflammation and colitis-associated colon cancer. Method: HCT 116 cells were pretreated with TUDCA and stimulated with tumor necrosis factor-α (TNF-α). Interleukin-8 (IL-8) expression was determined by real-time RT-PCR. IκB phosphorylation/degradation and intranuclear translocation of nuclear factor kappaB (NF-κB) was evaluated by western blotting. In the acute colitis model, mice were given 4% dextran sulfate sodium (DSS) for five days with or without TUDCA (50 mg/kg and 250 mg/kg per day). In the colitis-associated tumor model, mice were given a single intraperitoneal injection of azoxymethane, and then three cycles of 2% DSS for five days and two weeks of free water consumption. Results: TUDCA significantly inhibited IL-8 expression in HCT 116 cells stimulated with TNF-α. TUDCA inhibited TNF-α induced IκBα phosphorylation/degradation and NF-κB nuclear translocation. Administration of TUDCA significantly reduced the severity of DSS-induced murine colitis, as assessed by the disease activity index, colon length, and histopathology. Finally, TUDCA significantly reduced the development of colitic cancer in mice. Conclusion: Our results showed that TUDCA had anti-inflammatory effect both *in vivo* and *in vitro*; thus, TUDCA is a potential therapeutic agent for inflammatory bowel disease.

T.18. Colitogenic Th17 Cells may Help the Generation of Colitogenic RORγt^{-}Independent Classical Th1 Cells

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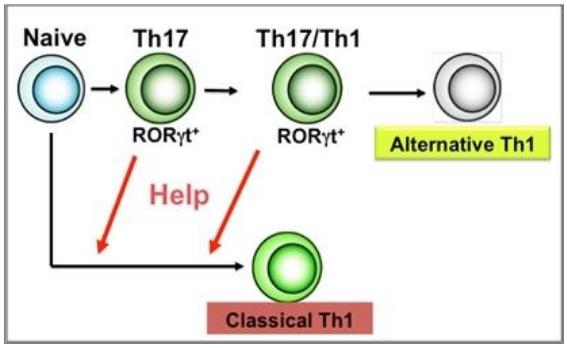
Background: Accumulating evidence suggests that both Th1 and Th17 cells are involved in the pathogenesis of chronic intestinal inflammation .We recently demonstrated that RORγt^{+}Th17 cells were progenitor cells for colitogenic alternative Th1 (aTh1) cells . However, it remains unknown how RORγt-independent classical Th1 (cTh1) cells are also involved in the pathogenesis of colitis. In this study, we focus on cTh1 cells in the development of T cell-dependent experimental colitis. Method: 1) RAG-2$^{-/-}$mice were transferred with CD4^{+}CD45RB^{high} T cells that were obtained from RORγt$^{-/-}$(RORγt-KO CD45RB^{high} T-RAG) or *in vitro*-manipulated RORγt-KO Th1 cells under Th1 polarized condition. (RORγt-KO Th1-RAG) 2) RAG-2$^{-/-}$mice were transferred with RORγt-KO CD4^{+}CD45RB^{high}T cells (Ly5.2) alone or co-transferred with wild type (WT) CD4^{+}CD45RB^{high} T cells (Ly5.1). Result: 1) RORγt-KO CD45RB^{high} T-RAG mice did not develop colitis, while RORγt-KO Th1-RAG mice developed colitis with the accumulation of Th1 cells in inflamed mucosa. 3) RAG-2$^{-/-}$mice co-transferred with WT and RORγt-KO CD4^{+}CD45RB^{high} T cells developed colitis with significant increase of Ly5.2^{+} RORγt-KO Th1 cells (cTh1) in inflamed mucosa as compared to non-colitic mice transferred with RORγt-KO CD4^{+}CD45RB^{high} T cells alone. Conclusion: colitogenic RORγt^{+} Th17 cells may function as helper cells for the development of colitogenic cTh1 cells.



T.19. Assessing the Role of CCR9 for T Cell Trafficking in Acute Small Intestinal Inflammation
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Under homeostatic conditions CCR9-deficient T cells are impaired in trafficking to the mucosa of the small intestine. Since massive infiltration of the intestine by pro-inflammatory T cells is the major underlying cause of inflammatory bowel diseases (IBDs), inhibition of CCR9-dependent T cell homing has been suggested as a potential treatment. To assess, whether CCR9 is required for the homing of effector T cells in acute small intestinal inflammation, we employed 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-1 cells) and adjuvant treatment. Interestingly, intestinal inflammation in IFABP-tOva mice could also be triggered following transfer of CCR9-deficient OT-1 cells. This suggests that CCR9 is not crucial for the homing of effector T cells, but that they employ alternative homing mechanisms in inflammation. In line with this, only a minority of IFNγ^{+} effector OT-1 cells actually expressed CCR9 on their cell surface. Instead, CCR9 was primarily expressed by IFNγ- OT-1 cells. Currently, we are addressing, how and why the CCR9 expression between the IFNγ^{+} and IFNγ- subsets is differentially regulated and how this influences the function of the respective T cell subsets.

T.20. Blockade of Death Receptor-3 Modulates Effector Function of CD4^{+} T Cells Isolated from Chronic Colitis Mouse Model
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TNF-like molecule (TL) 1a is a member of the TNF family of cytokines that is highly upregulated in the intestinal mucosa of Crohn's Disease (CD) patients and signals via the Death Receptor 3 (DR3) which costimulates T cell activation. Genome-wide association studies have implicated a role for TL1A in CD pathogenesis, which is highly supported by studies in animal models of inflammatory bowel diseases showing a reduction in pathology when neutralizing TL1A. In the current study we show by immunohistochemical staining of surgical resection material an increased number of DR3 immunopositive leukocytes in intestinal biopsies from CD patients when compared to healthy controls. Furthermore, we show that mesenteric lymph node CD4^{+} T cells, isolated from mice with chronic colitis, have increased proliferation and pro-inflammatory cytokine secretion when stimulated with recombinant TL1A in combination with anti-CD3. Finally, we show that blocking DR3 with an anti-DR3 fab fragment diminishes

both the proliferative and pro-inflammatory cytokine secretion from the activated $CD4^+$ T cells. These data suggests that TL1a/DR3 signalling could have an important role in driving inflammation in CD patients, and that blocking DR3 could be a novel therapeutic strategy.

T.21. Reversal of Murine Colitis and Fibrosis by Neutralizing TL1A Antibody: Potential Novel Therapy to Alter Natural History of Crohn's Disease

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Elevated expression of TL1A is found in inflamed gut mucosa, characterized by fibrostenotic disease and need for surgery in Crohn's Disease (CD) patients. Constitutive TL1A expression in mice led to proximal migration of colonic inflammation, relative rectal sparing of inflammation, ileitis and fibrostenosis under colitogenic conditions. In this study, using the adoptive T cell transfer chronic colitis model, treatment with neutralizing antibody to Tl1a mitigated murine colitis and reversed intestinal fibrosis back to original pre-inflamed levels. The anti-inflammatory and anti-fibrotic effect was associated with down-regulation of T helper (Th)-1 and Th-17 effector function and mediated by reversal of the fibrogenic program (reduced TGFβ and IGF1), leading to reduced numbers of fibroblasts and activated myofibroblasts. To assess the potential mechanism, we developed an *in vitro* primary fibroblast differentiation system and showed directly that DR3, the receptor for TL1A, is expressed on the activated myofibroblasts. Addition of TL1A to the primary fibroblasts increased collagen production, showing a direct effect of the TL1A/DR3 signaling pathway in the activation of the fibrogenic program. Modulation of TL1A/DR3 signaling may alter the natural history of Crohn's disease by treating both gut inflammation and fibrosis.

T.22. The Effect of Azithromycin, Metronidazole and Methylprednisolone on TNFα, TGFβ and TLR4 Expression in the Mucosa of Mice with Hapten-Induced *Experimental Colitis*

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Background: The aim of this study was to evaluate the effect of azithromycine, metronidazole and methylprednisolone on TNFα, TGFβ and TLR4 expression in colonic mucosa of mice with hapten-induced experimental colitis. Methods: *Experimental colitis* was induced in all the animals by receiving the challenge enema of 0.025 mL 0.2% DNFB solution in acetone and olive oil. Animals in study groups (10 per group) were treated with azithromycin, methylprednisolone and metronidazole. Mice in control group (n=10) received phosphate buffered saline (PBS), from day one to five, after induction of colitis. Immunohystochemical analysis (score 0-3) was performed for TNFα, TGFβ and TLR4 in the mucosa of all the animals. Results were considered significant at $P<0.05$. Results: Azithromycine and methylprednisolone significantly reduced mucosal expression of TNFα and TLR4, compared to mucosa of control groups of animals with experimental colitis, treated with PBS ($P<0.05$) and there was no difference. The expression of TNFα and TLR4 in mucosa of mice treated with metronidazole did not differ significantly to expression in control group. Expression of TGFβ was significantly increased in mucosa of mice treated with azithromycin, metronidazole and methylprednisolone, compared to control group ($P<0.05$). Conclusion: Azythromicine, an antibiotic with acceptable side-effects is capable of reducing the expression of proinflammatory markers in inflamed mucosa, pointing out to possible clinical implications, especially considering TLR4 as interesting target for treatment of intestinal inflammation.

T.23. Genetic Licensing Uncovers a Role for NK Cells in Immune Colitis by Co-Activation of $CD4^+$ T Cells

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While NK cells are mainly known for their cytolytic function, recent work in humans has uncovered diverse cytokine production states programmed by genetic licensing (differentiation induced by interaction of inhibitory NK receptor with their cognate MHC class 1 ligands). In humans, genetic evidence suggests that licensing is a risk factor for Crohn's Disease. However, limited and discrepant studies exist on the

relationship of NK cells to immune colitis. To biologically test this genetic association, this study evaluates the interaction between licensed NK cells and $CD4^+$ T cells using a mouse model of NK cell licensing. As in humans, licensed mouse NK cells co-cultured with $CD4^+$ T cells strongly augmented $CD4^+$ T cell proliferation. Both soluble and cell-cell interaction modes of co-stimulation was observed in $CD4^+$ T cells (unlike humans in which cytokine interactions predominate). Neutralizing antibody interrogation and model cytokine add-back experiments will be reported to identify the NK-T interaction molecules. Conclusion: Our results demonstrate that licensed NK cells augment the threshold of $CD4^+$ T cell activation in a manner that may biologically account for the genetic association of NK licensing to Crohn's Disease.

T.24. Tristetraprolin-Mediated mRNA Decay in Colitis and Colitis-Associated Cancer

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Intestinal homeostasis is achieved through a complex interplay between intestinal cells and commensal bacteria. Although proinflammatory gene expression is needed for host protection, prolonged and dysbalanced inflammation may lead to inflammatory bowel diseases (IBDs), including ulcerative colitis. This autoimmune disease enhances the risk to develop colitis-associated cancer (CAC). Stability and decay of mRNAs are fundamental parameters involved in inflammatory gene regulation. Many proinflammatory mRNAs contain AU-rich elements in their 3' untranslated regions interacting with RNA-binding proteins (RBPs). Tristetraprolin (TTP) a well characterized RBPs, exerting destabilizing effects via recruitment of mRNA degrading enzymes. We recently described that TTP targets 1/3 of unstable inflammation-induced mRNAs for degradation in macrophages. Therefore, TTP appears to be a key factor controlling immune homeostasis and aberrant expression of TTP is associated with different types of cancer. To decipher cell-specific roles for TTP in mucosal immunity we employed models of experimental colitis and CAC using mice with conditional TTP-deletion in myeloid and T cell subsets. *In vivo* endoscopic analysis revealed that TTP-mediated mRNA decay is fundamentally involved in the control of CAC development. The contribution of TTP-expression in the individual subsets (e.g. T cells, macrophages etc.) to the CAC development is currently scruntinized using conditional knockout mice.

T.25. Role of the Commensal Microbiota in Calibrating the Responsiveness of Systemic Innate Immune Cell Populations

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Natural killer (NK) cells are innate immune components that are required for efficient elimination of viral infections and tumor cells. We observed in our studies in germ-free (GF) mice that activation of NK cells at non-mucosal sites after injection of Toll-like receptor (TLR) agonists was strongly reduced in GF mice compared to conventional mice hold under specific pathogen-free (SPF) conditions. Adoptive transfer studies showed that NK cells isolated from GF mice were fully functional when transferred into SPF mice while NK cells from SPF mice lost their responsiveness in a GF environment. Thus, the observed defect in NK cell activation is not an NK cell-intrinsic defect but rather a consequence of the GF environment. We found that mononuclear phagocytes in GF mice were unable to produce pro-inflammatory cytokines including Type 1 IFNs in response to TLR ligands and were therefore impaired in their ability to prime NK cells. While signaling downstream of various pattern recognition receptors was normal in mononuclear phagocytes, binding of RNA polymerase II to the respective cytokine promoters was impaired. When measuring the presence of the permissive histone mark H3K4me3 at the promoters of pro-inflammatory genes, we found significantly reduced levels in DCs from GF mice. Our data reveal a previously unrecognized role of the postnatally colonizing microbiota to introduce chromatin level changes in the mononuclear phagocyte system which poises expression of central inflammatory genes to initiate a powerful systemic immune response required for immunity to viral infections. Our current goal is to uncover the exact mechanisms underlying the microbiota-induced alterations in chromatin structure.

T.26. Role of Retinoic Acid in CD4$^+$ T Cell Mediated Experimental Colitis
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Epidemiological studies of vitamin A-deficient populations have illustrated the importance of vitamin A and its metabolite all-trans retinoic acid (RA) in mucosal immune responses. In support of these findings, we and others have demonstrated a requirement for RA in the generation of gut-tropic T cells *in vivo* and in promoting IgA-secreting plasma cells differentiation *in vitro* (Iwata M. 2004; Svensson M. 2008; Mora JR. 2008). Evidence from the literature has also suggested various roles for RA in regulating mucosal T cell differentiation (Uematsu S. 2008; Mucida D. 2007; Coombes JL. 2007; Sun CM. 2007). Thus under homeostatic conditions RA has been proposed to have anti-inflammatory functions, by promoting the development of Foxp3$^+$ regulatory T cells (Tregs) in the periphery while restraining Th17 effector T cell differentiation. In contrast, RA was recently suggested to have a pro-inflammatory function in the gut upon pathogen exposure, promoting Th1/Th17 effector T cell responses (Hall JA. 2011). What role, if any, RA plays in the setting of intestinal inflammation however remains to be determined. Here using the T cell transfer model of colitis we have assessed the importance of RA signaling in CD4$^+$T cells in the development of intestinal inflammation. Naïve CD45RBhi CD4$^+$ T cells with a disruption of RA signaling were transferred into RAG1$^{-/-}$ mice and their proliferation, effector functions and migration potential were assessed, parallel to the monitoring of colitis development. An interesting feature of this model is that colitis development can be prevented by co-transferring regulatory (CD45RBlo) T cells together with the naïve T cells, allowing assessment of the direct contribution of RA specifically in Tregs or effector T cell development. Results from these ongoing experiments will be presented.

T.27. Investigating the Role of NLRC3 in T Cell-Mediated Immunity
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The NLR family consists of 22 and 34 members in human and mice, respectively. They play important roles in recognizing intracellular bacteria, promoting inflammatory responses, assembling the inflammasome complex, autophagy and metabolic stress. NLRC3 has been found to be highly expressed in T cells as opposed to other NLRs, which are mainly expressed in myeloid cells. In the present study, we investigated T cell-mediated immunity in NLRC3 deficient mice. We first characterized the kinetics of NLRC3 expression. Our data show that NLRC3 is downregulated within three hours of T cell activation and was linked to Ca^{++} influx. The development of EAE upon immunization with MOG/CFA appeared to be delayed in NLRC3$^{-/-}$ mice but the recovery phase of the disease was similar between NLRC3 KO and WT mice. Higher percentages of IFNγ$^+$ T cells were found in the brain of NLRC3$^{-/-}$ mice. In a T cell-transfer colitis model, we observed lower pathological scores in the colon of hosts reconstituted with NLRC3KO T cells. In conclusion, our data suggest that NLRC3 might play an important role in fine tuning T cell immune responses, particularly, in chronic immune responses such as colitis.

T.28. Animal Model in Testing of the Effect of Edible Plants' Extracts
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BALB/c mice were fed orally (15 mg/200µl/day) for two weeks with plant extracts of Nettle, Dill, Kale, Persimmon, Pomegranate, and Sideritis. *Lactobacilli* in mice gut content were not affected by Dill and Sideritis; stimulated by Pomegranate on day three, inhibited by Kale on days 3-14 days and by Persimmon on day seven; after wash-out period was no statistical differences observed. *Klebsiella pneumoniae* and *Enterobacter cloacae* were eliminated by feeding of extracts of Sideritis, Dill, Nettle, and Persimmon but not of Pomegranate and Kale. *Enterococcus faecium* and *E. faecalis* was stimulated significantly by Pomegranate. Bifidobacterium bifidum and *B. longum* were increased in colon of mice fed with extracts of Sideritis, Pomegranate and Kale. Interestingly, commensal *E. coli* was not affected by any of tested plants' extracts. All plants' extracts promoted of *Candida albicans* persistence in mice colon; only Kale had lowering its amount on 7-14 days, but not on 21-28 days and after wash-out period. Plants' extracts differently regulated IgA secretion by splenic and peritoneal cavity B1/B2 cells; changed the ratio

of CD45/CD45 RB, T4/T8/NK and $CD4^+CD25^+$ Treg cells in mesenteric lymph nodes, Payer patches and spleen. Cytokines produced locally were plant-specific.

T.29. IL-25 Alters Gut Microbiota and Limits Experimental Colitis

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Recent investigations reveal that IL-25 expression is downregulated in inflammatory bowel disease (IBD), and suggest that this IL-25 deficiency may result in further amplification of the inflammatory pathways in IBD. These data strongly suggest an important role for IL-25 in the regulation of intestinal inflammation in IBD. We examined the susceptibility of $IL\text{-}25^{-/-}$ and wild-type (WT) mice in a dextran sulfate sodium (DSS) model of intestinal inflammation. $IL\text{-}25^{-/-}$ mice exhibited increased susceptibility to DSS-induced intestinal injury, displaying significantly increased pathology. Of note, this increased susceptibility was transmissible to WT mice following cohousing with $IL\text{-}25^{-/-}$ mice. To obtain evidence that colitogenic gut microbiota contributes to the increased severity of DSS-induced colitis in the absence of IL-25, $IL\text{-}25^{-/-}$ and WT mice were treated with antibiotics prior to DSS administration. Taken together, these data suggest a role for IL-25 in preventing gut dysbiosis that contributes to IBD. Here, we examine how this results in gut inflammation, which will provide critical immunological targets for IBD therapy.

T.30. Impact of Chronic Salmonella Typhimurium ΔaroA Infection on Colitis Recovery and Microbiota in Nod-Deficient Mice

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Inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn's disease, are characterized by chronic intestinal inflammation and an altered microbiota, shaped by both genetic and environmental factors. To gain insights into these interacting elements, we induced chronic colitis in C57/Bl6 mice by infecting (10^8 CFUs by gavage) untreated and streptomycin-pretreated heterozygous and KO littermates of Nod1 and Nod2, as well as DKO, mouse strains with Salmonella Typhimurium ΔaroA. Mice were weighed on days 0, 3, 6, 14, 20, 27, 34, 41 and 49 and fecal samples were collected for bacterial analysis. After 7 weeks, intestinal tissues and contents were sampled to assess inflammatory status and bacterial community structure. Our preliminary results show that colitis-induced weight loss was delayed in streptomycin-pretreated DKO compared to single KOs and Hets, but that all mice began to gain weight by day 10. Long-term (seven weeks) recovery from infection was also slower in the DKO mice, which exhibited a delayed Th1 response with increased IFNγ expression in the cecum. Ongoing bacterial analysis will track changes in bacterial communities correlated with the development and resolution of inflammation in order to reveal aspects of the microbial disruption that might be related to IBD.

T.31. Ramelteon, a Melatonin Receptor Agonist, Ameliorates Trinitrobenzene Sulfonic Acid (TNBS)-Induced Colitis in Mice

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Background: Melatonin, which is a hormone produced by the pineal gland, is secreted in a circadian rhythm. Recently it has been shown that melatonin could have as regulators of inflammation in the gastrointestinal tract, and plays an important role as an antioxidant as well as a player in proper immune system. Ramelteon is a synthetic analog of melatonin that acts specifically on melatonin receptor 1A (MT1) and 1B (MT2), but the effect of ramelteon in intestinal inflammation remains unclear. The aim of this study was to investigate the effect of ramelteon on trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice. Materials and Methods: Acute colitis was induced with TNBS in male C57BL/6 mice. Ramelteon (30mg/kg) was intraperitoneally administered daily. The colonic inflammation was evaluated macroscopically, histologically, and biochemically. Results: MT1 expression was mainly localized in lymphoid cells, and was up-regulated in inflamed colonic mucosa. TNBS administration resulted increases in colonic damage score and neutrophil accumulation of the intestine, and pro-inflammatory

cytokines (TNF-α, IFN-γ) and chemokines (KC). In contrast, the treatment with ramelteon prevented these changes. Conclusion: Our results showed that ramelteon reduced the intestinal inflammation, and indicating that melatonin receptor may be a novel therapeutic target in the intestinal inflammation.

T.32. Deletion of the Pathogen Recognition Receptor Nod2 Affects the Resolution of Intestinal Inflammation in a Murine Model of T Cell Activation

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Crohn's Disease (CD) is characterized by activation of T cells which contributes to chronic intestinal inflammation. Studies have shown that Nod2 is functionally active in human T cells and modulates the immune response in murine models of intestinal infection and colitis. Given that mutations in NOD2 gene are linked to susceptibility to CD, we investigated whether absence of Nod2 alters T cell function *in vitro* and *in vivo*. Our *in vitro* data showed that Nod2 is expressed in murine $CD4^+$ T cells including both effector and regulatory subsets and this expression was inducible by stimulation with anti-CD3 and anti-CD28 monoclonal antibodies. T cell stimulation with the Nod2 ligand MDP induced nuclear cRel translocation, although it didn't modulate cytokine expression. *In vivo* T cell activation with anti-CD3 monoclonal antibody in $NOD2^{-/-}$ mice led to enteropathy equivalent to that seen in WT mice; however these mice had a delayed healing characterized by a decrease in recovery of villus height. Our data indicate that Nod2 is functional in murine $CD4^+$ T cells and although its expression does not alter T cell function, Nod2 appears important for the resolution of T cell induced intestinal inflammation.

T.33. Regulation of Colonic Inflammation by Commensal Flora in SAMP1/YitFc Mice

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We hypothesized that Tregs in germ free (GF) SAMP mice may be generated and enhanced by commensal bacteria, and that Tregs play a role in regulating the development of colonic inflammation. To test our hypothesis, we administered dextran sodium sulfate (DSS) to GF and SPF mice, as well as GF mice who were treated with oral fecal suspensions from SPF (colonized-GF mice). Following administration of 3.5% DSS for eight days, colonized-GF mice significantly lost weight and showed severe clinical symptoms by day five. However, they rapidly recovered, and by day eight there was no significant difference in clinical symptoms and histological scores (SPF = 12.6, colonized-GF = 12.6). In a separate group of experiments, administration of 4% DSS for five days to colonized-GF mice significantly increased histological scores (SPF = 11.83, colonized-GF = 14.40). In MLN cells of colonized-GF, a significant increase in $CD4^+CD25^+Foxp3^+$ cell subsets was detected on day eight compared to day five post-colitis induction. Immune responses generated by bacterial colonization rapidly induced Tregs in the periphery, with down regulation of DSS-induced colitis. Our study demonstrates that commensal flora is necessary for the normal development of Treg function and the regulation of DSS-induced colitis in SAMP.

T.34. High-Sucrose Purified Diet Supplemented with Fructo-Oligosaccharides and Isomalto-Oligosaccharides Failed to Reduce Inflammation in HLA-B27 Transgenic Rats

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Composition of cecal microbiota and inflammation development were studied after feeding purified AIN-76A or standard rat chow diets supplemented with or without fructo-oligosaccharides (FOS) or isomalto-oligosaccharides (IMO) in HLA-B27 transgenic rats, a validated model of inflammatory bowel disease. Effects of fibres and diets on intestinal inflammation were evaluated by histology and mucosal IL-1β. The results revealed that both FOS and IMO failed to reduce colitis in the presence of AIN-76A compared to transgenic rats fed chow diet. Quantification of dominant bacterial groups showed that copy numbers of bifidobacteria and Enterobacteriaceae were stimulated by FOS versus control and IMO treatment regardless of the background diet. Chow diet rather than fibre treatments, mediated a significant increase of clostridial clusters XI and XIVa, and butyrate-kinase compared to animals on AIN-76A. Higher

concentration of total short-chain fatty acids (SCFA) were observed in cecal contents of rats on chow diet compared to the purified diet. AIN-76A increased the relative proportions of propionate and branched-SCFA irrespectively the oligosaccharide treatment. In conclusion, the SCFA composition, particularly the relative concentration of branched-chain fatty acids, was highly correlated to inflammation. Although diet modifies cecal microbiota, our study indicates that these parameters are not associated with colitis reduction.

T.35. Effector and Regulatory T Cell Subsets Deficient in NOD1 or NOD2 Retain the Ability to Induce and Prevent Colitis

Ashleigh Goethel, Katharina Forster, Galliano Zanello, Dana Philpott, Kenneth Croitoru. University of Toronto, Toronto, ON, Canada

Crohn's disease (CD) is a multifactorial disease, involving genetic mutations, dysbiosis of the gut microbiome and environmental triggers. The strongest genetic association is with NOD2, a pattern recognition receptor that recognizes a component of the bacterial cell wall. Although NOD2 is expressed in T cells, the functional significance is still unknown. Given that T cells are important in the induction and regulation of intestinal inflammation, we aimed to determine if T cell subsets lacking NOD1 or NOD2 retain their ability to function in a T cell transfer model of colitis. Effector and regulatory T cell subsets were isolated from WT (B/6), NOD1 and NOD2 deficient mice, as well as mice deficient for both NOD1 and NOD2. These subsets were transferred into $RAG^{-/-}$ recipients and monitored for colitis development. NOD2 and NOD1 deficient effector T cells induced colitis to the same degree as WT effector T cells. NOD deficient regulatory T cells were also effective in preventing colitis. Additionally, feeding OVA induced development of $Foxp3^{+}CD25^{+}$ regulatory T cells in NOD deficient OT-II mice. These data suggest that NOD deficient T cell subsets are functionally normal in the context of colitis and oral tolerance. Funded by CCFC, CIHR, CAG and Mount Sinai Department of Medicine.

T.37. Complete Mucosal Healing Is Associated With Long-Term Remission In Ulcerative Colitis

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Introduction: Recently, mucosal healing (MH) has been addressed in the treatment of ulcerative colitis (UC). To explore the association of degree of MH with long-term clinical outcome, we performed a retrospective cohort study. Methods: Among 724 UC patients who underwent colonoscopy, 331 patients in clinical remission were enrolled. Demographics, clinical data, endoscopic activity, histological activity and clinical outcome were collected. Endoscopic activity was graded according to Mayo endoscopic score. Association between variables and clinical relapse was evaluated. Results: For endoscopic activity, 176 patients (53.2%) were Mayo score 0, 111 (33.5%) were score 1 and 44 (13.3%) were score 2. During follow-up period, 69 patients (20.8%) had clinical relapse. Patients with Mayo score 0 had significantly less clinical relapse (11.4%) compared with those with endoscopic score 1 (29.7%) or score 2 (36.4%) ($p<0.001$). Cox regression analysis demonstrated that endoscopic activity was significantly associated with clinical relapse. Hazard ratio of score 1 was 2.04 to score 0 ($p=0.021$). The Kaplan-Meier estimate of non-relapse rate in Mayo score 0 was significantly higher than that in score 1 or 2. Conclusion: The present study demonstrated that endoscopic remission (Mayo score 0) was associated with long-term clinical remission in UC.

T.38. TCRβ CDR3 Region Diversity in Crohn's Disease: Disease-Associated Sequences and Biomarker of Disease Activity

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Crohn's Disease (CD) patients share B and T cell reactivity to the same immunodominant microbial antigens. We hypothesized that TCR repertoire diversity reflects CD disease activity while containing shared disease-specific CDR3 sequences. RNA was isolated from peripheral CD4 T cells of CD (n=37)

and ulcerative colitis (n=18) patients and healthy controls (n=100); the CDR3 TCRβ chain region was PCR-amplified with immunorepertoire primer sets for Illumina-based Next-Gen sequencing (mean unique sequence number >0.8x10^6/sample). TCR diversity was measured by a validated summary statistic, D50. Active CD (n=18) had significantly less TCRβ CDR3 diversity than inactive disease (n=19), D50 14.9±4.4vs21.3±4.8 p<0.001. Of >26,000 unique TCRβ CDR3 amino acid sequences present at ≥100 copies, only 57 were present in ≥73-100% of CD and ≥71-100% of UC patients (24 sequences were common to both). Three CDR3 sequences were significantly associated with CD itself (73-82%vs 14-29%UC and 5-22%HC,p<0.05). One CDR3 sequence shared by 86% of UC was present in 45% of CD and 9% of HC. These data suggest that TCR diversity measures may be a biomarker of CD disease activity and that highly shared CDR3 sequences might reflect the effects of disease-specific immunodominant antigens and genetic influences on disease susceptibility.

T.39. Combined Genome-Wide DNA Methylation and mRNA Expression Profiling Defines a Crohn's Disease (CD) Patient Population with Predilection for Early Surgical Intervention

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Background: Dysregulated mucosal immune response to environmental factors in genetically susceptible individuals can trigger IBD. Although GWAS identified IBD-risk regions, epigenetic remodeling additionally modulates disease. In IBD, differential IFNG methylation correlated with enhanced IFN-γ secretion and seroreactivity to microbial antigens. Aim: Determine if genome-wide methylation and RNA expression in IBD identify disease biomarkers. Methods: Matched $CD3^+$ LP and PB T cells from 12 CD, 11 UC or eight normal donors were analyzed for differentially methylated regions (DMRs) and mRNA expression. Patients were stratified based on years from diagnosis to surgery. Results: DMRs comparing LP to PB T across multiple loci identified inflammatory/adaptive immune response genes. The percentage of DMRs is increased in IBD (UC 58%, CD 57%) vs. normal (48%, p<0.001). CD patients requiring early surgical intervention displayed distinct methylation and mRNA expression. In CD, but not UC or normal, a greater percentage of DMRs mapped within IBD-risk GWAS (62%) vs. non-GWAS loci (57%, p<0.001). Molecular pathways involving antigen presentation, cytokines and transcriptional regulators of differentially methylated and expressed genes were upstream and downstream of IBD-GWAS loci. Conclusion: These data suggest that molecular epigenetic and expression patterns may stratify CD patients into subgroups with a different natural course of disease.

T.40. Human Intestinal $Vδ2^+$ T cells Promote Mucosal Inflammation in Crohn's Disease and are Selectively Ablated by Azathioprine Therapy

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Human $Vγ9Vδ2^+$ (Vδ2) T cells are uniquely responsive to microbial phosphoantigens and can enhance IFNγ production by conventional colonic T cells, so we hypothesized that Vδ2T cells promote mucosal inflammation in Crohn's disease (CD). Cell suspensions prepared from peripheral blood and intestinal biopsies were stimulated with HDMAPP phosphoantigen and analyzed by flow-cytometry. Circulating Vδ2T cells in CD patients contained an elevated proportion of $β7^+$ 'gut-homing' cells (CD median 80.8%, controls 64.9%; p=0.010), and increased $CD69^+$ cells (p=0.006) that correlated with reduced Vδ2T cell frequency (ρ-0.661; p=0.0018), suggesting increased Vδ2T cell activation/trafficking to the gut. Blood Vδ2T cells did not express integrin CD103, but intestinal biopsy-derived Vδ2T cells from CD patients contained both $CD103^+$ and CD103- subsets that produced IFNγ and TNFα. Activated blood Vδ2T cells up-regulated CD103 when exposed to TGF-β1, suggesting that TGF regulates Vδ2T cell mucosal phenotype. There was an extensive, selective loss of Vδ2T cells in blood from CD patients receiving azathioprine therapy (p<0.001), and azathioprine impaired proliferation and cytokine production by intestinal Vδ2T cells *in vitro*. These data demonstrate that human intestinal Vδ2T cells exert pro-inflammatory effects in CD that are ablated by azathioprine therapy, which may help to resolve intestinal

inflammation, but could also confer increased risk of malignancy due to loss of tumor surveillance by Vδ2T cells in blood.

T.41. Dissecting the Role of Intestinal Epithelium in the Pathogenesis of Inflammatory Bowel Diseases

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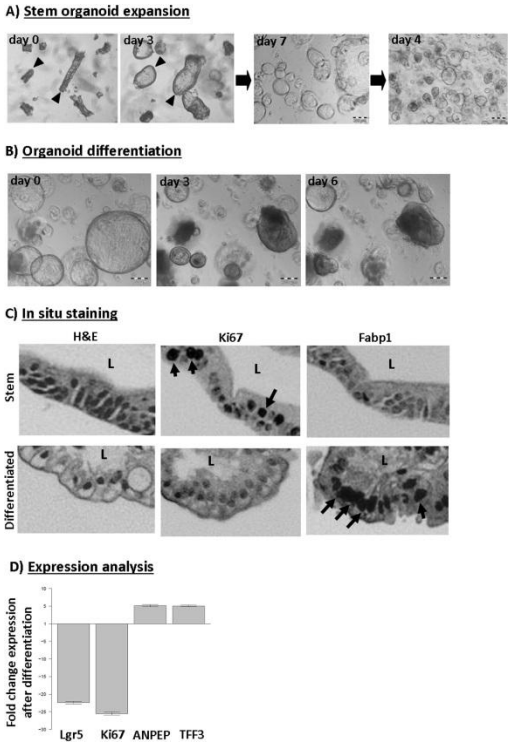

Crohn's Disease (CD) and ulcerative colitis (UC) are chronic inflammatory bowel diseases (IBD) of unknown aetiology. Alterations in the intestinal mucosal barrier contribute to the onset of these diseases; however, little data is available about the primary role of epithelial cell (EC) dysfunction in human IBD. Recently, a novel intestinal stem cell culture system has been established to study gastrointestinal EC physiopathology. By this approach we aim to investigate if a primary defect in intestinal EC function may drive the development of inflammation and associated complications in colonic CD and UC. To this end, biopsies from the sigmoid colon of IBD patients and non IBD controls are being collected. Isolated crypts are cultured and RNA from expanded stem and differentiated organoids is extracted for transcriptional analysis. Our results so far demonstrate that a stem cell signature is maintained in colonic organoids from healthy and diseased individuals and that a differentiation program can be induced in both groups. We show that the organoid culture system can be employed to expand stem cells and generate EC organoids from IBD patients. Our results can contribute to define the potential of stem cell-based therapy in the treatment of intestinal inflammation.

T.42. *In vitro* and *in vivo* Epithelial Repair Activities of M0, M1 and M2 Macrophages

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Macrophages play a critical role in wound repair. However, the specific role of the different macrophage subtypes in wound repair remains incompletely understood. The aim of this study was to compare wound repair activities of undifferentiated macrophages (M0), as well as of classically activated (M1) and alternatively activated (M2) macrophages. Repair activities of intestinal wound were evaluated using *in vitro* and *in vivo* models. All three macrophage subtypes enhanced wound closure *in vitro*, M2 macrophages demonstrating higher repair activities than M0 and M1 macrophages. Injection of M0 and M2 macrophages into mice with experimental DSS colitis significantly enhanced ulcer repair when compared to control mice. In contrast, injection of M1 macrophages did not affect ulcer repair. These results yield new insights into the wound repair capacity of different macrophage subsets. Notably, wound repair activity is not restricted to M2 macrophages, as suggested in the current literature.

T.43. Counter-Regulation of Eotaxin-3/CCL26 Expression by Th1 and Th2 Cytokines in Human Colonic Myofibroblasts

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Backgrounds: Eotaxins induce the trafficking of eosinophils to the sites of inflammation via CC chemokine receptor 3 (CCR3). In this study, we investigated eotaxin-3/CCL26 expression in the inflamed mucosa of patients with inflammatory bowel disease (IBD), and characterized the molecular mechanisms

responsible for eotaxin-3 expression in human colonic myofibroblasts. Methods: Eotaxin-3 mRNA and protein expression was evaluated by real time-PCR and ELISA, respectively. Results: Eotaxin-3 mRNA expression was significantly elevated in the active lesions of ulcerative colitis (UC) and Crohn's Disease (CD). In colonic myofibroblasts, Interleukin (IL)-4 and IL-13 significantly induced eotaxin-3 mRNA and protein expression. But Eotaxin-3 expression in intestinal epithelial cell lines (HT-29 and Caco-2 cell) was minimal even under the stimulation of IL-4 and IL-13. There was a significant positive correlation between mucosal eotaxin-3 and IL-4 mRNA expression in the active lesion of IBD mucosa. The IL-4 and IL-13-induced Eotaxin-3 mRNA expression was regulated by the STAT6 and SOCS1-mediated pathways. IFN-γ acts as a negative regulator on IL-4 and IL-13 induced Eotaxin-3 expression via STAT1 activation. Conclusions: Eotaxin-3 expression was specifically elevated in the active lesions of IBD, in particular UC. Eotaxin-3 derived from colonic myofibroblasts may play an important role in the pathophysiology of UC.

T.44. Evaluation and Quantification of Faecalibacterium Prausnitzii in the Gut Microbiota of Crohn's Disease

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Background and Aims: Dysbiosis is thought to be relevant to the etiology and pathogenesis of Crohn's Disease (CD). In this study, we investigated the abundance of *Faecalibacterium prausnitzii* in the gut microbiota of Japanese CD patients. Methods: 47 CD patients and 20 healthy controls were enrolled. Abundance of *F. prausnitzii* in fecal samples was quantified by real-time polymerase chain reaction. The gut microbiota profile was evaluated by T-RFLP analyses. Results: The abundance of F. prausnitzii decreased in CD patients compared with healthy subjects. Among CD patients, the Crohn's Disease Activity Index, C-reactive protein levels, and erythrocyte sedimentation rate were significantly lower, and serum albumin levels were significantly higher in the high *F. prausnitzii* group compared with the low group (divided by Tukey's test). T-RFLP analysis showed that fecal bacterial communities of CD patients differed from those of healthy individuals. The changes in simulated bacterial composition indicated that class Clostridia, including *genus Faecalibacterium*, was less abundant in CD patients as compared with healthy individuals. Conclusion: The decreased abundance of class Clostridia, including *F. prausnitzii*, may translate into a reduction of commensal bacteria-mediated, anti-inflammatory activities in the mucosa, which are relevant to the pathophysiology of CD.

T.45. *Citrobacter rodentium* Uses Pic, a Mucinase, to Subvert Mucosal Host Defenses in the Mammalian GI Tract

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Most enteric bacterial pathogens, including enteropathogenic *E. coli* (EPEC) and enterohemorrhagic E. coli (EHEC) ultimately target the intestinal epithelium, however to do so, they must interact with the overlying mucus layer. We investigated how *Citrobacter rodentium*, a mouse pathogen that mimics EPEC and EHEC interacts with intestinal mucus. We found that *C. rodentium* carries a homologue of Pic, a mucinase that has been implicated in bacterial virulence, and has also been proposed to have mucus secretagogue actions, i.e. increasing mucin release. To investigate the role of Pic in *C. rodentium* pathogenesis, we generated a Δpic C. rodentium strain and infected C57BL/6 mice. While wild-type *C. rodentium* infection caused no mortality, Δpic infected mice suffered upto 50 % mortality. Bacterial plating demonstrated 10-100 fold greater *C. rodendium* burdens in the Δpic infected mice, along with deeper penetration of bacteria into colonic crypts. We also noted epithelial adherent biofilms made up of *C. rodentium* and commensal microbes in the Δpic infected mice, along with impaired mucin secretion. Taken together, our data suggest that that Pic drives mucin secretion during infection which appears to help remove competing commensal microbes, and limit pathogen invasion of crypts to ensure host survival during *C. rodentium* infection.

T.46. Intestinal Fibroblasts in Crohn's Mucosa Condition Dendritic Cells to Drive the Generation of Inflammatory CD4$^+$ T Cells in Response to Enteric Bacteria

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Mucosal inflammation in Crohn's Disease results, in part, from a compromised intestinal epithelium that permits microbe penetration into the lamina propria and interaction with sub-epithelial cells. To elucidate the contribution of stromal cells to these processes, we show that isolated Crohn's intestinal fibroblasts exposed to Crohn's-associated bacteria (LF82) or bacterial products (CBir1 flagellin), released substantially more TGF-β and IL-6 ($p<0.001$) and more potently drove dendritic cell (DC) activation (↑CD86 and ↑HLA-DR), maturation (↑CD83) and pro-inflammatory activity (↑IL-23 release) compared to normal intestinal fibroblasts. We then showed that Crohn's intestinal fibroblast-conditioned DCs more strongly promoted CD4$^+$ T cell proliferation, IL-17 release and gut homing receptor CCR9 expression than DCs conditioned by normal intestinal fibroblasts. Together, these findings indicate that intestinal fibroblasts condition mucosal DCs, which after migration to the mesenteric lymph nodes, drive CD4$^+$ T cell differentiation and recruitment to the lamina propria and importantly that fibroblasts in Crohn's disease intestinal mucosa are more potent in performing these activities. Thus, intestinal fibroblasts regulate the function of immune cells in the intestinal lamina propria well beyond the previously recognized role of the fibroblasts and suggest a new target cell for the therapeutic interdiction of inflammatory processes in Crohn's Disease.

T.47. T Cell Receptor Repertoire Deep Sequencing Reveals Clonal Diversity and Overlap in Colonic Effector T Cells in Ulcerative Colitis

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We used TCR deep sequencing to identify the clonal diversity and overlap of phenotypically distinct T cell populations in anatomically distinct locations in ulcerative colitis (UC). When isolated from biopsies spatially separated along the colon of a UC patient, considerable overlap in TCR repertoire was seen among gamma delta T cells and α beta CD8$^+$ T cells. In contrast, a minority of colonic α beta CD4$^+$ T cells from different biopsies shared TCR sequences, indicating that CD4$^+$ clones are heterogeneously spread throughout the colon. When the surgically resected colons of UC patients were homogenized to eliminate this variability, more clonal overlap of CD4$^+$ T cells was seen between Inflamed and uninflamed colon segments among effector and Foxp3$^+$ regulatory T cells than among CD38$^+$ (activated) T cells. Likewise, less overlap was seen between mucosa and lymph nodes, demonstrating anatomic compartmentalization of T cells. The diversity of the TCR repertoire was also broader in the lymph nodes, except among CD38$^+$ T cells, which had a narrower TCR repertoire in all locations. UC was not associated with the narrowed clonal diversity in any regulatory or effector T cell subsets one might expect from the outgrowth or deletion of specific antigen-responsive clones.

T.48. Paradoxically Increased Foxp3$^+$ Regulatory T Cells in Ulcerative Colitis are not Activated CD4$^+$ Effector T Cells

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To determine if intestinal mucosal Foxp3$^+$ T cells of ulcerative colitis (UC) patients are the activated clones of otherwise Foxp3- effector cells, we sequenced the T cell receptors (TCRs) of CD4$^+$ populations from the inflamed versus uninflamed colon or the mesenteric lymph nodes (MLN) of three patients with UC, or the colons of three patients without UC. From each specimen, Foxp3$^+$ cells were sorted into fractions with and without Helios, while Foxp3-, CD45RA- cells were sorted into CD161$^+$ (including pro-inflammatory "Th17" cells), CD161- (excluding Th17's), and CD38$^+$ (activated) cells. The clonal diversity of Tregs was greater in MLN than colon, but was no different between patients with versus without UC.

More Helios$^+$ and Helios- Foxp3$^+$ cells were seen in inflamed than uninflamed colon. There was considerable TCR repertoire overlap between Helios$^+$ and Helios- Foxp3$^+$ cells, but this overlap was no different between patients with versus without UC. Less overlap was seen between Foxp3- effector T cells and Foxp3$^+$ cells in the colon, regardless of UC and inflammation. Much less overlap was seen in MLN, where Helios$^+$ Foxp3$^+$ nTregs shared almost no TCR repertoire with other populations. Hence most Foxp3$^+$ cells do not share a clonal origin with effector T cells.

T.49. Mediation of Immune Dysregulation in Ulcerative Colitis by Human Dendritic Cells: the Aryl Hydrocarbon Receptor as a Therapeutic Target

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Background: Activation of the aryl hydrocarbon receptor (AhR) ameliorates murine colitis via IL-22; intestinal AhR expression is lost in human ulcerative colitis (UC). Dendritic cells (DC) drive T cell responses, mediating UC pathology, but there is no data available regarding gut DC expression of AhR. Methods: Healthy human gut DC and DC from active UC patients (UC-DC) were isolated from colonic biopsies, and conditioned $^{+/-}$ AhR agonist 6-formylindolo (3,2-b)carbazole (FICZ). Cells were characterised by flow cytometry or co-cultured with T cells. Results: Human gut DC expressed AhR; expression was reduced in UC. UC-DC were more stimulatory for T cells than control-DC and stimulated higher levels of activation marker CD25 on T cells. UC-DC primed T cells with enhanced expression of gut-homing markers β7/CCR9, enhanced IL-10/IL-4/IFNγ production, but reduced TGFβ production. FICZ conditioning of gut DC reduced their T cell stimulatory capacity; stimulated T cells expressed lower levels of CD25, reduced expression of β7, reduced IL-10 production/T-β expression but increased production of TGFβ, IL-17 and IL-22. Conclusions: Targeting the AhR on gut DC in UC for therapeutic purposes may partially prevent enhanced T cell migration to intestinal sites and aberrant production of Th1 and Th2 cytokines, whilst promoting production of regulatory cytokines and epithelial barrier repair.

T.50. Epigenetic Profiling Reveals Involvement of Regulatory Elements in Inflammatory Bowel Disease

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Inflammatory Bowel Disease (IBD) is a multifactorial disorder occurring in genetically susceptible individuals. Research on the genetic components of IBD has mainly focused on protein coding genes, thereby omitting other functional elements in the human genome i.e. the regulatory regions. By performing acetylated histone 3 lysine 27 (H3K27ac) chromatin immunoprecipitation and sequencing (ChIP-seq), we identified tens of thousands of potential regulatory regions active in intestinal epithelium and immune cells. Altogether, 49 out of 163 SNPs in susceptibility loci for IBD identified by GWAS co-localize with an active regulatory region from intestinal epithelium or immune cells. This overlap is five-times larger than can be expected from random distributions. In addition we found that genomic variation in these SNPs often creates or diminishes known binding motifs, thereby possibly affecting the binding affinity of transcriptional regulators, and altering the expression of regulated genes. Notably, some of these affected sites alter binding motifs of transcription factors known to be involved in inflammatory response like AP1, PPARG, IRF, YY1 and FOXO. In summary, we provide evidence that non-coding regulatory regions that are active in immune cells and in intestinal epithelium may contribute to the IBD pathogenesis.

T.51. Retinoic Acid and the Differentiation of Intestinal Inflammatory CD14$^+$ Macrophages in Crohn's Disease

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Monocytes become either anergic or inflammatory CD14$^+$ macrophages (MΦ) in intestinal mucosa

contributing to local immune regulation. We hypothesised that these alternative differentiation fates are regulated by retinoic acid (RA) and that changes in RA generation are linked to the accumulation of inflammatory $CD14^+$ MΦ in Crohn's Disease (CD). MΦ generated from monocytes with GM-CSF *in vitro* were $CD14^+$ and produced TNFα upon LPS stimulation. Inhibition of RA receptor (RAR)α signalling with Ro41-5253 during differentiation reduced both CD14 expression ($p<0.001$) and TNFα production ($p=0.003$). Retinaldehyde dehydrogenase (RALDH) activity ($p=0.009$) and ALDH1A1/RALDH1 expression ($p=0.016$) were reduced in Ro41-5253-treated MΦ, indicating that the ability to oxidise retinal to RA is RARα-dependent and associates with an inflammatory phenotype. *Ex vivo* intestinal $CD14^+$ MΦ from healthy controls (n=8) had ALDH1A1-associated RALDH activity which was enhanced in active CD patients (n=11;$p=0.004$), alongside increased ALDH1A1 expression. Blood monocytes from CD patients (n=5) had reduced RALDH activity compared with healthy controls (n=6;$p=0.04$), indicating that MΦ RALDH activity is locally induced within the intestinal mucosa. RA is therefore a key factor in the differentiation of human inflammatory MΦ. Increased RA production by intestinal $CD14^+$ MΦ in CD may maintain their inflammatory properties and represents a novel therapeutic target.

T.52. Functional Consequences of a Novel IL-10 Receptor α Mutation on Innate and Adaptive Immunity in Early-Onset Inflammatory Bowel Disease

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Interleukin-10 (IL-10) plays a crucial role in orchestrating intestinal homeostasis. To date, its mechanism of action in the human intestine is not well studied. Here, we investigated the consequences of IL-10 receptor (IL-10R) deficiency on dendritic cell and T cell function in human. We describe a 10-year-old patient with a novel homozygous frameshift mutation in the IL-10R gene, who developed severe early-onset colitis and fistulising perianal disease in the first months of life. The IL-10R was absent in colonic biopsies and impaired IL-10-mediated STAT3 phosphorylation was found in cells from the patient. Monocyte-derived DCs released enhanced amounts of TNFα and IL-6 upon LPS stimulation and IL-10 failed to control IFNγ and IL-17 production by activated T cells *in vitro*. In agreement, lesional intestinal tissue taken at onset of disease contained high numbers of $IL\text{-}17^+$ and $T\beta t^+$ Th1 cells. Remission was achieved with thalidomide, intravenous immunoglobulin (IVIG) and colchicine. Interestingly, IVIG efficiently suppressed anti-CD3-driven IL-17 and IFNγ release while thalidomide inhibited LPS-mediated TNFα production by peripheral blood cells. Taken together, our study describes the consequences of a novel IL-10 receptor mutation and reveals that IL-10 controls the effector function of both dendritic cells and effector T cells in human intestinal disease.

T.53. Increased pSTAT1 and Altered Responses to Type 1 Interferons in CD4 T Cells from Non-Inflamed Intestinal Mucosa in Inflammatory Bowel Disease

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IFNα/β signalling in T cells has an increasingly recognised role in intestinal homeostasis. In mice, IFNβ maintains Treg function and is protective against colitis. We hypothesised that IFNα/β contributes to intestinal homeostasis in humans via STAT signalling in T cells. As assessed by phosflow analysis, the frequency of phospho-STAT1 (pSTAT1) expressing $CD4^+$ T cells was significantly greater in cells isolated from non-inflamed mucosal biopsies of IBD patients (n=30) than from healthy controls (n=16; $p=0.03$). $pSTAT1^+$ve CD4 T cells were more frequent in non-inflamed areas compared with paired inflamed areas (n=18, $p=0.02$). pSTAT1 did not associate with expression of the TH1 transcription factor T-β. There were no differences in expression of pSTAT3, pSTAT5 or unphosphorylated STAT1. "Gut-homing" peripheral blood memory/effector T cells ($CD3^+CD4^+CD45RA\text{-}\beta7^+$) from IBD patients or controls showed no differences in pSTAT expression, suggesting a local gut effect on pSTAT1. Antibody neutralisation of IFNβ during a 48-hour culture reduced pSTAT1 expression and increased IFNγ production (n=15, $p=0.02$); IL-10 production was decreased in control T cells (n=5, $p=0.04$) but increased in non-inflamed IBD samples (n=5, $p=0.03$). Therefore, IFNβ may contribute to intestinal homeostasis via STAT1

signalling in intestinal CD4$^+$ T cells in humans. Dysregulation of this pathway may contribute to IBD.

T.54. Lipopolysaccharide-Induced Genes, Which are Tolerised in Healthy Control Blood-Derived Macrophages are Up-Regulated in Inflammatory Bowel Disease Gut Macrophages

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Background and Aims: Blood-derived macrophages from healthy donors can undergo a tolerization process, in which certain immune response genes are suppressed upon repeated stimulation with bacterial-derived lipopolysaccharide (LPS). Since mucosal tolerance to the gut flora appears to be lost in inflammatory bowel disease (IBD), we assessed the expression of these tolerisable genes in gut macrophages of IBD patients. Methods: Blood-derived macrophages from six healthy donors were cultured for 24 hours with LPS, washed and re-stimulated with LPS for two hours. The expression of 86 immune genes was analyzed by RT-PCR array. We then assessed the expression of genes which underwent tolerization in CD33$^+$ lamina propria mononuclear cells (LPMCs) isolated from the inflamed colonic mucosa of 11 IBD patients and from normal colon of 10 healthy control subjects. Results: Among the 41 genes which underwent tolerization in blood-derived macrophages, tumor necrosis factor (TNF)-a, interleukin (IL)-6, IL-24, colony stimulating factor (CSF)-1, CSF-2, inhibin b A (INHBA) and, unexpectedly, IL-10 were up-regulated in CD33$^+$ IBD LPMCs compared to control subjects. Conclusions: A specific subset of pro-inflammatory genes, such as TNF-a, IL-6 and INHBA, fails to undergo tolerization in CD33$^+$ LPMCs from IBD patients. This may explain the abnormal immune response against the gut flora which triggers and sustains intestinal inflammation.

T.55. Triggering Receptor Expressed on Myeloid Cells 1 (TREM-1) Activation Increases the Production of Pro-Inflammatory Cytokines by Inflammatory Bowel Disease Mucosa

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Background and Aim: Intestinal macrophages play an important role in the pathogenesis of inflammatory bowel disease (IBD). Inflamed IBD mucosa contains high numbers of CD33$^+$CD68$^+$ macrophages overexpressing Triggering receptor expressed on myeloid cells 1 (TREM-1). We explored the *ex vivo* effects of a TREM-1 activating antibody on the intestinal immune response in IBD. Material and Methods: The expression of CD68, CD33 and TREM-1 was analysed by flow cytometry on lamina propria mononuclear cells isolated from inflamed colon of 11 IBD patients and normal colon of eight control subjects. Inflamed IBD biopsies were cultured *ex vivo* with or without an activating anti-TREM-1 monoclonal antibody, and the production of interleukin (IL)-1b, IL-6 and IL-8 was determined by ELISA. Results: The percentage of mucosal CD33$^+$CD68$^+$ macrophages was significantly higher in IBD compared to control subjects. TREM-1 expression by mucosal macrophages was significantly higher in IBD compared to control subjects. TREM-1 activation significantly increased IL-1b, IL-6 and IL-8 production by IBD biopsies cultured *ex vivo*. Conclusions: TREM-1 is overexpressed on IBD mucosal macrophages, and its activation amplifies pro-inflammatory cytokine production. Further studies using chromatin immunoprecipitation assays are under way in order to establish whether TREM-1 overexpression in IBD may derive from epigenetic changes.

T.56. Microbial Antigen-Specific Memory T Cells in Crohn's Disease

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Experimental models have led to the theory that chronic inflammation, as seen in Crohn's Disease (CD), results from a loss of tolerance towards commensal microbiota in genetically susceptible individuals. In line with this, antibodies specific for microbial components are found in 50% of CD patients, which

indicates that a memory T cell response might be generated as well. However, there is still little evidence in human disease proving the later. We have investigated T cell reactivity to microbial antigens in peripheral blood of CD patients and of healthy individuals. We analysed T cell proliferation by thymidine incorporation and determined their cytokine production by intracellular staining and ELISA. We were able to detect microbial antigen specific-T cells in both healthy individuals and CD patients. CD patients showed increased T cell proliferation towards some microbial antigens such as Fla2, FliC and YidX. Interestingly, each antigen elicited different T cell phenotypes. CD patients presented higher percentages of Th1, Th17 and Th1-Th17antigen-specific T cells compared to healthy individuals. Altogether, our results provide evidence for a microbial-specific memory T cell response in CD patients and suggest that these cells may play a role in sustaining disease.

T.57. Interleukin (IL)-17A Homodimer Reduces Pro-Inflammatory Cytokine Production by Inflammatory Bowel Disease Mucosa Cultured *ex vivo*

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Background and Aims: Interleukin (IL)-17A, which is up-regulated in inflammatory bowel disease (IBD) mucosal lesions, and IL-17F may form IL-17AA and IL-17FF homodimers or IL-17A/F heterodimers. The role of each IL-17 dimer in IBD is unknown; therefore we studied the effects of IL-17AA, IL-17FF and IL-17-A/F in ulcerative colitis (UC) and Crohn's Disease (CD) mucosa. Methods: Inflamed colonic biopsies from 17 IBD patients (6 UC and 11 CD) were cultured *ex vivo* with IL-17AA, IL-17FF or IL-17A/F (1 ng/ml). Mucosal myofibroblasts isolated from the inflamed colon of 4 CD and 4 UC patients were cultured with increasing concentrations (1-100 ng/ml) of each dimer. IL-8 and IL-6 were measured in culture supernatants by ELISA. Results: IL-17AA, but not IL-17FF, significantly reduced both IL-6 and IL-8 production by inflamed IBD biopsies cultured *ex vivo*, whereas IL-17A/F decreased IL-8 release by IBD mucosa. No difference was observed between CD and UC. Neither IL-17AA, nor IL-17FF, nor IL-17A/F exerted any effect on IL-6 and IL-8 production by IBD myofibroblasts. Conclusion: IL-17AA exerts an anti-inflammatory action on inflamed IBD biopsies cultured *ex vivo*. Its action is not mediated by myofibroblasts; therefore further studies are underway to ascertain which cell type is the main target of IL-17AA in IBD mucosa.

T.58. The Role of Human Neutrophil Elastase and Its Inhibitor Elafin in Ulcerative Colitis

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Background: In ulcerative colitis (UC) neutrophils infiltrating the inflamed mucosa release human neutrophil elastase (HNE), causing matrix degradation, as well as its specific inhibitor, elafin. We aimed to evaluate elastase and elafin production in active UC, and to investigate the modulatory effect of elafin on mucosal proteolytic activity. Methods: Intestinal biopsies from patients with active UC (18) and healthy controls (12) were homogenised and analysed for proteolytic activity and elafin concentration (ELISA). The *in vitro* effect of different protease inhibitors (elafin, marimastat, AAVP) on proteolytic activity of mucosal homogenates was determined. Biopsies from inflamed UC mucosa were cultured *ex vivo* for 24 hours with or without elafin and the effect on proteolytic activity was evaluated. Results: Mucosal samples from patients with active UC display significantly higher proteolytic activity and elafin levels than healthy controls. The *in vitro* addition of elafin, marimastat and AAVP significantly diminishes proteolytic activity. Elafin reduces proteolytic activity of inflamed UC biopsies cultured *ex vivo*. Conclusion: Colonic mucosa from UC patients show higher elastinolytic activity compared with healthy controls. Elafin has a restorative effect on the elastinolytic activity of UC mucosal homogenates. We observe a beneficial modulatory effect of elafin on human gut tissue, suggesting a possible therapeutic role of elafin in UC.

T.59. Innate Immune IL-10 Receptor Signaling Regulates Intestinal Mucosal Homeostasis

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Background: While recent data show that IL-10R signaling is important on T cell populations to prevent IBD, there is no data on its role on innate immune cells in maintaining mucosal homeostasis. We hypothesized that innate IL-10R signaling is a key regulator in the intestine of immune responses. Results: We generated IL-10Rb-RAG double KO (DKO) mice that were viable and did not develop spontaneous colitis. However, transfer of total WT $CD4^+$ T cells into DKO mice caused rapid severe intestinal inflammation, associated with Th1-Th17 immune responses. Similarly, when $CD4^+$ T cells were transferred into RAG KO mice reconstituted with DKO bone marrow, severe colitis resulted, indicating that the loss innate IL-10R signaling was responsible for the phenotype. $CD4^+$CD45RBhigh transfer also elicited colitis in DKO mice, was not ameliorated by co-transfer of CD45RBlow cells, and was associated with a significant decrease in generation of inducible Tregs. Mechanistically, we demonstrate that macrophage differentiation in the LP of DKO mice is altered, even in the absence of colitis, with a significant decrease in tolerogenic subsets. Conclusion: Innate IL-10R signaling is a key regulator of intestinal mucosal homeostasis. Loss of signaling might alter differentiation of innate cells towards pro-inflammatory subsets, leading to colitis.

T.60. IFN-ɣ-Independent Intestinal Inflammation in Mice with T Cell Specific Deficiency of the Transcriptional Regulator B Lymphocyte-Induced Maturation Protein 1 (Blimp-1)

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Blimp-1 is a zinc finger-containing transcription factor expressed in several cell lineages, including B and T lymphocytes. The gene encoding Blimp-1 (PRDM-1) has been linked by Genome Wide Association Studies (GWAS) with several chronic inflammatory conditions, including Inflammatory Bowel Disease (IBD). We have previously described that T cell-specific deletion of Blimp-1 (Blimp-1CKO mice) results in spontaneous development of severe colitis associated with the accumulation of $IL\text{-}17A^+$ and $IFNg^+$ $CD4^+$T cells, however the mechanisms underlying the development of chronic inflammation in the Blimp-1CKO mice are not fully understood. Here we show that genetic deletion of IFNg in the Blimp-1CKO mice did not rescue the inflammatory phenotype observed in these mice. Moreover, while $IFNg^{-/-}$ naive T cells did not cause disease when adoptively transferred to $Rag1^{-/-}$ mice, IFNg/Blimp-1 double knock out cells caused severe colitis, associated with increased production of the inflammatory cytokines IL-17A and IL-17F, indicating that IL-17 family cytokines could be the main mediator of intestinal inflammation in the Blimp-1CKO mice. We are currently in the process of analyzing Blimp-1-and IL-17A double knockout mice to determine the role of IL17A in the inflammation that develops in the Blimp-1CKO mice.

T.61. Enhancement of Colonic Mucosal Repair and Protection by FGF15/19 Derived from Myofibroblasts

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Background: FGF15, human ortholog of FGF19, has been reported to express in small intestine and control bile acid secretion. However, little is known about the roles of FGF15 to colonic epithelial cells. In the present study, we determined the roles of FGF15 to colonic epithelial cells in view of inflammation. Methods: Mouse colonic myofibroblast (VUPF1) cells were treated with TNF-α and FGF15 mRNA expression was measured. Mouse colonic epithelial cells (YAMC) were treated with FGF19 recombinant protein and we measured cellular proliferation, and restitution. Cellular viability and apoptosis treated with H2O2 were measured as well. Human colonic mucosal biopsy specimens, normal control and ulcerative colitis patients, were used to detect FGF19 expression. Results: TNF-α increased FGF15 mRNA expression in VUPF1 cells. The proliferation, restitution, of YAMC were significantly enhanced by FGF19. Cellular damage and apoptosis induced by H2O2 were significantly inhibited by FGF19. mRNA expression of FGF19 was increased in colonic mucosa at the patients of ulcerative colitis. Conclusion: FGF15/19 was secreted by colonic myofibroblasts and increased by colonic mucosal inflammation. FGF15/19 promotes proliferation, enhances restitution, inhibits apoptosis of colonic epithelial cells. Taking together, FGF15/19 may play an important role of colonic epithelial homeostasis and the pathogenesis of ulcerative colitis.

T.62. AIEC Pathobiont Instigates Chronic Colitis in Susceptible Hosts by Altering Microbiota Composition

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Background: Inflammatory bowel diseases are driven by an aberrant mucosal immune response to the gut microbiota. We recently demonstrate that colonization of adherent-invasive *E. coli* (AIEC) during microbiota acquisition results in chronic colitis in mice that lacked the flagellin receptor TLR5 (T5KO). That such colitis persists after AIEC is cleared suggests that AIEC instigates chronic inflammation, and that it requires TLR4 and NLRC4 suggest it may involve alterations in levels of LPS and/or flagellin. AIM: Examine if AIEC alters microbiota composition and levels of bioactive LPS and flagellin. Methods: Germ-free mice were inoculated with AIEC strain LF82 and placed in standard housing to allow more complex microbiota to displace AIEC. Inflammatory marker lipocalin-2, total bacterial load, microbiota composition, and fecal levels of LPS and flagellin were measured. Results: Transient colonization by AIEC in WT mice did not alter inflammatory markers, bacterial loads, nor microbiota composition. In contrast, transient AIEC colonization of T5KO mice resulted in chronic inflammation, which correlated with an altered microbiota having loss of species diversity and higher levels of bioactive LPS and flagellin. Conclusion: AIEC may instigate chronic inflammation in susceptible hosts by altering gut microbiota composition having inherently greater ability to activate innate immunity/pro-inflammatory gene expression.

T.63. Food Additives Promote Intestinal Inflammation in Susceptible Host

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Background: Inflammatory bowel diseases (IBD) are driven by aberrant mucosal immune response to the gut microbiota. While genetics contribute to disease susceptibility, environmental factors are also determinants. Dramatic increase in IBD incidence over the last half-century amidst relatively constant host genetics supports a pivotal role for an environmental driver that might be associated with modern society. Aim: We aimed to test if modern food production regimes could disturb the host-microbiota relationship. We examine the hypotheses that emulsifying agents, which are commonly added to processed foods, could promote intestinal inflammation by altering the composition of the gut microbiota and/or its interaction with the intestine. Methods: IL10KO mice were treated with the commonly used emulsifying agents Carboxymethylcellulose (CMC, 1%) or Polysorbate-80 (P-80, 1%). Inflammatory marker lipocalin-2, microbiota composition, and mucus layer integrity were analyzed. Microbiota from each group were transferred to WT germ-free mice. Results: Both CMC and P-80 component promoted colitis in IL-10KO mice. The microbiota transfer from emulsifying agent treated mice to germfree mice is sufficient to transfer low-grade intestinal inflammation. Conclusion: These data highlight the hypothesis that routinely used food additives could be related to the increased incidence of IBD.

T.64. RIP2 Kinase Inhibition Reduces Inflammatory Cytokine Production in *ex vivo*-Cultured Inflammatory Bowel Disease Biopsies

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The loss of epithelial barrier integrity is a common feature of inflammatory bowel disease (IBD). Disrupted barrier function allows bacteria to penetrate from the gut lumen, driving inflammation through activation of host pattern recognition receptors (PRRs). Although it is unclear which PRRs are most important in this process, accumulating evidence points to a role for the cytoplasmic PRRs NOD1 and NOD2, which signal through RIP2 kinase to activated NF- κB. We therefore utilized the highly potent and selective RIP2 inhibitor GSK214 to examine the function of RIP2 in spontaneous cytokine production by *ex vivo*-cultured inflamed mucosal biopsies isolated from Crohn's disease and ulcerative colitis patients. Treatment with GSK214 potently inhibited production of IL-1β, IL-6 and TNF-α in this system. Approximately 70% of patient cultures responded to GSK214 in a dose-dependent fashion, which was similar to the response

rate observed for the corticosteroid prednisilone. Data will also be presented on the effect of GSK214 on activation of RIP2 as measured by Ser176 autophosphorylation. Our results highlight the importance of RIP2 kinase in promoting intestinal inflammation, and suggest that RIP2 inhibitors may have therapeutic potential in the treatment of IBD.

T.65. Down-Modulation of Pro-Inflammatory Cytokines in Experimental Colitis and in Inflammatory Bowel Disease by a Narrow Spectrum Kinase Inhibitor

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Background and Aims: Mitogen-activated protein kinases are crucial regulators of mucosal pro-inflammatory cytokine expression in both experimental colitis and inflammatory bowel disease (IBD). We evaluated the effects of a narrow spectrum kinase inhibitor (NSKI-1) on IBD inflamed mucosa and on mouse colitis. Methods: NSKI-1 (1-100ng/ml) or BIRB796 (1µg/ml) was added to biopsies or anti-CD3/anti-CD28-stimulated lamina propria mononuclear cells (LPMCs) isolated from inflamed gut of 19 IBD patients. Interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor (TNF)-α were evaluated in 24-hour culture supernatants by ELISA. Colitis was induced in $Rag2^{-/-}$ mice by naïve T cell transfer, and colonic explants were cultured *ex vivo* with or without NSKI-1 (1-100ng/ml) and assessed as above for pro-inflammatory cytokine release. Results: NSKI-1, but not BIRB796, inhibited IL-1β, IL-6, IL-8 and TNF-α production by inflamed IBD mucosal explants cultured *ex vivo*. Furthermore, TNF-α release by anti-CD3/anti-CD28stimulated LPMCs was reduced in a dose-response manner by both NSKI-1 and, less potently, BIRB796. NSKI-1 inhibited in a dose-response manner IL-1β, IL-6, TNF-α, interferon (IFN)-γ and IL-17A production by explants of inflamed mouse colon. Conclusions: NSKI-1 showed consistent down-regulatory effects in both mouse and human *ex vivo* and *in vitro* models of intestinal inflammation, and may be a promising candidate for IBD treatment.

T.66. The Green Tea Polyphenol EGCG Impacts Secretion and Expression of Tight Junction (TJ)-Regulating Cytokines and the Expression of Key TJ Proteins in Human Intestinal Epithelial Cells

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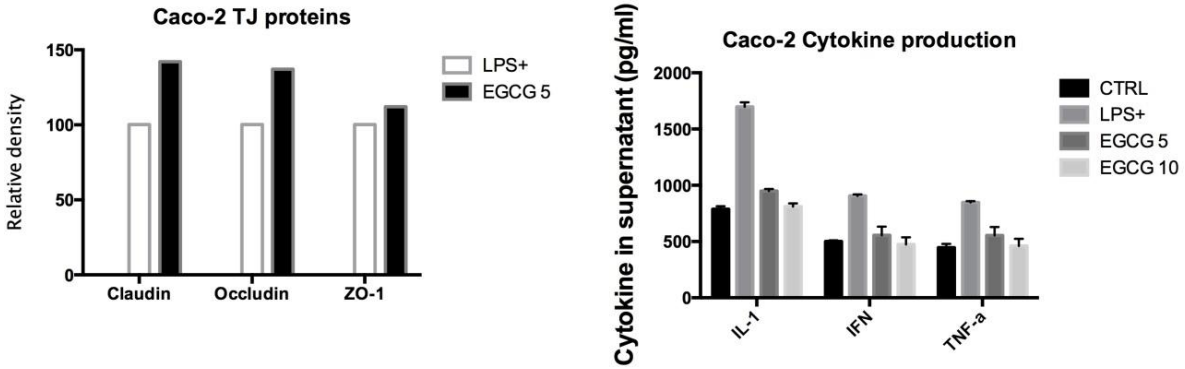

Background: The green tea polyphenol (-) epigallo-catechin 3-gallate (EGCG) has demonstrated therapeutic potential for inflammatory bowel disease (IBD) and impacts cytokine production by human immune cells, particularly interleukin (IL)-1β, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α. Specific Aim: Determine impact of EGCG on key tight junction (TJ) proteins after LPS exposure. Experimental Design: Caco-2 cells ($1x10^6$ cells/well) were incubated with LPS in the presence of EGCG (0-10 ng/µl) at 37°C and 5% CO2 for 24 hours. Supernatants were collected to assess related cytokines. Cells were processed to evaluate TJ protein and RNA expression by RT-PCR, with unstimulated cells as controls. Results: EGCG 5µg/mL maximally reduced inflammatory cytokine production while boosting TJ protein ZO-1, claudin and occludin production and mRNA expression. Cytokine reductions were not due to loss of cell viability, as determined by mitochondrial reduction of MTT and Trypan blue exclusion.

Conclusion: EGCG exerts a positive effect on epithelial barrier function by both reducing production of TJ loosening pro-inflammatory cytokines as well as enhancing TJ protein expression. These effects result in enhanced epithelial integrity and provide evidence that EGCG may provide a preventative effect against barrier disruption, which could serve as a useful property for a prophylactic agent.

T.67. Differential Compartition of Immune Cells in the Internal and External Foreskin Tissue is Associated with the Sensitivity to HIV-1 Infection

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Ex vivo model of foreskin has demonstrated that the inner foreskin is more susceptible to HIV-1 infection than the outer foreskin. The mechanism remains elusive. In this study, density and distribution of HIV potential target cells and the cell types of lymphoid tissues were visualized by microscopy. The expression of CD4, CCR5 and CXCR4 were quantified on HIV potential target cells in epidermis and dermis of foreskins by flow cytometry separately. We observed that the epidermis of inner foreskins was more enriched in $CD4^+$ T cells and Langerhans cells with co-expression of CCR5 and/or α4β7 but not CXCR4 than that in its outer counterparts; Lymphoid aggregates, composed of $CD4^+$ T cells, macrophages and DCs, were more concentrated and closer to epithelial surface in inner than outer foreskins. Overall, the discrepancy of HIV receptor/coreceptor expression was mainly in the epidermis but not the dermis between inner and outer foreskins. Lymphoid aggregates in dermis of foreskins were more concentrated and closer to epithelial surface in inner foreskins than outer foreskins may also contribute to the different sensitivity to HIV.

T.68. Interleukin 15 Modulates the Balance Between Bcl-2 and Bim via Jak3/1-PI3K-Akt-ERK Pathway to Promote $CD8\alpha\alpha^+$ Intestinal Intraepithelial Lymphocyte Survival

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Interleukin 15 (IL-15) is an essential survival factor for $CD8\alpha\alpha^+$ intestinal intraepithelial lymphocytes (iIELs) *in vitro* and *in vivo*. However, the IL-15-induced survival signals in primary $CD8\alpha\alpha^+$ iIELs remained elusive. Although the level of Bcl-2 in $CD8\alpha\alpha^+$ iIELs positively correlates with the expression of IL-15Rα in the intestinal epithelial cells, overexpression of Bcl-2 only moderately restores $CD8\alpha\alpha^+$ γδ iIELs in $IL\text{-}15^{-/-}$ mice. Here we found that IL-15 promptly activated a Jak3-Jak1-PI3K-Akt pathway that led to the up-regulation of Bcl-2 and Mcl-1. This pathway also induced a delayed but sustained ERK1/2 activation, which was necessary for the maintenance of Bcl-2 level and resulted in the phosphorylation of BimEL at Ser65. The latter event facilitated the dissociation of Bim from Bcl-2 without affecting Bim abundance. Using adoptive cell transfer approach, we found that overexpression of Bcl-2 or removal of Bim in $CD8\alpha\alpha^+$ iIELs promoted their survival in $Il15ra^{-/-}$ mice. Taken together, IL-15 promotes $CD8\alpha\alpha^+$ iIEL survival by both increase of Bcl-2 level and dissociation of Bim from Bcl-2 through activation of a Jak3-Jak1-PI3K-Akt-ERK1/2 pathway.

T.69. Identification of an IL-15 Responsive Bipotential NK/T Cell Precursor in the Human Intestinal Epithelium

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Duodenal intraepithelial lymphocytes (IEL) are composed of T cells, NK cells and an enigmatic CD3-CD56- IEL subset of unknown origin and function, which is particularly prominent in children. The latter population contains a lineage-negative (Lin-) $CD7^+CD103^+CD127\text{-}CD34\text{-}$ subset (Lin-IEL), a likely precursor of aberrant IEL found in refractory celiac disease. The human thymus also harbors cells with this Lin-IEL phenotype (Schmitz et al, GUT 2012). We now report that thymic and duodenal Lin-IEL are highly similar and express IL-2/15Rβ, CD244 and CD160, are partially $NKp46^+$ and lack CD16 and CD5. Also, Lin-IEL can express CD3 ε, γ and ζ intracellularly. Upon co-culture with stromal cell line OP9-DL1, duodenal and thymic Lin-IEL differentiated into NK cells and T cells. For thymic Lin-IEL, this process was

notch-independent and survival depended on IL-15. Furthermore, clonal thymic Lin-IEL could develop both into NK cells (CD3-CD56brightNKp46^{+}) and T cells (CD3^{+}TCRαβ^{+}). These results indicate that in the human thymus and duodenum highly similar Lin-IEL populations exist, that express T and NK cell-associated markers and contain bipotential NK/T cell precursors. We speculate that the duodenal Lin-IEL cells are thymus derived and have the ability to differentiate into T or NK cells in response to local stimuli.

T.70. The Role of TGFβ Signaling in the Development of Unconventional T Cells
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The gastrointestintal (GI) tract presents challenges not faced at other sites, immune regulation must be maintained concomitant with tonic inflammatory signaling. One way this is achieved is through the presence of unique T cell populations nestled between epithelial cells called intra-epithelial lymphocytes (IELs). Populations of IELs are similar to peripheral T cells, but others are unique to the GI epithelium. One unique population is TCRαβ^{+}CD8αα^{+}, which develop via TGFβ-dependent mechanisms. Another is TCRαβ^{+}CD4^{+}CD8α^{+}, whose development also requires TGFβ-signaling, as TGFβ induces CD8α expression on CD4^{+} T cells. Both these IEL exhibit regulatory functions as they reduce colitis severity upon transfer. Here we examine the developmental pathway of TCRαβ^{+}CD4^{+}CD8α^{+} IEL, probing the molecular events downstream of TGFβ. Canonical TGFβ-signaling is mediated by Smad-proteins; here we show these are important for the development of CD8α bearing CD4^{+} T cells. In the absence of Smad3 TGFβ-mediated CD8α induction is reduced, yet loss of Smad4 has little effect, indicating Smad3 mediates its effects on CD8α gene transcription in the absence of the co-Smad, Smad4. These data begin to elucidate the molecular events that drive CD8α expression on CD4^{+} T cells and also shed light on the developmental pathway of a potentially regulatory IEL population.

T.71. Muc2 Limits Pathogen Burdens and Epithelial Barrier Dysfunction During *Salmonella Typhimurium* Colitis
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To study how *Salmonella typhimurium* causes gastroenteritis, we used a colitis model relying on streptomycin to displace intestinal commensal microbes, resulting in heavy pathogen colonization of cecal tissues and severe inflammation. Although intestinal mucus is the first line of defense in the GI tract, its role against *Salmonella* is still unclear. The mucus barrier is made up of the highly glycosylated mucin Muc2, which is secreted by goblet cells. Muc2 is glycosylated by several enzymes, for example, Core 3-O derived glycans are synthesized by Core 3 β1,3-N-acetylglucosaminyltransferase (C3GnT). Mice lacking these glycans still produce the Muc2 protein, but display a thinner mucus barrier. By comparing *Salmonella* induced colitis and mucin dynamics in Muc2 deficient ($^{-/-}$), C3GnT$^{-/-}$ and C57BL/6 mice, we observed that mucin secretion increased during infection in C3GnT$^{-/-}$ and C57BL/6 mice, with *Salmonella* found within the mucus layer. In contrast, Muc2$^{-/-}$ mice carried heavier cecal pathogen burdens and suffered increased epithelial barrier disruption compared with C57BL/6 mice, leading to high rates of morbidity and mortality. C3GnT $^{-/-}$ mice carried WT level pathogen burdens but developed exaggerated barrier disruption like Muc2 $^{-/-}$ mice, suggesting that the mucus layer controls *Salmonella* intestinal burdens, whereas Core 3 glycosylation controls epithelial barrier function.

T.72. Apoptotic Cell Based Therapy in the Treatment of Inflammatory Bowel Diseases
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Inflammatory Bowel Disease (IBD) is characterized by an abnormal expression of proinflammatory cytokines and impairment in regulatory T cells (Treg). It has been described that apoptotic cell (ApoCell) injection can create an immunomodulatory environment through anti-inflammatory cytokines and Treg induction. Therefore, apoptotic cell therapy has been efficient to modulate inflammation, prevent autoimmune disease onset, as well as induce tolerance in experimental models. Thus, based on these immunomodulatory properties, we evaluated such cell-based therapeutic approach to treat ongoing IBD

in a mouse model. IBD was induced by intra-rectal administration of TNBS in eight weeks Swiss mice. apoptotic cell, generated from X-ray irradiated thymocytes, were intraperitoneally administered 24 hours after the TNBS challenge. Control mice receive either TNBS vehicle, or TNBS and 5-ASA. When mice with IBD were injected with apoptotic cell, they showed lower IBD clinical scores, and significantly improved body weight gain compared to mice injected with TNBS only. This weight gain was similar to IBD mice treated with 5-ASA. The first results concerning mechanisms involved in tolerance seem to show a role of IL-10 more important than that of Treg. Such results are encouraging to propose a new therapeutic approach for the treatment of IBD in drug-resistant patients.

T.73. Gamma IFN Production in *Clostridium Difficile* Infection

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Introduction: *Clostridium difficile* infection (CDI) is a global challenge. 15-30 % of CDI patients have recurrence after completion of initial antibiotic treatment and up to 65% of these recur. Little is known about what cellular immune responses are key to CDI resolution. Hypothesis: γIFN plays a role in CDI clearance and/or recurrence. Methods: Patients were identified as initial (never had CDI) or recurrent (one documented CDI within two weeks to six months). Healthy and case controls (comorbid patients) were also examined. All samples were obtained after informed consent was signed. We examined the ability of each patient's PBMC to produce γIFN by flow cytometry and cell culture. Results: Expression of γ IFN was found in four separate PMBC populations: (a) $CD3^+$ $CD4^+$ (b) $CD3^+$ $CD8^+$ (c) CD3-$CD8^+$, and (d) CD3-CD8-. There are distinct quantitative and qualitative differences in γIFN producing PBMC subsets between case controls, initial and recurrent patient groups. Similarly, there were quantitative differences in the amount of γIFN found in the cultured supernatants, both in the SLP and PHA stimulated cultures between patient groups. Conclusions: There is a different balance in the cell-mediated γIFN response and ability to produce γIFN between case control patients, initial and recurrent *C. difficile* patients.

T.74. Immune Response to *Taenia solium Calreticulin* During Experimental Taeniosis in the Golden Hamster

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Taenia solium is a tapeworm that causes two diseases in humans. Research focuses mainly on the metacestode that causes neurocysticercosis, an important parasitic disease of the central nervous system. However, the tapeworm carrier is a risk factor for acquiring neurocysticercosis and little is known about the immunity induced in the small intestine. Calreticulin is a ubiquitous protein involved in cellular $Ca2^+$ homeostasis, present in excretion/secretion products in several helminth infections and shown to induce predominantly Th2 responses. We cloned and expressed *T. solium calreticulin* (rTsCRT) as a functional recombinant $Ca2^+$-binding protein. We also standardized a model of hamster tapeworm infection and analyzed if rTsCRT induces humoral and cellular immune responses, both locally and systemically. Our results indicate that there is no proliferative response by mesenteric lymph node cells, except at 20 days post-infection when IL-4 is expressed and tapeworm expulsion initiates. Splenocytes proliferate upon stimulation and IL-4 and IL-5 levels increase along the 30 days of infection. Additionally, rTsCRT induces a robust expression of the regulatory cytokine IL-10 in spleen and lymph node cells. These data suggest that there is a time- and microenvironment-dependent cytokine expression profile during *T. solium* infection and that rTSCRT is a potential immunomodulatory agent.

T.75. Membraneous Cells of the One Humped Camel (Camelus Dromedarius)

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Membraneous cells (M cells) constitute a very thin epithelial barrier located among the follicle-associated epithelium covering the domes of Peyer's patches. These cells have characteristic ultrastructural features that vary according to the species. The unique camel Peyer's patches persist in old age. The present study aimed to localize and describe M cells in the dromedary camel. Specimens from the ileal Peyer's patches of 10 male camels were fixed in 4% phosphate buffered glutaraldehyd. Semithin sections were prepared and examined by light microscopy; ultrathin sections were prepared and examined by

transmission electron microscope. Several M cells were distributed among enterocytes. Their cytoplasm was elevated above that of neighboring enterocytes. Depending on the number of apical processes, M cells were variable in appearance, ranging from M cells without any processes and M cells with a few short processes to M cells with several short processes. In general, M cells were rich in mitochondria an have several cytoplasmic pockets contained lymphocytes or macrophages. The M cells were tightly connected to the neighboring enterocytes by junctional complexes of tight junctions, desmosomes, and interdigitations. The variable morphological appearance of camel M cells may be related to different stages of development or different functional stages.

T.76. Correlation Between *Mycobacterium Avium* Subspecies Paratuberculosis Infection and Colitis

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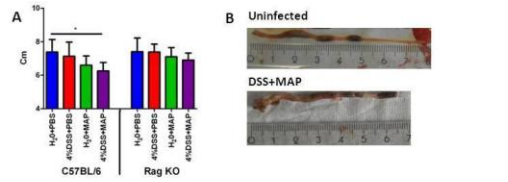

Figure 1. DSS-treated MAP infected mice showed colon length reduction after secondary challange only in wild type mice, not in Rag2-/- mice. A. colon length measurement (cm), B. representative colon are shown

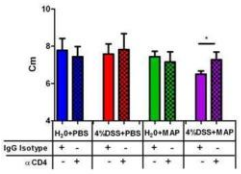

Figure 2. CD4+ T cells depleted DSS-treated MAP infected mice showed no colon length reduction after secondary challange. IgG isotype = control, αCD4 = anti CD4

An association of Mycobacterium avium subspecies paratuberculosis (MAP) with Crohn's Disease, an inflammatory bowel disease in human, has been discussed because of the similar pathophysiology with Johne's disease, a chronic gastrointestinal disease of ruminants caused by MAP. In this direction, the lack of suitable mouse model has limited for detailed study about the disease. We hypothesized that MAP could contribute to exacerbate the existing inflammatory bowel disease. In our previous studies, the higher progression of infection and worsened colitis was shown in Dextran Sulfate Sodium - treated MAP infected mice (DSS^+MAP). The exacerbation of the infection was evident from the increasing liver and spleen weight, enlargement granuloma in liver and delayed colon recovery in DSS^+MAP mice (Suwandi, et al, in preparation). In further study, we found that after secondary infection with MAP, DSS^+MAP mice showed shortening of colon length as well as diarrhea. Increasing number of B and T cells in mesenteric lymph nodes were observed after secondary infection. Furthermore, $Rag2^{-/-}$ and $CD4^+$ T cells depleted mice did not show the same effect as wild-type mice. Other mycobacterium species, *M. avium* also failed to induce similar effects, which might be due to different tropism. Taken together, MAP exacerbates the existing colitis and the adaptive immune system contributes to this effect.

T.77. Elucidating the Determinants of Protective Norovirus Immunity

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Volunteer studies have clearly demonstrated that the prototype norovirus, Norwalk virus, fails to elicit protective immunity. In contradiction to this result, recent pandemic norovirus strains appear to elicit herd immunity that drives the evolution of the virus. One possible explanation for these apparently contradictory results is that specific human norovirus strains interact with the host immune system differentially. To test this hypothesis, we utilized two murine norovirus strains called MNV-1 and MNV-3. We demonstrated that MNV-3 induced robust protective immunity against both homologous and heterologous re-challenges, whereas MNV-1 failed to elicit significant protective immunity. Both $CD4^+$ T cells and B cells were essential to MNV-3 protective immunity whereas Type 1 interferon signaling, type II interferon, and $CD8^+$ T cells were not. Furthermore, MNV-3 induced higher antibody titers than MNV-1 and MNV-3, but not MNV-1, virus-specific serum antibody conferred partial protection when used in passive transfer experiments. We also uncovered a helper-independent role for $CD4^+$ T cells in MNV-3 protection. We are testing whether Th17 cells or cytolytic $CD4^+$ T cells account for this layer of protection. Altogether, our findings have revealed remarkable norovirus strain-specific disparities in protective immunity induction and have shed light on the determinants of norovirus protective immunity.

T.78. Tenofovir 1% Gel Causes Strong and Broad Changes to the Mucosal Transcriptome in a Phase I Rectal Microbicide Trial

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Topical vaginal pre-exposure prophylaxis with tenofovir has shown efficacy in preventing HIV transmission in one of two trials. The discrepant results are likely explained by product adherence and/or unintended biological effects of tenofovir on the mucosa. In this Phase 1 randomized, double-blind, placebo-controlled trial, we determined the global transcriptome effects of daily tenofovir 1% or nonoxynol 9 (N9) 2% gels on the rectal mucosa of eight healthy men per study arm by mRNA microarrays. Tenofovir results were confirmed by PCR in seven additional subjects. After seven days of use, tenofovir gel suppressed 505 genes and induced 137, whereas N9 gel suppressed 56 and induced 60, with little overlap between the affected genes. Tenofovir gel suppressed genes more strongly than N9 gel ($p<0.0001$), including many transcription factors, anti-inflammatory factors such as IL-10, and genes important for mucosal remodeling and mitochondrial function. Chemokine genes, as well as some T and B cell markers, increased significantly. Tenofovir gel caused mitochondrial dysfunction, as evidenced by a marked decrease in mitochondrial gene transcription ($p<0.001$) and ultrastructural changes. Tenofovir's pronounced effects on mucosal immunity and barrier homeostasis may modify antiretroviral efficacy over time. Moreover, the breadth of the changes raises potential concerns about mucosal safety.

T.79. Dietary Oils Modulate the Host Immune Response and Colonic Tissue Damage Following Citrobacter Rodentium-Infection in Mice

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Inflammatory bowel disease (IBD) is a chronic intestinal inflammatory disorder of multi-factorial origin, in which changes in diet that favor high n-6 and low n-3 fatty acids have been implicated. The present study addressed whether dietary n-6 and n-3 fatty acids alter severity of colonic mucosal responses to *Citrobacter rodentium* infection in mice. Mice were fed diets identical in all nutrients except fatty acids, with as a percent energy, 15% 18:2n-6 and <0.06 % 18:3n-3 (Safflower oil; SO), 4.2% 18:2n-6 and 1.9% 18:3n-3 (Canola oil; CO), or 1.44% 20:5n-3, 4.9% 22:6n-3, 0.32% 18:2n-6 and 0.12% 18:3n-3 (Fish oil; FO) for three weeks prior to *C. rodentium* infection. Dietary fatty acids had no effect on colonic *C. rodentium* burden, however, at 10 days post-infection, mice fed FO had reduced histological damage score with lower infiltration of macrophages and neutrophils in colonic mucosa, compared to mice fed SO ($P <0.05$), and lower number of apoptotic cells in colonic mucosa ($P <0.01$). Moreover, FO decreased expression of IFNγ, IL6, IL-17A and TGFβ, and increased IL-10 expression in infected mice ($P <0.05$). This study demonstrates that dietary 18:2n-6 exacerbates and 20:5n-3 and 22:6n-3 reduce severity of mucosal immune response to an enteric pathogen infection.

T.80. ETEC Colonization Factors Modulate the Antigen Presentation Function of Porcine Intestinal Mononuclear Phagocytes

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Enterotoxigenic E. coli (ETEC) are still a major cause of traveller's diarrhea and intestinal infections in children in less affluent countries. In addition, ETEC infections lead to postweaning diarrhea in piglets and result in significant economic losses in swine production. This intestinal pathogen displays colonization factors or fimbriae on its surface enabling the colonization of the small intestinal epithelia. In swine, F4 and F18 fimbriae are frequently associated with ETEC-induced diarrhea. As opposed to F4 fimbriae, oral immunisation with F18 fimbriae fails to protect animals from a challenge infection. Besides structural differences and a different uptake mechanism by the intestinal epithelium of F18 (M cell dependent transport) and F4 fimbriae (M cell and enterocyt-dependent transport), these fimbriae could differently modulate the function of intestinal phagocytes. Indeed, F18 fimbriae drastically diminished the antigen

presentation capacity of mononuclear phagocytes (MHCII$^+$SIRPαhi) isolated from the small intestinal lamina propria. As F18 fimbriae bind glycosphingolipids and presumably disrupt lipid raft formation, the impaired antigen presentation ability of the intestinal phagocytes could result from a F18 fimbriae-mediated deformation of the immunological synapse. These results could accelerate the development of an improved F18$^+$ ETEC vaccine and hint at novel immune evasive mechanisms employed by bacterial pathogens.

T.81. Intestinal Expression of the Blood Group-Related Glycosyltransferase B4galnt2 Influences Susceptibility to *Salmonella Typhimurium* Infection

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Glycans on mucosal surfaces play an important role in host-microbe interactions. We previously demonstrated that intestinal epithelial expression of the glycosyltransferase β-1,4-N-acetylgalactosaminyltransferase 2 (B4galnt2) influences the composition of the intestinal microbiota. To determine whether B4galnt2 glycans influence host susceptibility to enteric pathogens, we investigated *Salmonella Typhimurium* (S.T.) induced gastroenteritis in presence and absence of B4galnt2 expression. Although intestinal S.T. colonization was enhanced in B4galnt2 deficient mice, they developed significantly less pathology in the cecum compared to wild type mice one day post-infection. Diminished cecal inflammation inB4galnt2 deficient mice was associated with significantly lower numbers of tissue infiltrating CD68 and CD3 positive cells and less mRNA expression of inflammatory cytokines monocyte chemotactic protein-1 and interleukin-6 compared to wild type. Furthermore, changes in the intestinal microbiota correlated significantly with the development of inflammation and the associated B4galnt2 genotype. Further experiments will be performed to investigate the direct interaction of S.T. with B4galnt2 glycans. Detailed analysis of B4galnt2 dependent changes in the intestinal microbiota will reveal species dependent contributions to host susceptibility for S.T. infection. In summary, our results demonstrate that variation in intestinal glycosylation patterns alters susceptibility to diseases such as infectious gastroenteritis.

T.82. High and Low Loads of Cecal Colonization by *Salmonella Enteritidis* in Chickens Triggers Distinct Immune Kinome Profiles

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Salmonella enterica serovar Enteritidis are facultative intracellular bacteria that cause disease in numerous species. *Salmonella*-related infections originating from poultry and/or poultry products are a major cause of human foodborne illness, and *S. Enteritidis* is the leading cause worldwide. Despite the importance of *Salmonella* to human health and chickens being a reservoir, little is known of the response to infection within the chicken gastrointestinal tract. Using chicken-specific kinome immune peptide arrays we compared a detailed kinomic analysis of the chicken gut immune response in birds with high and low *Salmonella* loads. Four-day-old chicks were challenged with *S. Enteritidis* (105cfu) and cecal content and a section of jejunum collected on days 4, 7, 10, 14, 17, 24 and 37 post-infection (pi, [n=5]). *Salmonella* colonization was enumerated and birds with the highest and lowest loads were selected for kinomic analyses. A small number of peptides were differentially phosphorylated between birds with high and low *Salmonella* loads including IGF2R, Pyk2, VIM and BLNK. Identification of specific proteins associated with increased resistance against *S. enteritidis* provides breeders additional biomarkers to identify birds naturally more resistant to this important foodborne pathogen potentially reducing the need for antibiotics and creating a safer food supply for the consumer.

T.83. Elastase Regulates Type 2 Immune Response to Enteric Nematode Infection

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Neutrophil elastase is important to the pathogenesis of IBD and adversely impacts healing and remission.

The aim of this study was to determine the contribution of elastase to the protective immune response to nematode infection, which features an influx of mast cells, a reported source of elastase, rather than neutrophils. Elastase deficient (ELko) or WT (C57BL/6) mice were infected with Nippostrongylus brasiliensis (Nb) and studied nine days later. Expression of elastase, mMCP-1 (mast cell marker), and LY6G (neutrophil marker), IL-4, IL-13 and the M2 marker, arginase-1, was determined by real-time PCR in small intestine. Smooth muscle contractility was assessed by suspending 1 cm sections in organ baths. Mucosal permeability was assessed in muscle-free mucosae mounted in Ussing chambers. Nb infection in WT mice induced 100-fold increase in mMCP-1 and a 21-fold increase in elastase expression, but no changes in LY6G. When compared to WT mice, ELko mice had accelerated Nb expulsion coupled with significantly ($p<0.01$) augmented elevation in IL-4, IL-13, and arginase-1 expression. Both strains showed similar increases in smooth muscle hypercontractility and epithelial permeability. These data indicate that during nematode infection, elastase negatively regulates the Th2 response and could be a novel target for modulating Type 2 immunity.

T.84. Early Initiation of Combined Anti-retroviral Therapy (c-ART) Protects HIV-1 Infected Individuals from the Alteration of the Treg/Th17 Profile in the Gut

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In HIV infection, gut depletion of specific T cell populations, such as IL-17$^+$CD4$^+$T cells (Th17) and regulatory T cells (Tregs), may significantly impact microbial translocation which is claim to be associated with systemic inflammation and a higher risk of mortality. Here, we show a decrease of rectal Th17 cells of individuals naïve of c-ART (NT, n=6; 1.3±0.5%) or who have initiated c-ART during chronic phase of infection (CHI, n=14; 2.8±0.2%) as compared to HIV-negative individuals (HIV-, n=7; 4.0±1.4%)(P=0.01 and P=0.005 respectively). IL-22$^+$T cells were significantly decreased in NT (5.0±1.8%) but were restored in CHI patients (9.5±0.9%) as compared to HIV-(10±1.8%). In contrast, patients who initiated ARV at early phase of HIV-1 infection (PHI, n=11) maintained a normal range of IL-17 and IL-22 secreting T cells (3.7±0.4% and 7.6±1.3% respectively). The frequency of Treg was the same in all groups. Importantly, as compared to the Treg/Th17 balance in HIV-control (0.2±0.02), the Treg/Th17 ratio remains increased in CHI and in NT (0.4±0.05 and 0.9±0.3, P=0.01) while PHI patients maintained a normal ratio (0.3±0.02). ARV initiation at PHI leads to a better Treg/Th17 restoration as compared to CHI despite a control of HIV replication. These results provide a strong rationale for ARV initiation at the PHI.

T.85. SIgA Enhances Acinetobacter Baumannii Gastrointestinal Colonization

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Multi-drug resistant Acinetobacter baumannii is of major concern for both hospitals and military personnel. In addition, reports suggest a link between gastrointestinal colonization and persistent infection. While predominantly associated with respiratory infections, persistence of the organism within mucosal compartments may be due to degradation of secretory IgA (SIgA), the primary humoral defense mechanism associated with mucosal tissues, resulting in formation of free secretory component (SC). Counterintuitively, we also have observed the requirement of SIgA in *A. baumannii* colonization and infection using C57BL/6: IgA$^{-/-}$ knockout and wild type (WT) counterparts, with mice deficient in IgA resistant to A. baumannii gastrointestinal infection compared to WT. Full body live *in vivo* imaging using bacteria labeled with a near-infrared fluorescent dye further supported these findings. Additionally, intestinal tissue sections collected from IgA$^{-/-}$ mice exhibited significantly ($p<0.001$) reduced bacterial adherence compared to WT, although IgA$^{-/-}$ mice retained substantial bacterial adherence. These results, in combination with our *in vitro* assays examining *A. baumannii* degradation of SIgA, suggest that SC may mediate *A. baumannii* mucosal colonization. Ongoing studies to identify the enzyme(s) responsible for degradation of SIgA by *A. baumannii* may lead to identification of new drug targets to treat these multi-drug resistant infections.

T.86. Dietary Induced Vitamin D3 Deficiency Increases Susceptibility to *Citrobacter Rodentium* Infection

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Vitamin D deficiency is linked to an increased risk of developing inflammatory-mediated diseases, including bacterial infections and inflammatory bowel disease (IBD). Pathogenic strains of Escherichia coli are important causes of infectious diarrhea, with EPEC contributing to ~1 million infant deaths per year in developing nations. Mucosa associated *E. coli* has been found in greater numbers in IBD patients compared to healthy controls, and these microbes have been shown to play a role in driving inflammation. Since pathogenic strains of *E. coli* do not colonize mice, researchers rely on the related mouse-specific *Citrobacter rodentium* (Cr). It is hypothesized that VD3 deficiency will increase susceptibility to Cr infection. Weanling C57Bl/6 mice were fed either VD3 deficient or sufficient diets for five weeks and then infected with Cr. At day 10 pi, VD3 deficient mice had 10x higher cecal bacterial burdens, indicating higher colonization of Cr. Histologically, the ceca of VD3 deficient mice were most damaged, with increased ulceration, edema, hyperplasia, and cell sloughing. VD3 deficient mice also had higher cecal expression of pro-inflammatory cytokines, IL-1β and IL-6 (qPCR). These results show that dietary VD3 is protective during Cr infection; however further studies are required to clarify how VD3 protects the host.

T.87. Impaired Innate Immunity to Enteric Viral Infection Following Exposure to a Helminth Parasite

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In regions where helminth infections are endemic there are high rates of exposure to enteric viral pathogens including rotavirus, hepatitis A virus and norovirus. Despite our understanding that helminth-induced Th2 cytokine responses can antagonize cytolytic antiviral CD8 T cells, the innate immune mechanisms underlying this impairment are poorly defined. Here, we employ a helminth co-infection system with murine norovirus (MNV) (a murine model of intestinal norovirus infection) to investigate how fundamental alterations in innate immune cell populations affect the generation of protective antiviral T cell responses in the gut. Macrophages isolated from helminth-infected animals displayed an alternatively activated phenotype and produced high levels of Relmα, YM1 and arginase. The presence of alternatively activated macrophages (AAMacs) in helminth co-infected mice was associated with increased susceptibility to MNV infection. Using novel tetramer reagents to track the CD4 and CD8 T cell response to murine norovirus (MNV), we demonstrate that the increased presence of AAMacs resulting from prior helminth infection impairs MNV-specific T cell responses and diminishes antiviral immunity. Collectively, these studies provide novel insight into how helminth infection can impair antiviral immunity and suggest that vaccine strategies targeting innate immune pathways could improve vaccine efficacy of in the context of helminth co-infection.

T.88. Crosstalk Between IL-22 and IL-18 Mediates Small Intestine Inflammation During *Toxoplasma Gondii* Infection

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Toxoplasma gondii infects 30% of the human population with potential complications in pregnant women and immunodeficient people. In a murine model of oral infection by *T. gondii*, both IL-18 and IL-22 have been shown to enhance necrosis in the ileum. IL-18 is well characterized as a pro-inflammatory cytokine that enhances Th1 responses, a major driver of the immune response to *T. gondii*. On the other hand it was previously unclear how IL-22 exacerbates inflammatory responses in this model. Here we describe a positive feedback loop in the production of IL-18 and IL-22, leading to a massive inflammation of the ileum. Using IL-18 deficient mice, we show that IL-18 induces IL-22 production from T cell subsets and intra-epithelial lymphoid cells during *T. gondii* infection. Moreover, injection of recombinant IL-18 in mice directly increases mRNA and protein levels of IL-22 in mouse ileum. More interestingly, we find that IL-22

in turn triggers the production of IL-18 from epithelial cell in the ileum, which completes the auto amplification loop between IL-18 and IL-22. In summary, our findings demonstrate for the first time a bi-directional cross talk between IL-22 and IL-18 in the small intestine that facilitates the development of *T. gondii*-induced immunopathology.

T.89. Morphine Treatment Increases Citrobacter Rodentium Virulence and Disrupts Infection Induced IL-17a Immune Response in Mice

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Opioids induce immunosuppression and bowel dysfunction leading to increased susceptibility to bacterial and opportunistic infections (*The American Journal of the Medical Sciences* 2012, 343:277). It is unclear how opioids modulate bacterial virulence and mucosal host defense against intestinal infection. *Citrobacter rodentium* is a natural mouse pathogen that models intestinal infection by enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) in humans and causes attaching and effacing (A/E) lesions and colonic hyperplasia (*Journal of Clinical Microbiology* 2000, 38:4343). The expression of most virulence genes in *C. rodentium* is controlled by a regulator, Ler, a member of the histone-like nucleoid structuring (H-NS) protein family (PNAS 2004, 101:3597). Our study shows that morphine treatment resulted in increased expression of the virulence factor, Ler. Furthermore, we observed increased infiltration/adherence of bacteria to the gut epithelium by fluorescence in situ hybridization (FISH) on intestinal cryostat sections. Meanwhile, *C. rodentium* intestinal infection induced IL-17a expression was attenuated in morphine treated animals compared to placebo treated groups. This is the first study to demonstrate that morphine modulates virulence factor-mediated adhesion of pathogenic bacteria and induces disruption of mucosal host defense during *C. rodentium* intestinal infection in mice.

T.90. Differential STAT3 Signaling in Innate Lymphoid Cells Mediates Host Defense Against Mucosal Infection

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STAT3 inhibitors can target STAT3-dependent tumorigenesis but patients often develop diarrhea from unknown mechanisms. Our data show that inhibiting STAT3 increases morbidity and mortality after intestinal *Citrobacter rodentium* infection, which depends on both innate and adaptive immunity. As a transcriptional factor, STAT3 controls RORγt expression on Th17 and presumably innate lymphoid cells (ILCs). We have unexpectedly revealed that STAT3 regulates RORγt expression in Th17 but not ILCs. Furthermore, we observed that STAT3 in RORγt$^+$ ILCs, but not in RORγt$^+$ Th17 cells, was crucial for protection from infection. We further demonstrated that activated STAT3 could directly bind the Il22 locus. Moreover, exogenous IL-22 rescued the mice conditionally deficient of STAT3 in RORγt$^+$ lymphocytes from lethal infection. Together, our data show that cancer therapies that utilize STAT3 inhibitors increase the risk for pathogen-mediated diarrhea through direct suppression of IL-22 from gut ILCs rather than the adaptive Th17 cells.

T.91. Lack of Skin Th17/Th22 CD4$^+$ T Cells and Antimicrobial Peptides (AMP) in Lesions of Hidradenitis Suppurativa (HS) Leads to a Vicious Circle Perpetuating Chronic Inflammation

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The medical treatments in use for HS (antibiotics and immunosuppressive agents) point out to a deregulated immune response against the microflora and lead us to investigate the hypothesis of a dysbalance between the Th17/IL-22/Treg axis in HS. In the blood, HS patients exhibited a higher frequency of CD4$^+$IL-17A$^+$(2.1%) and CD4$^+$IL-22$^+$(1.3%) as compared to controls (1.2% and 0.5%, respectively) ($P<0.05$) whereas no differences in the frequency of Treg or CD4$^+$T cells expressing skin homing receptors were found. Stimulation of PBMC from HS with flagelline or Staphylococcus aureus led to a higher production in supernatants of IL-17, IL-22 and IFN-g as compared to controls. In the skin,

expression of RORgt mRNA was decreased in HS (n=9) contrasting with a higher expression of Foxp3 mRNA as compared to controls (n=12). HIC confirmed an infiltration of Treg but not Th17 cells in HS. A low expression of AMP mRNAs (LL-37,hBD2,PS100A7) was found in HS skin lesions. Despite high frequency in the blood of functional Th17/Th22 T cells, these cells are rare at the sites of HS skin inflammation and local production of AMP, key trigger of T cell homing, remains low. These results point out a vicious circle perpetuating chronic inflammation in HS.

T.92. Inflammatory Cytokines Specifically Regulate Endoplasmic Reticulum Stress in Mucosal Secretory Cells During Infection and Inflammation

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Endoplasmic reticulum (ER) stress is implicated in the aetiology of mucosal inflammatory diseases. The affected tissues in these diseases contain highly secretory cells that are most likely to be affected by ER stress. However, the contribution of inflammation in initiating/exacerbating ER stress has remained unclear. We have evidence that unequivocally demonstrates that inflammatory cytokines either directly induce or suppress ER stress in secretory cells. *In-vivo* TH1/TH17-focussed responses to mucosal pathogens result in ER stress and secretory cell failure, whereas in TH2-focussed responses secretory cell protein production is high without ER stress. An *in-vitro* screen showed that IFNγ, IL-17A/F, IL-23, IL-24 and IL-33 drive protein misfolding/ER stress and block mucin biosynthesis in intestinal/respiratory goblet cells via several distinct mechanisms. In contrast, IL-10 and IL-22 suppressed cytokine-induced and chemically-induced ER stress and allowed continued mucin biosynthesis in a STAT1/STAT3-dependent manner, *in-vivo* and *in-vitro*. We propose that cytokine-induced ER stress has evolved as a mechanism to prevent viral protein synthesis by secretory cells. However, in sterile inflammatory disease these pathways can exacerbate disease, e.g., mucosal mucus depletion exposes the epithelium to microbes which contributes to the progression of disease. Therefore targeting ER-stressor or replenishing ER-suppressor cytokines could restore protein production in mucosal diseases.

T.93. GD3 Ganglioside Augments Foxp3$^+$ T Regulatory (Treg) Cell Responses in a Rat Model of Necrotizing Enterocolitis (NEC)

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Background: NEC is the most devastating gastrointestinal emergency in newborns. It is characterized by an acute uncontrolled inflammatory response to bacterial colonization, leading to intestinal necrosis. While preterm birth, infection, and early formula feeding have all been identified as major risk factors for NEC, the pathogenesis of this disorder is unclear, and effective treatment strategies remain elusive. Gangliosides, glycosphingolipids rich in colostrum and membrane microdomains, promote enterocyte growth and differentiation, and modulate Th1/Th2 response. Using an *in vitro* NEC model, gangliosides have been shown to ameliorate intestinal injury. However, their potential role in modulating immunoregulatory mechanisms in NEC is unknown. We evaluated the effects of dietary GD3, the most predominant ganglioside in rat neonatal intestine, on clinicopathological expression and ileal Foxp3$^+$ Treg immune responses in a rat model of NEC. Methods: Newborn rats were gavage-fed formula (NEC) or formula supplemented with 15 ug/ml GD3 (GD3-NEC). Dam-fed littermates served as controls (DF). NEC was induced by asphyxia and cold stress. At 96 hours, ileal gross and histological changes were evaluated. Relative ileal cytokine profiles, Foxp3 expression and Foxp3$^+$ cell numbers were analyzed. Results: GD3 decreased the incidence, gross and histopathological severity of NEC. Ileal Foxp3 expression and Foxp3$^+$ cell numbers were significantly decreased in NEC group compared with DF. GD3 significantly increased ileal Foxp3 expression and Foxp3$^+$ cell numbers, in association with marked upregulation of Th2 chemokines, tissue inhibitor of metalloproteinases 1 (TIMP-1), IL-1 receptor antagonist (IL-1ra). Conclusion: Our data suggest that dietary GD3 protects newborn rats from NEC, in part, by augmenting mucosal Foxp3$^+$ Treg immune responses.

T.94. CD45 is Protective in Dextran Sodium Sulphate-Induced Colitis
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CD45 is a leukocyte specific tyrosine phosphatase that severely affects lymphocyte development and activation by regulating Src family kinases. CD45 also regulates macrophage adhesion and TLR-induced proinflammatory cytokine production in dendritic cells. Here, we investigated the function of CD45 in innate and adaptive immune responses in the gut using a model of dextran-sodium sulfate (DSS)-induced intestinal inflammation. CD45 deficient mice (CD45KO) have increased susceptibility to colitis compared to wild-type mice. Despite having greatly reduced T cell numbers in the periphery, CD45KO mice have equivalent T cell numbers in the lamina propria both prior to and post colitis. These T cells showed increased IFN-γ and IL-17A production. To determine if this was a T cell intrinsic or extrinsic effect, we generated CD45-recombinase activating gene 1 deficient mice (CD45RAGKO) that lack T and B cells. Like CD45KO mice, CD45RAGKO mice were more susceptible to DSS-induced colitis compared to RAGKO mice. CD45RAGKO mice have increased expression of IL-23 in the colon. This suggests that the lack of CD45 on antigen-presenting cells leads to hyperproduction of IL-23 upon microbial stimulation which contributes to the increased activation of effector cells that drive the inflammatory response in the gut.

T.95. Attenuation of Lipopolysaccharide-Induced Lung Inflammation by Glucosamine
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Acute inflammation is often noted during acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). Glucosamine is known to act as an anti-inflammatory molecule. The effect of glucosamine on acute lung inflammation and its associated mechanisms remain unclear. The aim of the present study was to address how glucosamine plays an anti-inflammatory role in acute lung inflammation *in vivo* and *in vitro*. Using the LPS intratracheal instillation-elicited rat lung inflammation model, pulmonary edema and PMN infiltration, as well as the production of TNF-α, IL-1β, cytokine-induced neutrophil chemoattractant (CINC)-1, macrophage inflammatory protein (MIP)-2 and nitric oxide (NO) in the bronchoalveolar lavage fluid (BALF) and the cultured medium of BALF cells, were found to be attenuated by glucosamine. Expression of TNF-α, IL-1β, IFN-γ, CINC-1, MIP-2, monocyte chemotactic protein (MCP)-1 and inducible NO synthase (iNOS) in LPS-inflammed lung tissue was also suppressed by glucosamine. Using a rat alveolar epithelial cell line L2, cytokine mixture (cytomix)-regulated production and mRNA expression of CINC-1 and MIP-2, NO production, protein and mRNA expression of iNOS, iNOS mRNA stability and iNOS promoter activity were all noted to be inhibited by glucosamine. Furthermore, glucosamine reduced LPS-mediated nuclear factor (NF)-κB signaling by decreasing IκB phosphorylation, p65 nuclear translocation and NF-κB reporter activity. Overexpression of the p65 subunit restored the inhibitory action of glucosamine on cytomix-regulated NO production and iNOS expression. Taken together, glucosamine appears to act as an anti-inflammatory molecule in LPS-induced lung inflammation, at least in part by targeting to the NF-κB signaling pathway.

T.96. Ghrelin Receptor Modulates CD4 T Cell Function During Intestinal Inflammation
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Introduction: Recent work suggests that the orexigenic hormone ghrelin have potent anti-inflammatory properties in preclinical model of intestinal inflammation. The effects of ghrelin are mediated via the growth hormone secretagogue receptor (GHSR). The expression of this receptor has been reported on T cells, thus we decided to investigate the effect of ghrelin on T cell during colitis. Methods: T cell transfer chronic colitis was induced in the $Rag1^{-/-}$ knockout mice by adoptive transfer of naïve CD4 T helper cells from wild-type (WT) or $GHSR^{-/-}$ knockout mice. Development of colitis and the degree of intestinal inflammation was monitored over time. Results: The lack of the ghrelin receptor on CD4-transferred T cells significantly worsened the course of colitis in $Rag1^{-/-}$ mice. Indeed, $Rag1^{-/-}$ mice reconstituted with $GHSR^{-/-}$ CD4 Ths (GHSR→Rag1) showed colitis symptoms significantly earlier than $Rag1^{-/-}$ mice reconstituted with WT CD4 Ths (WT→Rag1). In line, GHSR→Rag1 mice displayed increased body

weight loss and higher diarrhea score compared to WT→Rag1. In addition, GHSR→Rag1 mice had significantly increased spleen weight and shortening of the colon compared to WT→Rag1. Histological analysis showed a higher lesion score in GHSR→Rag1 mice compared to WT→Rag1 mice as well as higher level of inflammatory cytokines in the gut. Conclusions: Our observations strongly suggest that ghrelin may significantly ameliorate experimental chronic colitis by modulating T helper effector cell function in the gut.

T.97. Longitudinal Analysis of the Impact of Commercial Sex Work in HIV Exposed Seronegative (HESN) Sex Workers from Nairobi, Kenya

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Background: The Pumwani Sex Workers Cohort, Kenya, contains a small sub-group of women who are intensively exposed to HIV-1 yet remain uninfected. HIV Exposed Seronegative (HESN) sex workers naturally harbour low levels of immune activation. Sex work results in exposure to strong stimulants and affects the level of immune activation. A better control of mucosal immune activation in response to these stimulants may limit targets availability in the genital tract and prevent the establishment of a productive HIV infection. Methods: Cervical and peripheral lymphocytes from HESN, newly enrolled negative and HIV-positive sex workers that interrupted sex work for 4-8 weeks were collected before and after the break period and 6-9 months following the resumption of commercial sexual activities. Activation markers were measured by flow cytometry. Results: We observed a reduction of immune activation markers during the sex work break among HIV-positive women. In the vaginal mucosa of HESNs, levels of immune activation remained constant while they increased after resuming sex work in new negative controls to reach higher levels compared to HESNs. Conclusion: A better capacity to maintain low levels of immune activation in the vaginal tract following work interruption may contribute to protect HESNs from acquiring HIV infection.

T.98. The Role of Th17 and VEGF in LPS Induced Rhinitis Model

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Lipopolysaccharide (LPS) is a cell wall component of gram negative bacteria and is important for pro-inflammatory mediators. We aimed to establish a rhinitis model using ovalubumin (OVA) and LPS and to evaluate the role of IL-17 and VEGF in pathogenesis of LPS induced non-eosionophilic rhinitis. Balb/C mice were sensitized with OVA and 1ug or 10ug of LPS, and challenged with intranasally with OVA and multiple parameters of rhinitis were evaluated to establish the LPS induced rhinitis model. IL-17 knock-out mice were used to check if this LPS induced rhinitis model were dependent on IL-17. Finally, pan-VEGFR inhibitor, SU-5416, was administered to see the effect of VEGF in this model. Results: In LPS induced rhinitis model, eosinophil infiltration was decreased, neutrophil infiltration was increased in the nasal mucosa, and systemic and nasal IL-17 and interferon-gamma were increased as compared with OVA induced allergic rhinitis model. Those findings were LPS dose dependent. In IL-17 knockout mice, those phenotypes (neutrophil infiltration, IL-17 and interferon-gamma) were reversed, showing IL-17 dependency of LPS induced rhinitis. VEGF inhibitor, SU-5416 inhibited symptom score, neutrophil infiltration, systemic and nasal IL-17 increase in LPS-induced rhinitis model, suggesting mediating role of VEGF in LPS induced rhinitis. Conclusion: We established a LPS induced rhinitis model, which is dependent on IL-17. VEGF played an important mediating role in that model.

T.99. Combined CCR7 and RORγt-Deficiency Causes Transition from an Autoimmune-Prone State to Fatal Autoimmune Disease

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The balance between immunity and tolerance is regulated by numerous molecular factors including

nuclear hormone and homeostatic chemokine receptors. Members of both families are involved in functional lymphoid organogenesis and lymphocyte homeostasis. We investigated the combined function of the nuclear hormone receptor RORγt and the chemokine receptor CCR7 in spontaneous progression from an autoimmune-prone state to lethal mucosal autoimmune disease. Double-deficient $Ccr7^{-/-}Ror\gamma t^{-/-}$ mice succumbed to acute mucosal inflammation with massive inflammatory cellular infiltrates, pro-inflammatory cytokine and autoantibody production, and wasting disease. Antibiotic-treatment reduced the gut microflora and abrogated the development of mucosal inflammation. RORγt-deficiency alone imposes an autoimmune-prone state associated with spontaneous germinal center formation, an increased proportion of follicular B helper T cells, and autoantibody production. Commensal bacteria and a confined tissue-specific inflammatory milieu supported by CCR7-deficiency serve as complementary trigger to initiate the lethal pathophysiologic process in $Ccr7^{-/-}Ror\gamma t^{-/-}$ mice.

T.100. Pre-Treatment with ω3 Fatty Acid Allevietes Mucositis in BALB/C Mice

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Mucositis is a common side effect to cancer treatment and may predispose to intestinal damage. The administration of immunomodulators such as ω3 fatty acid should be an effective treatment to minimize those effects. The aim of this study was to evaluate the effects of ω3 fatty acids in an experimental model of mucositis. The experiments were procedure with BALB/c. Groups: Control (CTL), Mucositis (M), $Control^{+}\omega3$ ($CTL^{+}\omega3$) and $Mucositis^{+}\omega3$ ($M^{+}\omega3$). The animals of ω3 group received a diet prepared with ω3 fatty acid (50% of total lipid content in the diet) during ten days before mucositis induction. Others mice received conventional AIN93G. On the tenth day the animals received an intraperitoneal injection of 200mg/kg of 5-FU (fuorouracil) for mucositis induction. After 72 hours, permeability was analyzed using DTPA labeled with Tc-99m. Intestines were collected for histology analysis and cytokine measurement. Intestinal permeability was higher in M compared with CTL, but similar in M and $M^{+}\omega3$. Histological data showed maintenance of villous height in M^{+} ω3. The treatment decreased IL-6 production in ileum. There was no significant difference in the IL-10, INF-γ and IL-4 levels. Mucositis changes in the intestinal mucosa and increase permeability. The pre-treatment with ω3 appears to alleviate the intestinal damage.

T.101. Angiotensin II Receptor Blockade Inhibits TGFBRII Signaling in Epithelial Cells

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We reported enteropathy in patients on angiotensin receptor blocker ARB therapy. ARBs may inhibit the effects of TGFb in fibroblasts. Treatment of fibroblasts with losartan, an ARB, inhibits this TGFbRII-induced translocation of psmad2/3 to the nucleus. Aims: To examine the cell types responsible for injury and if ARBs inhibit TGFb-induced PSMAD2/3 translocation in human intestinal epithelial cells. Methods: Duodenal biopsies were fixed in formalin and paraffin embedded (FFPE) and stained for CD4, CD8 and Foxp3. Cultured CaCo-2 cells were treated with TGFb. Treatment of Caco-2 cells with losartan was done at 30 minutes before or simultaneously with TGFb treatment. Results: Extensive inflammatory infiltration was present in the epithelium, lamina propria, and occasionally the crypts. This resolved after discontinuation of olmesartan. $CD4^{+}$ cells and $CD8^{+}$ cells comprised the majority of the infiltrate, and $CD8^{+}$ cells outnumbered the $CD4^{+}$ cells in all three regions. There was no change in the number of $CD4^{+}$ cells or $Foxp3^{+}$ cells following discontinuation of olmesartan. Pre-treatment of CaCo-2 cells with losartan 30 minutes before TGFb was added, inhibited nuclear translocation of psmad2/3. Simultaneous treatment of CaCo-2 cells with both TGFb and losartan did not effectively block nuclear translocation of psmad2/3. Conclusion: This retrospective study suggests that the inflammation seen in ARB associated enteropathy is a T cell based disorder with predominately CD8 cells and occurs despite abundant $Foxp3^{+}$ cells. Losartan, an ARB can effectively block the nuclear translocation of psmad2/3 that is normally induced by TGFbRII signaling; however, it must be given before TGFB application in epithelial cells. TGFb inhibition may be a potential mechanism to explain ARB associated enteropathy.

T.102. Opioid-Mediated Inhibition of Visceral Inflammatory Pain by Colitogenic CD4$^+$ T Lymphocytes

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Inflammatory bowel diseases (IBD) are characterized by an uncontrolled inflammatory response to colonic luminal content involving both innate and adaptive immunity. Thus, a dysregulated response of CD4$^+$ T lymphocytes against microbiota highly contributes to the development of IBD. We have recently shown that effector CD4$^+$ T lymphocytes generated in response to microbe-derived antigens alleviate somatic inflammatory pain, through the local release of opioids. Here, we investigated whether colitogenic CD4$^+$ T lymphocytes that accumulate within inflamed colon also produce opioids and are able to counteract inflammation-induced visceral pain. For this purpose, we used the chronic colitis model induced by the transfer of naïve CD4$^+$CD45RBhigh T lymphocytes into immune-deficient mice. We show that colitogenic CD4$^+$ T cells including Th1 and Th17 lymphocytes but not macrophages and epithelial cells isolated from inflamed colon six weeks after T cell transfer, expressed high level of endogenous opioids. The local release of opioids by colitogenic CD4$^+$ T lymphocytes was responsible for a significant reduction of inflammation-associated visceral hypersensitivity as assessed by colorectal distension. Thus, Colitogenic Th1 and Th17 which promote intestinal inflammation and colonic tissue damage exhibit an opioid-mediated analgesic activity which paradoxically reduces abdominal pain.

T.103. Recruitment of Effector CD4$^+$ T Lymphocytes into Inflamed Intestine Reduces Visceral Hypersensitivity in Mice

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It has been reported that visceral pain sensitivity is increased in mice exposed five days to Dextran Sodium Sulfate (DSS) (acute colitis), while it remained unchanged in mice treated with three cycles of DSS (chronic colitis). The higher density of CD4$^+$ T lymphocytes within mucosal tissue in chronic form of the DSS-induced colitis argued for an endogenous regulation of visceral sensitivity by mucosal T lymphocytes. Here, we demonstrate that mobilization of opioid-producing effector CD4$^+$ T lymphocytes within the intestinal mucosa during the acute phase of DSS-induced colitis significantly reduces visceral hypersensitivity. BALB/c mice were transferred with syngeneic naïve transgenic anti-ovalbumin (OVA) CD4$^+$ T lymphocytes and then immunized against OVA to actively generate opioid-producing effector CD4$^+$ T cells. Six weeks later, acute colitis was induced by adding DSS to the drinking water of the mice. Given that local opioid release by effector CD4$^+$ T cells requires the presence of cognate antigens, OVA was added together with DSS. Our results show that DSS-induced visceral hypersensitivity was significantly reduced in mice fed with OVA but not with BSA as assessed by colorectal distension. Thus, our study shows that recruitment of effector CD4$^+$ T cells into inflamed intestine decreases visceral inflammatory pain.

T.104. An Elastolytic Protease is Expressed and Secreted by Human Colonic Epithelium and Could Participate to IBD Damage

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Proteases have a crucial role in the persistence of chronic inflammatory responses of the gastro-intestinal tract. We have previously shown that colonic biopsies from IBD patients released higher elastolytic activity together with a lower expression of ELAFIN, an endogenous elastase inhibitor, as compared to healthy biopsies. The imbalance between proteases and their inhibitors appears to be crucial in the development of Inflammatory Bowel Diseases (IBD). In situ zymography reveals that enterocytes from healthy human colon is a major source of elastolytic activity, which is greatly enhanced in IBD conditions. Immunostaining shows that a candidate elastase is expressed in epithelial cells from colonic crypt. Notably, this protease is secreted into the lumen. In IBD, candidate elastase expression is increased in the remaining epithelium. In addition ELAFIN tightly inhibits this protease. Therefore, as ELAFIN expression is decreased in epithelial cells from IBD patients, we hypothesized that uncontrolled candidate

elastase participates to higher elastolytic activity in IBD mucosa. Our result highlights the role of epithelial cells as major actors in the dysregulation of balance between proteases and their inhibitors in IBD.

T.105. Differences Between IL-17A and IL-17RA Blockade in Psoriasis and Asthma and the Role of IL-17C

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IL-17 family cytokines have been implicated in a number of autoimmune diseases. In the skin, IL-17A produced by Th17 cells, γδ T cells and innate lymphoid cells is a key driver of pathogenesis in psoriasis. In contrast, IL-25 has been associated with promotion of Type 2 immunity and diseases of mucosal surfaces, such as asthma. IL-17RA is a necessary receptor for multiple IL-17 family cytokines, including IL-17A, IL-17F and IL-25. Recently, it has been shown that IL-17C also requires IL-17RA, plus the IL-17 receptor family member IL-17RE, for its function. To explore the role of IL-17C we over-expressed IL-17C in mice using hydrodynamic DNA injection. IL-17C-induced serum G-CSF was inhibited with an IL-17RA-specific antibody and lost in mice lacking IL-17RA or IL-17RE confirming that IL-17RA and IL-17RE are necessary for IL-17C-induced responses. We have gone on to assess the contribution IL-17C and IL-17RE make to inflammation in both the skin and lung, using a TPA treatment model of psoriasis and the murine OVA model of asthma. Results will be presented.

T.106. HpEurope, Not HpMauri, Genotype Dominates in *H. Pylori*-Infected Easter Island Residents with Gastrointestinal Symptoms

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Phylogenetic analysis using multilocus sequence typing (MLST) for housekeeping genes has become a fundamental tool to trace human migrations carrying ancestral *H. pylori*. In Polynesia, where the HpMauri genotype dominates, Easter Island offers a unique opportunity to characterize regional variation in *H. pylori* strains in Polynesia's most eastern island. Eleven Rapa Nui people with gastrointestinal symptoms were infected *H. pylori*. Gastric biopsies were evaluated for histology, and *H. pylori* for CagA, vacA alleles and genotype using PCR and MLST. Six patients were infected with *H. pylori*: four had mild chronic gastritis, one intestinal metaplasia and one focal glandular atrophy. Five patients had *H. pylori* from which bacterial DNA was isolated: all isolates contained CagA sequences, and three isolates contained the vacA s1 allele, one the m2 allele and four the m1 allele. Surprisingly, MLST showed that *H. pylori* strains from all the Easter Island patients corresponded to *H. pylori* of European, not Mauri, origin, similar to the dominance of HpEurope in continental Chile. We speculate that HpEurope was introduced by the Dutch when they colonized Easter Island in 1722. More robust diversity in HpEurope may have allowed this genotype to out compete the indigenous HpMauri genotype. Supported by Fondecyt #1100654 and #1085232.

T.107. G-Protein Regulatory (GPR) Motif in Activator of G-Protein Signaling 3 (AGS3) Protein Regulates SDF1α-Induced MUC1 Gene Expression

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Mucus overproduction and airway obstruction are common features in airway mucosal inflammation. The mechanism by which SDF1α induces MUC1 overexpression, however, has not been fully explored. The aims of this study were two-fold; firstly, to examine the Activator of G-protein signaling (AGS) 3-dependent mechanism by which SDF1α reduces MUC1 gene expression, and secondly, to identify specific molecules which could suppress SDF1α-induced MUC1 expression at a G-protein coupled receptor level. Here, we suggest that SDF1α induces MUC1 gene expression via CXCR4 receptor. In addition, we showed that AGS3 plays as a suppressor for SDF1α-induced MUC1 and TNFα gene expressions by regulating with Gαi. More interestingly, G-protein Regulatory (GPR) motif in AGS3 bound to Gαi and decreased MUC1 expression, whereas increased TNFα expression. In addition, GPR mutation (DDQR→DDAR) increased MUC1 expression, but decreased TNFα, IL-6, and IL-8 expressions. We

suggest that GPR mutation peptide play as suppressive compound to decrease airway inflammation. These results give additional insights into the molecular mechanism of negative regulation of mucin overproduction and enhance our understanding of mucus hypersecretion during airway mucosal inflammation.

T.108. Probiotic Bacterium, *Lactobacillus Gasseri* SBT2055-Induced β-Defensin Prevents Porphyromonas Gingivalis-Medicated Periodontal Disease

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Probiotic strains of bacteria have extensively been studied for their potential therapeutic effects. However, the precise mechanisms by which they modulate the immune system are poorly understood. Periodontal disease has been suggested as a potential risk factor for systemic diseases including cardiovascular diseases, diabetes, and osteoporosis. In this study, we assessed the role of probiotic, *Lactobacillus gasseri* SBT2055 (*L. gasseri*) for the prevention of periodontal disease caused by *Porphyromonas gingivalis* (*P. gingivalis*). *L. gasseri* was orally administered 21 consecutive days before *P. gingivalis* infection (Treated). Mice without oral *L. gasseri* treatment were employed as controls (Non-treated). Thirty days after the last infection with *P. gingivalis*, *L. gasseri* treated mice showed significant reduction of alveolar bone loss when compared with controls. Further, infiltration of lymphocytes in fibrotic tissues was diminished in gingival tissues of *L. gasseri*-treated mice. Interestingly, the level of β-defensin-specific mRNA expression was significantly increased in oral mucosa of *L. gasseri*-treated mice which persisted additional 15 days post *P. gingival* infection. These results suggest that oral *L. gasseri* treatment up-regulates β-defensin production in the oral mucosa for the prevention of periodontal disease.

Poster Session: Friday, July 19

F.1. Immune Responses to Vaccinia Virus-Induced Lung Pathogenesis *in vivo*

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Vaccinia virus is a large DNA virus that infects many cell culture *in vitro* and animal species *in vivo*. Although the virus has been used widely as a vaccine, the cell entry pathways are complex in nature and varied among different virus strains and cell types. Recently, we showed that vaccinia A25/A26 proteins are determinants for virus entry into HeLa cells through endocytosis (i.e. wild-type virus) whereas virus mutants deleting these two proteins entered HeLa cells through plasma membrane fusion (i.e. ΔA26 virus). Here we want to compare two vaccinia virus for their virulence *in vivo.* First, we have investigated the immune responses in bronchial lavage fluid (BALF) extracted from lungs of the infected animals at different days after wild type vaccinia virus infections. FACS analyses showed an increase of CD8 T cells in BALF at 6-10 days p.i. and slow a decrease of CD11c-positive dendritic cells/macrophages. Inflammatory cytokines such as IL-6 and TNF-α were detected. In addition, IHC showed that abundant viral Ag staining were detected in the respiratory organs of mice infected by wild type virus. Virus titers were detected in lungs, spleens, livers and brains isolated from the infected mice in order to understand the contribution of viral A26 protein in virus-induced pathogenesis. Next, we will infect mice with ΔA26 mutant virus and analyze the pathology, virus titers and immune responses triggered by ΔA26 virus. These results will aid in our understanding of viral factors contributing to in vivo pathogenesis in hosts.

F.2. Enteric Neurons Mediate the Anti-Inflammatory Effect of the Vagus Nerve During Intestinal Inflammation

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Background: The cholinergic anti-inflammatory pathway has been proposed as a key mechanism by which the brain, through the vagus nerve, modulates the immune system. Vagus nerve stimulation (VNS) reduces intestinal inflammation. Here we investigated the neural circuit involved and the cells targeted by

the anti-inflammatory vagal effect in the gut. Methods: The effect of VNS on surgery-induced inflammation was investigated in wild-type, splenic denervated, Rag-1$^{-/-}$ and α7 nicotinic receptor (α7nAChR) deficient bone marrow chimera mice. Anterograde tracing of vagal efferents was performed to reveal the intestinal cells targeted by the vagus nerve. Using live $Ca2^{+}$ imaging, the interaction between enteric neurons and resident macrophages was studied. Results: VNS attenuates surgery-induced intestinal inflammation in wild type, splenic denervated and T cell deficient mice. This effect is mediated by α7nAChR expression on intestinal immune cells, as VNS was ineffective in α7nAChR deficient bone marrow chimera mice. Anterograde tracing reveals synapses of the vagus nerve with cholinergic neurons in close contact with resident macrophages. Stimulation of enteric neurons reduces ATP-induced activation of resident macrophages expressing α7nAChR. Conclusion: Our data demonstrate that the anti-inflammatory effect of VNS in the gut is independent of the spleen, but is mediated by cholinergic enteric neurons directly interacting with intestinal macrophages.

F.3. Prevention and Cure of Rotavirus Infection via Activation of Dendritic Cell TLR5 and NLRC4
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Viral infections, most of which cannot be treated or prevented, cause and/or promote many of the world's most pressing health problems. Consequently, we investigated a novel strategy to prevent and treat viral infection, namely activating endogenous pathways of innate immunity by administration of the bacterial protein flagellin, which is known to be a dominant target of the immune system at mucosal surfaces. Using murine rotavirus (RV) infection as a model of an acute diarrhea-inducing infection in young mice and a chronic infection in immune-deficient mice, we observed flagellin treatment could prevent or eliminate ongoing RV infection. Such protection was independent of adaptive immunity and interferon (Type 1 and 2), which is thought to be the major mediator of antiviral immunity, while requiring both known flagellin receptors, Toll-like receptor 5 (TLR5) and Nod-like receptor C4 (NLRC4), whose expression by dendritic cell was necessary and sufficient to mediate flagellin-induced protection against RV. Importantly, flagellin-mediated blockade of infection dramatically attenuated the severe diarrhea induced by RV in young mice. Thus, we report a novel means to prevent a leading cause of mortality in children. Moreover, the means by which flagellin prevents and treats infection may prove applicable to treating other acute and/or chronic viral infections.

F.4. Two Distinct Populations of Antigen-Presenting Cells in Human Distal Colonic Mucosa: Langerhans Cells Expressing CD1 and Macrophages Expressing CD209
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Mucosal surfaces, including those of the colon and rectum, are the first line of defense against many pathogens, but the antigen-presenting cells (APCs) that are the key regulators of innate immunity are not well-defined in these key tissues. Here we define the phenotypes and distribution of APCs in mucosae of normal human sigmoid colon and rectum. By immunohistochemistry, Langerhans cells ($CD1a^{+}/CD207^{+}$) were detected in the periphery of glands and sparsely scattered in the submucosa similarly in both tissues. However, the largest APC population was a macrophage-like population expressing CD14, CD68 and CD163. Confocal microscopy revealed co-localization of CD209 (DC-SIGN) with this macrophage-like population but not with dendritic cells. These results demonstrate the previously unconfirmed presence of Langerhans cells in these tissue compartments, and a predominance of macrophage-like APCs that express CD209. This information has key implications for the pathogenesis of transmission of infectious diseases such as HIV-1, as well as primary immune diseases involving the gut.

F.5. The Neurotransmitter GABA Exacerbates *Clostridium Difficile*-Associated Colitis and Alters Innate Immune Responses
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Background: *Clostridium difficile* infection (CDI) is a major cause of nosocomial antibiotic-associated

diarrhea and colitis. While identifying biomarkers of disease, we discovered an association between GABA, the major inhibitory neurotransmitter in the central nervous system, and disease recurrence. In addition to its role in the CNS, GABA also has an inhibitory role in the immune system. Objective: To determine the role of GABA in CDI pathogenesis. Methods and Results: C57BL/6 mice were treated with an antibiotic mixture $^{+/-}$ GABA in their drinking water and infected orally with *C. difficile*. Cecal and colons were collected and examined for histopathology and gene expression. Administration of GABA exacerbated CDI- induced mucosal inflammation and damage, whereas inhibition of GABA with picrotoxin reduced disease severity. To explore functional mechanisms, CACO-2 cells were stimulated with *C. difficile* toxin, LPS, or flagellin in the absence or presence of GABA. In contrast to its suppressive effects on LPS and flagellin stimulation, GABA significantly enhanced toxin induced secretion of chemokines involved in leukocyte recruitment and adhesion to the intestinal endothelium. Conclusion: Exogenous GABA promotes CDI disease pathogenesis by modulating innate immune responses. Decreasing GABAergic activity may be a new approach to treating and preventing CDI associated disease.

F.6. Examination of Intestinal Eosinophil Phenotype and Function During Inflammation and Steady State

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The intestinal lamina propria contains relatively high numbers of eosinophils during steady state, as well as inflammation. Eosinophil accumulation during inflammation is particularly pronounced during parasitic infections and certain inflammatory diseases of the gastro-intestinal tract, such as eosinophilic oesophagitis and inflammatory bowel disease (IBD). Despite this fact the function of gastro-intestinal eosinophils remains largely unknown. We have isolated and purified eosinophils from the large and small intestine of both mice and humans in healthy as well as diseased states by MACS and FACS. Our preliminary data suggests that there is much more plasticity among eosinophils with regards to phenotype, localization and function than previously believed. Indeed, eosinophils appear to be localized to different anatomical compartments in patients with IBD, and express different surface receptors than their blood-borne counterparts. Furthermore, murine eosinophils secrete distinct combinations of cytokines in response to various stimuli *in vitro*. Thus, it appears that both mouse and human eosinophils are far more varied than the dogma portrays them to be.

F.7. Type 1 Interferon Indirectly Induces Natural Killer (NK) Cell Interferon (IFN)-γ Production During Herpes Simplex Virus (HSV) Type 2 Infection

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HSV-2, alongside HSV-1, is the predominant cause of recurrent genital herpes and one of the most prevalent STIs worldwide. NK cells are primary responders to HSV-2 infection and release IFN-γ to control replication. Type 1 IFN is necessary for NK cell activation, though the mechanism is under debate and unknown during HSV-2 vaginal infection. We observed that NK cells lacking Type 1 IFN receptor ($IFNAR^{-/-}$), while unable to produce IFN-γ in $IFNAR^{-/-}$ mice, produced IFN-γ when transferred into an alymphoid recipient with functional Type 1 IFN signalling. This suggests that Type 1 IFN receptor is not required on NK cells for their activation, but is required in the environment. Dendritic cells may be involved as early IFN-γ production was reduced upon depletion of $CD11c^{+}$ cells. Administration of IL-15 complexed with IL-15Rα to $IFNAR^{-/-}$ mice, was unable to induce NK cell IFN-γ production during HSV-2 infection, suggesting that IL-15 is not involved in activating NK cell IFN-γ production. Overall, NK cells do not respond directly to Type 1 IFN for their activation during HSV-2 infection. Furthermore, this indirect mechanism of activation involves $CD11c^{+}$ cells, but not IL-15. In understanding this activation mechanism, we can develop therapeutics and vaccination strategies against HSV-2.

F.8. Properdin Plays a Protective Role in Intestinal Inflammation

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Background: Properdin plays multiple roles including as a pattern recognition receptor (PRR) for the removal of damaged cells, and to stabilize the C3 convertase of the complement alternative pathway. As

split products of the alternative complement pathway influence T cell differentiation and proliferation we hypothesized that properdin inhibition would reduce T cell-dependent colitis. Methods: Properdin/IL-10 double deficient mice were prepared and proved fertile. Colitis was accelerated in these and IL-10 deficient mice using piroxicam added to their food for 14 days. Intestinal inflammation, colon mediator levels (ELISA) and cleaved Caspase 3 (Western blot) were measured. Splenic $CD4^+$ T cells were stimulated *ex vivo* and cytokines measured. Results: Double deficient mice experienced significantly greater colon shortening, increased numbers and length of ulcers, increased levels of cleaved Caspase 3 and higher colonic and stimulated splenic T cell IFN- γ levels compared to IL-10 deficient mice. Double deficient mice had greater numbers of mucosal macrophages and $CD4^+$ T cells but not neutrophils. Blood from both strains was negative for bacteria. Conclusion: Contrary to our hypothesis, blocking properdin exacerbated the symptoms of colitis characterised by greater histological damage and apoptosis likely mediated by higher levels of IFN-γ. The role of properdin as a PRR in colitis seems more important than its role in regulating complement activation.

F.9. The Defining Role of the Mucin Muc2 in Paneth Cell Granule Secretion

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Paneth cells are granulated secretory epithelial cells localized to the base of small intestinal crypts, where they protect the mucosa. Although Paneth cells are known to produce the mucin Muc2, it is unclear whether Muc2 plays a role in the function of these cells. Previous studies have observed an electron-lucent layer surrounding each granule within the cell. Our results conclude that this layer is comprised of Muc2 and is completely absent in $Muc2^{-/-}$ mice. Loss of Muc2 leads to abnormalities in Paneth cell structure, with increased numbers of granules found closely packed together and in a disorganized manner compared to wild-type cells. Additionally, the $Muc\ 2^{-/-}$ Paneth cells exhibit decreased expression of various antimicrobial peptides. While $Muc2^{-/-}$ Paneth cells do not demonstrate signs of ER stress, they do show a noticeable accumulation of cytoplasmic autolysosomes. We thus conclude that in the absence of Muc2, the ordered secretion of Paneth cell granules is reduced, while the autophagic recycling of granules is increased to compensate. Together, these findings point to a key role for Muc2 in facilitating the secretory/antimicrobial properties of Paneth cells, and help clarify how Paneth cell function is regulated to promote intestinal mucosal health.

F.10. Dectin-1 Expressing Intestinal Macrophages Are Controlled by Retinoic Acid

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Retinoic acid (RA), the active metabolite of vitamin A, plays an essential role in the mucosal immune responses. Several cell types in the intestinal mucosa are involved in maintaining homeostasis, among which macrophages. Depending on their microenvironment, various macrophage subsets can be distinguished of which the artificial classification into the classically activated (caMø) or pro-inflammatory and alternatively activated (aaMø) or anti-inflammatory macrophages are the two extremes of a broad spectrum. Here we have addressed how RA influences macrophage differentiation. *In vitro* studies with murine bone marrow showed that RA induced macrophages to differentiate towards a phenotype that is most related to aaMøs. RA was shown to inhibit IL-12 expression in caMøs, whereas the expression of Arginase-1 and Dectin-1, both specific for aaMøs, were induced upon RA stimulation. Furthermore, our data showed that ligation of Dectin-1 enabled macrophages to rapidly switch their cytokine expression from an anti-inflammatory to a pro-inflammatory profile, and that intake of vitamin A was needed for Dectin-1 expression. Our results show a crucial role for RA in maintaining the intestinal macrophage phenotype, which is needed for both immunity and tolerance.

F.11. Influenza A Virus Infection Induces a Unique Nasal Innate Immune Response Associated with Dominant Secretion of Type III Interferon

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Interferon (IFN)-related innate immune system might be critical for controlling viral infection. Recent studies have started to reveal indispensable antiviral immune activities of Type 3 IFNs in respiratory epithelium. Our goal of this study is to explore the role of type III IFNs after influenza A virus (IAV) infection in antiviral innate immune response of nasal epithelium. Our results demonstrated that viral titer and mRNA level of IAV increased in normal human nasal epithelial (NHNE) cells after infection. In concert with viral titer, we found that the generation of IFNs, such as Type 1 IFN and Type 3 IFNs were induced until three days after IAV infection. These changes translated into increased activation of the transcription factor STAT1, STAT2 and increased expression of interferon-responsive genes (MX1, 2-5 OAS1, IP10 and CXCL10). Interestingly, viral titer and mRNA level were significantly higher in case of knock-down of type III IFNs' secretion. In addition, induction of type III IFNs was closely correlated with IFN-related innate immune responses against IAV infection in NHNE cells. Our findings suggest that production of Type 3 IFNs be primarily responsible for controlling IAV infection and were involved in induction of IFN-stimulating innate immune response in nasal epithelium.

F.12. The Role of Factors of Immunity in Hypertrophy of Adenoid Vegetations

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In fact, the adenoids tend to shrink after early childhood, and by the teenage years they often almost disappear completely. The aim of this investigation was the study of gene expression of molecules of innate immunity in the epithelial cells of the nose in children with adenoid hypertrophy vegetations. Materials and Methods: Was examined 147 patients with a diagnosis of adenoid hypertrophy vegetations II-III degree. The expression of TLR2, TLR4, TLR9, HNP1, HBD1 and HBD2 genes was detected by method of RT-PCR with TaqMan zonds. The epithelial cells of the nasal cavity of healthy children express the genes TLR2, TLR4, TLR9, HBD1, HBD2, HNP-1. In the epithelial cells of the nose in children with adenoid hypertrophy vegetations we found the hyperexpression of TLRs. The expression of gene TLR2 increased by 7.8 times (P=0,006), TLR4- in 17.1 times (P=0,019), TLR9- at 10.3 times. (P=0,005). At that time the expression of HBD2 reduced by 14 times (P=0,005).Thus, there is an imbalance in the mechanisms of protection of a mucous membrane of the nasal cavity in children, which shows the endoscopic adenotomy. Identified imbalance in the recognition and effector molecules of innate immunity may be the cause, which leads to a decrease in the protection of the nose.

F.13. The Role of the BAI1-ELMO1 Engulfment Pathway in the Induction of the Autophagy and Host Inflammatory Responses

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Recent studies indicate a link between autophagy and phagocytosis but little is known about the role of engulfment of bacteria their clearance and subsequent host cellular responses. BAI1 (Brain Angiogenesis Inhibitor 1)-ELMO1 (EnguLfment and cell Motility 1) pathway is involved in the apoptotic cell engulfment and bacterial attachment/internalization. Here we hypothesized that the BAI1-ELMO1-mediated engulfment of enteric bacteria regulates autophagy and host inflammatory responses. Phagocytes depleted of ELMO1 expression by shRNA (J774 cells) as well as macrophages isolated from ELMO1 KO or wildtype mice were exposed to Salmonella. Induction of autophagy was monitored by western blot for LC3B expression. ELMO1 depleted macrophages showed reduced expression of LC3B after Salmonella infection. ELMO1 was detected in the phagosome after subcellular fractionation. Cytokine proteome profiler array showed reduced pro-inflammatory cytokines in ELMO1 depleted macrophages. Phosphorylation of ERK1/2 and p38 MAPK is also down-regulated in ELMO1 shRNA cells while an ERK1/2 inhibitor impaired ELMO1-dependent autophagy and TNF-α production. These findings indicate a

new role for the BAI1-ELMO1 engulfment pathway in the induction of autophagy and the regulation of host inflammation.

F.14. Osteopontin Prevents the Onset of Th1 Immune-Mediated Colitis by Regulating Innate Immune Responses

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Background: Osteopontin (OPN) was reported to play an important role in the development of Th1 immune responses. However, the role of OPN in the pathogenesis of immune-mediated colitis remains unclear. Aim: To investigate the role of OPN in the development of immune-mediated colitis using Interleukin-10 knockout (IL-10 KO) and OPN×IL-10 double KO (OPN/IL-10 DKO) mice. Methods: We examined the expression of OPN in the colonic tissues of IL-10 KO mice by immunohistochemistry. Inflammation was assessed by blinded histologic score and gene expressions of pro-inflammatory cytokines in the colonic tissues of IL-10 KO and OPN/IL-10 DKO mice. IL-12p40 secretions from bone marrow-derived macrophages (BMDMs) were measured by ELISA. Result: The expression of OPN was coincident with CD11b-positive cells in the colonic tissues of IL-10 KO mice. Histologic inflammation in OPN/IL-10 DKO mice was increased even at 4-weeks-old compared to IL-10 KO mice (2.0 vs. 9.0, $p<0.05$). Gene expressions of TNF-α and IL-6 in OPN/IL-10 DKO mice were significantly higher than that of IL-10 KO mice. IL-12p40 secretion from OPN/IL-10 DKO BMDMs was significantly higher than that of IL-10 KO BMDMs. Conclusion: OPN, which might regulate innate immune responses, prevents the onset of Th1 immune-mediated colitis.

F.15. Characterization of miRNA Carried by Exosomes in Human Semen

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Exposure to infected semen is the primary route of HIV transmission. Our finding that trillions of microvesicles are secreted into each ejaculate led us to explore the exosomal compartment of semen as a source of immunoregulation in the recipient mucosa. Ejaculates contained on average $2.2x10^{13}$ exosomes, which rapidly entered vaginal Langerhans cells. Exosomal RNA was isolated and in 6 separate semen donors, small RNA from 20-40 nucleotides and from 60-100 nucleotides was sequenced. Sequencing results were compared to RT-PCR analysis of select transcripts. SE samples carried at least 162 different miRNAs in common, present in both mature and precursor forms, as well as a large fraction of Y RNA. The miRNA content of the 6 biological replicates was highly correlated. For many of the most abundant miRNAs found in seminal exosomes (e.g. miR-let7b, miR-23b miR-375), immunomodulatory activities have been reported or predicted. Using miRNA target gene-transfected THP1-NCI cells as surrogates for mucosal antigen-presenting cells, we are currently testing whether SE deliver functional miRNA to cells in sufficient quantities to regulate mRNA targets. Understanding how seminal exosomes transfer immunoregulatory messages between people may help to improve vaccination strategies against sexually transmitted infections.

F.16. A Safe and Non-Self Immunogenic Mucosal Adjuvant by Modifying Cholera Toxin A1 Subunit Conjugated with Protein Transduction Domain

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The cholera toxin (CT) as a mucosal adjuvant has been well documented with a variety of vaccine candidates in animal models. However, intact CT is highly toxic to use as a mucosal adjuvant in humans. Here we present a new approach to develop a safe and effective mucosal adjuvant from CT by replacing the B subunit of CT with protein transduction domain (PTD) which has been known to deliver fused proteins efficiently into cells by non-specific binding to cell surfaces. To enhance the cellular uptake, we attached PTD at both N-and C- termini of the CT A1 subunit (TCTA1T) and compared the toxicity and adjuvant activity of TCTA1T with CT by co-administration with ovalbumin. Intranasal (i.n.) delivery of

TCTA1T with ovalbumin significantly augmented Ag-specific both systemic and mucosal immune responses which were comparable to that of CT. The *in vivo* cytotoxic T lymphocyte activity elicited by TCTA1T was much higher than that by the mutant TCTA1T (TmCTA1T) which has inactive ADP ribosyltransferase (Ser63→Lys) activity. In addition, co-administration of influenza M2 protein with TCTA1T resulted in complete protection against influenza virus challenge. Moreover, it is also noteworthy that TCTAlT, unlike CT, was non-toxic in various toxicity tests and non-immunogenic to itself. These results suggest that TCTA1T might be useful as a safe and effective mucosal adjuvant.

F.17. Mucosal Surfaces Deferentially Respond to the Two Distinct TLR4 Ligands: FimH and LPS
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We have recently discovered that FimH, a bacterial Type 1 fimbria, is a ligand for TLR4 and a potent inducer of mucosal innate immunity. Importantly, unlike LPS, FimH requires TLR4, but not MD-2 and/or CD-14 to induce signaling. Here we first characterized mucosal innate responses to these two distinct TLR4 ligands; FimH and LPS. We have found that vaginal, rectal, and nasal mucosa are unresponsive to LPS, but highly responsive to FimH. However, uterine and lung mucosa are responsive to both LPS and FimH. Interestingly, LPS responsiveness was associated with the presence of soluble CD-14 (sCD-14). This suggested that presence of microbiota may lead to the absence of sCD-14 and unresponsiveness to LPS. Surprisingly, this was not the case as there was no detectable sCD-14 in vaginal, intestinal, or nasal washes from germ free mice. In addition, we have shown that mucosal delivery of sCD-14 restores their responsiveness to LPS. FimH, but not LPS, can activate human primary genital epithelial cells. Interestingly, these cells become highly responsive to LPS in the presence of sCD-14. Our data suggest that mucosal surfaces differentially respond to TLR4 ligands and sCD-14 regulates sensitivity of mucosal epithelial cells to LPS or commensal microflora.

F.18. Diesel Exhaust Augments Airway Eosinophil Activity in Response to Allergen in Humans
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Our objective was to determine whether diesel exhaust (DE) augments allergen-induced eosinophilia and eosinophil activation *in vivo* in the human lung. Methods: 16 volunteers participated in a blinded crossover experiment between two conditions (filtered air (FA) and 300µg PM2.5/m3 of DE), randomized and counter-balanced to order, with a four-week washout period between exposures (Fig.1). One hour following exposure, diluent-controlled segmental allergen challenge was performed. Two days post-exposure initiation, bronchoalveolar lavages (BAL) was collected in both the allergen-affected and control regions. Pairwise t-tests were performed to compare effects of contrasting conditions (DEA = diesel exhaust $^{+}$ allergen; DES = diesel exhaust $^{+}$ saline; FAA = filtered air $^{+}$ allergen; FAS = filtered air $^{+}$ saline). Results: Eosinophilia was significantly higher in FAS vs FAA (1.1% versus 10.2% respectively; p = 0.04) but there was no significant difference between DEA and FAA). BAL eosinophil activation was significantly higher in DEA vs DES (mean MFI of $CD69^{+}$ eosinophils = 2889 versus 1298 for DEA versus the sum of DES $^{+}$ FAA respectively; p = 0.001). Conclusions: Though it appears not to enhance the allergenic effect on lung eosinophilia, DE has a synergistic effect on eosinophil activation in response to allergen in previously-sensitized individuals.

F.19. Natural Cytotoxicity Receptors Provide Co-Stimulatory Signals for Human RORγt^{+} Innate Lymphoid Cells
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RORγt^{+} innate lymphoid cells (ILC) have a pivotal role in homeostasis, tissue repair and early innate defenses in mucosal tissues due to their capacity to produce IL-22. *In vitro*, IL-22 secretion by human ILC is induced by combinations of cytokines and TLR ligands. However, these are soluble factors while *in vivo* ILC activation is likely to be a local process induced by damage-associated cell surface proteins. To identify cell surface molecules capable of activating human ILC we analyzed the ability of ligands for the

Natural cytotoxicity receptors (NCR), NKp30, NKp44 and NKp46 to induce IL-22 production. Using agonist antibodies against individual NCRs in combination with TLR2 ligand Pam3Cys or with IL-23 we show that NCRs are co-stimulatory receptors on ILC. In addition, B7H6, a membrane-bound ligand for NKp30, is expressed on intestinal epithelial cell lines. To determine if B7H6-expressing intestinal epithelial cells are capable of activating ILC we performed co-cultures with tonsil-derived NCR^+ ILC. In the presence of TLR2 ligands, NCR-dependent activation of ILC was observed, revealing the first membrane-bound molecule involved in ILC activation. As NCR ligands are stress-induced molecules on normal cells, NCR ligation will allow for spatial control of ILC activation in response to intestinal damage.

F.20. Monitoring Mucus Production in a 3D-Model of Human Airway Epithelium to Develop Novel Chronic Obstructive Pulmonary Disease Therapeutics

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Pathological mucus production is a characteristic of chronic obstructive pulmonary disease (COPD). The altered mucus physiology observed in COPD patients contributes to chronic bronchitis and obstruction of expiratory flow, both defining features of disease. Specifically, mucus abnormalities such as hypersecretion and increased viscosity can result in impaired mucociliary clearance, microbial colonization, and mucoid impaction (mucus plugs). Such complications are rare in the lumen of healthy lungs, which are lined with ciliated epithelial cells and regulated numbers of mucin-producing goblet cells that help flush out microbes and particulates. In contrast, diseased lung epithelium exhibits a decrease in ciliated cells, goblet cell hyperplasia, increased mucus production and impaired mucus hydration. Given that opportunistic infections and airway occlusion are major contributors to lung function decline in COPD, restoring healthy mucus composition is an attractive pharmacological targeting strategy. Here, we model human lung morphology and mucus production *in vitro* by culturing primary bronchial epithelial cells at the air-liquid interface (ALI), a technique necessary for proper goblet cell differentiation. We then use this model as a tool to develop several mucin quantification and detection methods.

F.21. Soluble Toll-Like Receptor 2 (sTLR2) is Significantly Elevated in HIV-1-Infected Breast Milk and Inhibits HIV-1 Infection and Inflammation

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The majority of HIV-exposed breastfeed infants remain uninfected despite repeated exposure to virus over weeks to years and this has been linked to innate factors in breast milk. Soluble Toll-like receptor 2 (sTLR2) is found at a high level in human milk. Our results demonstrate that sTLR2 significantly reduced cell-free R5 HIV-1 infection and this effect could be neutralized following treatment with anti-TLR2 antibodies. Further, sTLR2 significantly reduced pro-inflammatory IL-8 production in cells exposed to cell-free virus and this was reversed by addition of sTLR2 antibodies. Next, we showed significantly increased sTLR2 in milk of HIV-infected Nigerian women compared to uninfected Nigerian and Canadian women. The level of sTLR2 in milk correlated with p24 virus levels. We also found significantly increased expression of numerous TLRs in breast milk cells from HIV-infected compared to uninfected Nigerian women. Additionally, IL-15 and the chemokine RANTES were significantly elevated in breast milk from uninfected compared to HIV-infected Nigerian women. Importantly, we showed that sTLR2 directly binds to specific HIV structural proteins. Our data indicate that sTLR2 decreases pro-inflammatory responses and directly inhibits cell-free HIV-1 infection by binding to HIV-1 proteins. Thus, sTLR2 may play a critical role in prevention of mother-to-child HIV transmission.

F.22. ELAFIN Alters Immune Cell Recruitment in Inflamed Intestine

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The expression of ELAFIN, a natural protease inhibitor normally expressed in intestine is down-regulated in patients suffering from Inflammatory Bowel Disease (IBD). This lost of ELAFIN expression is associated with an increase in elastolytic (elastase-like proteolysis) activity in colonic tissue. We have

recently reported, in models of acute (dextran sulfate sodium (DSS)) and chronic (adoptive transfer of CD45RBhighT cells) colitis in mice, that oral administration of lactic acid bacteria (LAB) engineered to express and deliver ELAFIN, markedly reduces intestinal inflammation. Here, we investigated the effect of in situ ELAFIN delivery on recruitment and distribution of mucosal immune cells including macrophages, neutrophils and T lymphocytes. We show that, in both mouse colitis models, ELAFIN-secreting LAB administration reduced the number of macrophages and T lymphocytes within inflamed intestine but did not alter neutrophils infiltration. This differential effect of ELAFIN on immune cell subsets was associated with a distinct alteration of chemokine profile. While the expression CCL5 was dramatically decreased, CCL2 and KC was unchanged. Thus, these results suggest that, in addition to its protease inhibitor activity, ELAFIN display immunoregulatory properties.

F.23. Elevated TLR9 and TNFα Expression in Cervical Cells is Correlated with Herpes Simplex Virus Type 2 Infection During Pregnancy

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Genital herpes virus infection is the frequent reason of pregnancy complications. Toll-like receptor 9 (TLR9) recognizes viral DNA. In present study we investigated gene expression of TLR9 and TNFαa in HeLa cells during herpes simplex virus Type 2 (HSV-2) infection. We determined that TLR9 and TNFα gene expression was increased in time- and dose-dependent manner in infected cells. We also investigated gene expression of TLR9, NF-κB and TNFα in cervical epithelial cells in pregnant women with normal pregnancy and pregnant women with genital herpes virus infection. Levels of TLR9, NF-kB and TNFα gene expression were significantly higher in group of pregnant women with the viral infection.

F.24. Human Milk Oligosaccharides Inhibit Multiple Pro-Inflammatory Processes in Human Fetal Intestinal Tissue

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Breast-feeding infants have lower risk of inflammatory enteric diseases than infants fed artificial diets. Several human milk components have been described as anti-inflammatory, but none account for the full activity of whole milk. Oligosaccharides are collectively one of the largest constituents of human milk, but their ability to attenuate inflammatory processes in intact human intestinal mucosa had not been defined. Human milk oligosaccharides (HMOS) inhibited inflammation induced in organ culture of immature human intestinal mucosa. Inflammation was stimulated by administration of flagellin (TLR5 agonist), polyinosinic-polycytidilic double stranded RNA (TLR3 agonist), and IL-1β (IL-1β receptor agonist), and expression of proinflammatory signaling proteins were measured. Western blot revealed that IFN-gamma, IL-6, MIP-1, RANTES and TNF-α/beta were greatly attenuated. ELISA indicated that IL-8 secretion was also reduced. Basal mRNA levels of 84 molecules involved in inflammatory responses were screened by RT-PCR array. Of these, 68 were different from untreated controls after HMOS treatment. Noteworthy is the 29 fold increase of IL-10 mRNA, because IL-10 is an anti-inflammatory cytokine. Thus, HMOS may be a major anti-inflammatory component of human milk that contributes strongly to the ability of breastfeeding to reduce the risk of inflammatory conditions in the immature infant.

F.25. An Important Role for Toll-Like Receptor 1 Signaling in the Regulation of Mucosal Immunity

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The intestine is a complex organ that maintains tolerance to food antigens and commensal microbiota while able to mount inflammatory responses against pathogens. The ability to restrain tolerogenic responses, while permitting inflammation, requires communication between commensal bacteria, intestinal epithelial cells and immune cells. Disruption or improper signaling may lead to the development of inflammatory diseases. Toll-like receptors (TLR) recognize conserved molecular motifs of

microorganisms and are important for maintaining tolerance to commensal microbiota, as well as inducing inflammation against pathogens. Our group has a number of recent discoveries indicating a critical role for TLR1 in regulating the responses to intestinal inflammation. Using a model of enteric infection in TLR1$^{-/-}$ mice we observed defects in the ability of dendritic cells to induce TH17 immunity and the epithelium for antimicrobial peptide and chemokine expression. The absence of TLR1 also results in the inability to control commensal microbiota leading to dysbiosis which conveys a pro-inflammatory signature and increases susceptibility intestinal injury when transferred to germ-free mice. Altogether, our work shows an important role for TLR1 signaling in dendritic cell, epithelial cell and microbiome regulation which will have important consequences for susceptibility to gastrointestinal infection, chronic intestinal inflammation and inflammatory bowel disease.

F.26. Dysregulated Neutrophil Function Promotes Chronic Intestinal Parasite Infection

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Clearance of intestinal helminth infection and acquired resistance is dependent on an appropriate Type 2 immune response. In the Trichuris muris (Tm) infection model in mice, resistant strains expel worms via a Th2 cell-mediated response whereas susceptible strains produce high levels of Th1 cell-associated cytokines and become chronically infected. Myeloid-cell specific deletion of SHIP1 (SHIP$^{\Delta LysM}$) in a normally resistant mouse strain results in chronic infection because of inappropriate production of IL-12 in the intestine and mesenteric lymph nodes (mLNs) in Tm-infected mice. Although Ship1-deficient macrophages overproduce IL-12 and thus likely contribute to the Tm-susceptibility of SHIP$^{\Delta LysM}$ mice; surprisingly, deletion of SHIP1 in the neutrophil lineage alone (SHIP$^{\Delta PMN}$) is sufficient to render a resistant strain susceptible. Chronic worm burden in SHIP$^{\Delta PMN}$ mice is associated with elevated IL-12, IFNγ and decreased IL-13 production in the intestine and mLNs. These data suggest that regulation of neutrophil function by SHIP1 is required for parasite clearance in response to Tm infection. Although neutrophils are rapidly recruited to the intestine following Tm infection, neutrophils are not required for clearance of parasites. However, our results suggest that dysregulated neutrophil activity can contribute to chronic intestinal parasite infection.

F.27. Morphine and HIV-1 Modulation of Toll-Like Receptor and Interleukin-17 Receptor Signaling in Lung Mucosa

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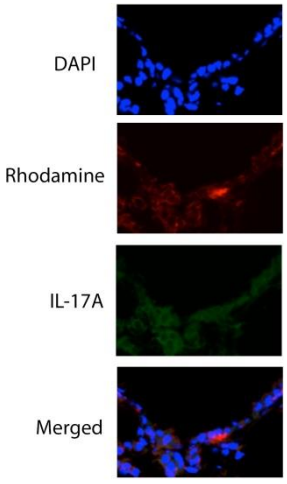

Chronic morphine and HIV-1 independently correlate with high incidence of opportunistic respiratory infections. Chronic Morphine, by itself, has been shown to increase the pathogenic load in lung infections. Together, both factors lead to an early immunosuppression, followed by a Toll-like receptor (TLR) induced persistent inflammation, mediated by interleukin-17 (IL-17). Although current literature seems to implicate circulating immune cells for this inflammation, our studies show a robust TLR2 mediated IL-17 response in murine lungs and there is a strong indication that the bronchial epithelium being the source of the cytokine. Under normal circumstances, an opportunistic infection brings about a tightly regulated balance between TLR and IL-17 receptor (IL-17R) mediated signaling resulting in an initial cytokine (primarily IL-17) burst, followed by dampening of the signal. This balance is lost in the context of chronic morphine and/or HIV-1 infection. Our studies suggest putative cross talk between the TLR2 and IL-17R pathways, mediated by the first messenger/adaptor molecules (MyD88 and Act1/CIKS respectively), which could potentially explain the tight control of inflammation in the lungs, otherwise disrupted by chronic morphine and/or HIV-1.

F.28. *In vitro* Infection of Bovine Monocytes with Mycoplasma Bovis Delays Apoptosis and Suppresses Production of IFN-γ and TNF-α but Not IL-10

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Mycoplasma bovis (M. bovis) is one of the major causative pathogens of the bovine respiratory complex disease (BRD) that is characterized by enzootic pneumonia, mastitis, pleuritis and as polyarthritis. *M. bovis* enters and colonizes the bovine respiratory epithelia through inhalation of aerosol from contaminated air. The nature of the interaction between *M. bovis* and cells of innate immunity is not well understood. We hypothesized that *M. bovis* invades blood monocytes and regulates cellular function to support its persistence and systemic dissemination. We applied the use of bovine peptide kinome arrays to identify cellular signaling pathways that could be relevant to *M. bovis*-monocyte interaction *in vitro*. We then validated these pathways using functional, protein and gene expression assays. Here, we show that *M. bovis* inhibits both spontaneous and staurosporine-driven apoptosis through activation of NF-κβ, caspase-9 blockage and increased expression the gene encoding the anti-apoptotic protein Bc-xL. We also report that *M. bovis* infected monocytes do not produce IFN-γ and TNF-α although the spontaneous production of IL-10 is not affected. Our findings suggest for the first time that *in vitro*, *M. bovis* takes over the cellular machinery of bovine monocytes to prolong bacterial survival and to possibly facilitate subsequent systemic distribution.

F.29. Changes in Inflammatory Cytokine-Levels in the Sera of Patients with Parainfluenza Virus Type 1-Infection

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Human parainfluenza virus is one of the main causative agents of respiratory tract infections. Human parainfluenza virus Type 1 has been reported to frequently infect the epithelial cells of the respiratory tract and induce lower respiratory tract infections, bronchitis and pneumonia, in immune-compromised hosts. Here we report an epidemic of infection by the virus that occurred in a hospital ward that houses patients with disabilities. Most of the patients infected with this virus experienced high fever for almost one week. One-third of the patients showed symptoms of bronchitis. Half of the patients showed increase in the number of blood monocytes at the initial stage of the infection. The serum levels of several inflammatory cytokines (IL-6, IL-8, IP-10 and MCP-1) were also high in the patients. Inflammatory cytokines has been reported to be released from the virus-infected cells, and these cytokines induce immunity in the host against the virus. These observation of increase in the number of blood monocytes and changes in the level of inflammatory cytokines in sera probably indicate that the parainfluenza virus Type 1 infection is related to the macrophage/monocyte system of the respiratory tract, in the initial stage of the infection. We further analize the levels of antibodies in blood, and the relationships between the virus-infection and the antibodies in sera.

F.31. Natural Killer T Cell Adjuvanted Inactivated Swine Influenza Virus Vaccine Enhances the Viral Load and Suppresses the Host Immune Response to Pandemic 2009 H1N1 Virus Challenge in Pigs

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Swine influenza virus (SIV) causes an acute respiratory disease in pigs, and pigs are infectable by both avian and mammalian influenza viruses. Activation of NKT cell induces heterologous protection against influenza viruses in rodent models. We discovered CD1d-restricted NKT cell in pigs. Our goal was to determine the efficacy of UV-inactivated bivalent SIV vaccine, comprising of triple reassortant zoonotic H1N1 (Sw/OH/24366/07) and H3N2 (Sw/OH/04) viruses; coadministered intranasally with NKT cell adjuvants, phosphatidylinositolmannosides-2 (PIM2) and α-Galctosylceramide. In vaccinated homologous H1N1 virus challenged pigs, reduced viral load in the lungs associated with increased IFN-γ^+ lymphocytes

in the lungs and tracheobronchial lymph nodes by ELISPOT, and an increase in IFN-γ$^+$CD8$^+$ and IFN-γ$^+$ γδ T cells was observed. Further, increased specific IgA and HI antibody response in the BAL and enhanced lung NK cytotoxicity was detected. However, in vaccinated pandemic 2009 H1N1 virus challenged pigs, enhanced nasal viral shedding and lung viral load was detected. Immunologically, reduced frequency of total lymphocytes, CD8$^+$ T cells, and frequency of total IFN-γ$^+$ T cells was detected in the lungs. In conclusion, NKT cell adjuvanted inactivated SIV vaccine in pigs enhanced the pandemic 2009 H1N1 replication and dampened the anti-viral immune response. Grant support - NPB and OARDC. Immunologically, reduced frequency of total lymphocytes and CD8$^+$ T cells, and reduced frequency of total IFN-γ$^+$ T cells was detected in the lungs. In conclusion, NKT cell adjuvanted inactivated SIV vaccine in pigs enhanced the pandemic 2009 H1N1 replication and dampened the anti-viral immune response. Grant support - NPB and OARDC.

F.32. Enterocytes Infected by *T. Gondii* Secrete Pro-Inflammatory Molecules in an Nf-kB Independent Mechanism

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Toxoplasma gondii (T.gondii) belongs to the Apicomplexa protozoan parasite. *T.gondii* tropism is extremely large since it is able to infect any type of eukaryote cells and any mammal. *T.gondii* naturally enters the organism via the oral route, which precedes its passage through the intestinal barrier to reach internal organs of the body. In several mammals and primates, ileal inflammation is a pathological feature of *T.gondii* infection. In humans, a study has shown an association between IBD and *T.gondii* positive serology, suggesting that *T.gondii* infection could be a risk factor for gut inflammation or vice versa. Since intestinal epithelial cells are the first cells interacting with the parasite *in vivo*, we focused our study on the pro-inflammatory events occurring in parasite-infected enterocytes. We first showed that enterocytes seemed more resistant to *T.gondii* infection than fibroblasts, which are the classical cellular model used for *T.gondii* studies. Moreover, we showed that some pro-inflammatory cytokines and chemokines are secreted by enterocytes in response to infection, with no apparent evidence of Nf-kB activation. This study highlights the primary innate responses of the epithelial barrier against *T.gondii* infection.

F.33. Cigarette Smoke Increases MUC5AC Protein Expression Through the Activation of Shp2 Enzyme

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Cigarette smoke (CS), the major cause of chronic obstructive pulmonary disease, contains a variety of oxidative components that were implicated in the regulation of Src homology Domain 2-containing protein tyrosine Phosphatase 2 (Shp2) activity. MUC5AC is the major inducible mucus gene in the airway. However, the regulation of Shp2 enzyme to MUC5AC protein expression in chronic obstructive pulmonary disease pathogenesis remains unclear. We investigated the role of Shp2 enzyme in blockading CS-induced MUC5AC expression. Shp2 levels were assessed *in vivo* and *in vitro*. Mice (C57BL/6) or pulmonary epithelial cells (NCI-H292) were exposed to CS or cigarette smoke extract (CSE) to induce acute injury and MUC5AC expression. Lungs of smoking mice showed increased levels of Shp2, compared with those of controls. Treatment of lung epithelial cells with CSE showed elevated levels of Shp2 associated with the increased MUC5AC expression. Selective inhibition or knockdown of Shp2 resulted in decreased MUC5AC expression in response to CSE treatment in pulmonary epithelial cells. In comparison with CS-exposed wild-type mice, selective inhibition or conditional knockout of Shp2 in lung epithelia reduced MUC5AC expression and acute lung injury in CS-exposed mice. *In vitro* biochemical data correlate CSE-mediated MUC5AC expression with Shp2-regulated epidermal growth factor receptor/Grb-2-associated binders/MAPK signaling. Our data suggest an important role for Shp2 in the pathological alteration associated with CS-mediated inflammation. Shp2 may be a potential target for therapeutic intervention for MUC5AC expression in CS-induced acute lung injury.

F.34. Bronchipret Increases Mucociliary Clearance Activity via Attenuating MUC5AC Expression
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Bronchipret, an oral preparation containing an extract of thyme and primula is frequently used for the therapy of chronic and acute bronchitis. The aim of the study was to assess its efficacy for mucociliary clearance in mice model. Bronchipret 300~600 mg/kg significantly enhances mucociliary clearance and airway mucopolysaccharide concentration in LPS-induced airway inflammation in mice. MUC5AC mRNA expression in lungs were measured by Q-PCR and MUC5AC level in bronchoalveolar lavage fluid (BALF). Bronchipret at 30 or 100 mg/kg significantly reduces MUC5AC mRNA expression and MUC5AC protein level in LPS induced-mice. Bronchipret 30~300 mg/kg significantly reduces airway mucopolysaccharide concentration in LPS induced-mice. In cigarette exposed-mice bronchitis model, treatment with Bronchipret at 30 or 100 mg/kg significantly reduces MUC5AC mRNA expression and MUC5AC protein level. Bronchipret 100~300 mg/kg significantly increases mucociliary clearance, and reduces airway mucopolysaccharide concentration. In ovalbumin-induced mice asthma model, treatment with Bronchipret at 10, 30 or 100 mg/kg significantly reduces MUC5AC mRNA expression. Bronchipret at the dose of 30 and 100 mg/kg markedly attenuated ovalbumin-induced goblet cell hyperplasia and mucus hypersecretion within the bronchi in the lungs as compared with that in model mice. Additionally, Bronchipret at 300mg/kg and 600mg/kg exhibited an increase in eliminating phlegm activity on tracheal phenol red secretion in mice. These results demonstrated a potent expectorant activity of Bronchipret in infection model, cigarette exposed model and asthma model in mice.

F.35. Early Cytokine Responses in Respiratory Syncytial Virus Infection are Dependent on Type I Interferon Signalling
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Respiratory syncytial virus (RSV) is the most common cause of bronchiolitis and hospitalisation of infants. Approximately 70% of infants are infected with RSV during the first two years of life and in some cases this can be associated with recurrent childhood wheezing and increased predisposition to asthma. Currently, there is no effective vaccine against RSV. Severe bronchiolitis is associated with polymorphisms in several innate immune response genes, in particular many that control the interferon (IFN) system. Innate IFNs (Type 1 and 3) are the first line of defense against viral infections and are produced very early upon direct recognition of the virus. They are important for inducing antiviral responses and to activate cells of the innate and adaptive immune systems. The mechanisms of how these cytokines regulates host resistance and immunopathology during RSV infection is poorly understood. Using IFNAR1 knockout mice we have found that the lack of IFN signalling increase viral load and pathology (weight loss) during RSV infection. In addition, the production of the other IFNs and, surprisingly, proinflammatory cytokines and chemokines are totally dependent on signalling through the IFNa/b-receptor. Thus, IFNs and IFN-receptor signalling are crucial for the early cytokine induction in the lung after RSV infection.

F.36. Ship1 Regulates Neutrophil Contribution in Allergic Airway Inflammation
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Introduction: The hallmarks of allergic airway inflammation (AAI) includes a Th2-biased immune response resulting in the influx of eosinophils and increase in IgE production. Although neutrophils comprise the majority of circulating leukocytes in humans and are prevalent in cases of severe asthma; they have an under-appreciated role in regulating immune response in atypical AAI. Ship1 is an inositol phosphatase that negatively regulates PI3K pathways associated with leucocyte migration, activation and survival. We intend to examine the role of Ship1 in regulating neutrophil function in a mouse model of AAI. Methods: We used Ship1fl/fl mice crossed onto neutrophil elastase Cre-expressing mice to specifically delete Ship1 expression in neutrophils. Mice were then subjected to a house dust mite (HDM) model of AAI that mimics

several features of clinical asthma. Results: Interestingly, the loss of Ship1 on neutrophils resulted in an increase in disease severity, with increased lung tissue damage and leukocyte infiltration into the airways. Deletion of Ship1 in neutrophils resulted in reduced Th2 and Th1 cytokine production and reduced IgE levels, suggesting that Ship1 attenuates pathological neutrophil functions and that neutrophils have an underappreciated immunomodulatory role in AAI.

F.37. Relationship Between Virus Clearance and Induction of Nasal IgA After Infection of Ferrets with H3N2 Influenza Virus Derived from Clinical Specimens

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Ferrets (Mustela putorius furo) are a very useful animal model for human and avian influenza, because clinical features are associated with those seen in human disease. However, the relationship between virus clearance and IgA induction in the nasal cavity after influenza virus infection in ferrets are not known. In this study, the kinetics of virus-specific IgG and IgM in the serum and IgA in upper respiratory tract, were investigated. Two clinical isolates of H3N2 virus were used for infection. Ferrets were infected with intranasally administered virus suspension and virus titers and antibody titers were determined. A remarkable high virus titer in the nasal washes was observed immediately after infection and stayed for five days before decreasing rapidly. Specific IgG and IgM antibody responses in serum increased slowly, peaked on day 14 and were then preserved. However, IgA antibody responses in nasal washes were increased already after five days, peaked on day nine, and decreased afterwards. Since the start of the decrease in viral titers coincides with the increase in IgA titers, these results suggest that rapid IgA induction in the upper respiratory tract strongly contributes to virus clearance.

F.38. Mucosal Immunization with Recombinant Influenza Hemagglutinin Protein and Poly Gamma-glutamate/Chitosan Nanoparticles Induces Protection Against Highly Pathogenic Influenza A Virus

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Intranasal administration of recombinant influenza hemagglutinin (rHA) antigen and inactivated virus with nanoparticles (NPs) composed of poly-γ-glutamic acid (γ-PGA)/chitosan which is safe, natural materials, and able to target the mucosal membrane as a mucosal adjuvant, could induce a high degree of protective mucosal immunity in the respiratory tract. Intranasal immunization with mixture of rHA antigen or inactivated virus and PC NPs induced not only a high anti-HA IgA response in lung and IgG response in serum but also an influenza virus-specific cell-mediated immune response. Also, PC NPs could function as a potential mucosal adjuvant when it was compared with the well-known mucosal adjuvant, cholera toxin (CT). Intranasal administration of rHA antigen or inactivated virus with PC NPs protected mice against challenge with a lethal dose of the highly pathogenic influenza A H5N1 virus. These results suggest that use of PC NPs with a subunit antigen of influenza produced by prokaryotic expression system that has several solutions against the current influenza vaccines that represents the commercialized current influenza vaccines can promote an effective protection and may be useful in clinical applications as a safe and potent mucosal adjuvant. (This research was supported by National Agenda Project grant from KRCFST (KGM 0821113).)

F.39. Potent Mucosal Immunization with Surface-Displayed CTA1-Fused sM2 Protein on Lactobacillus Casei Induces Protection Against Highly Pathogenic Influenza A Virus

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For safe and effective delivery of viral antigens to the mucosal immune system, we have used a surface antigen display system for lactic acid bacteria using the PgsA of Bacillus subtilis and prepared *Lactobacillus casei* expressing recombinant fusion proteins comprised of PgsA and conserved matrix Protein 2 from Influenza virus with or without cholera toxin subunit A1 (CTA1) which as a potent mucosal adjuvant. Surface localization of the fusion protein was verified by cellular fractionation,

immunofluorescence microscopy and flow cytometry. Oral and nasal inoculations of recombinant *L. casei* into mice induced not only high levels of serum IgG and mucosal IgA, but also M2-specific cell-mediated immune response. Especially, conjugation of CTA1 increased the systemic IgG, lung and intestinal IgA and cell mediated immune responses. More importantly, intranasal administration of Lactobacillus casei expressing CTA1-sM2 protected mice against challenge with a lethal dose of the highly pathogenic influenza A H5N1 virus and diverse influenza viruses (H1N1, H5N2, H7N3, H9N2). These results indicate that mucosal immunization with recombinant *L. casei* expressing CTA1-sM2 on its surface provides an effective means for eliciting broad protective immune response against influenza virus [This research was supported by National Agenda Project grant from KRCFST (KGM 0821113)].

F.40. Bovine Disialylganglioside GD3 Inhibits Human Respiratory Syncytial Virus Infection of HEp-2 Cells

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Aims: Breast milk consumption offers protection against respiratory syncytial virus (RSV) infection in infants. The aim of this study was to explore inhibition of RSV by bovine milk components, a major source for infant formulas. Materials & Methods: Several bovine milk compounds (disialylganglioside GD3, 3' sialyllactose, sialic acid and lactose) were pretreated with the virus and tested for inhibitory activity by plaque counts, RSV-N gene expression, and RSV-G protein level in HEp-2 cells. Glucosylceramide, precursor for GD3 was used as control. Results & Discussion: Among the compounds tested, disialylganglioside GD3 exhibited concentration dependent inhibition with maximal effect at 100 ug/ml (63.4 uM). GD3 reduced RSV plaque formation by 85.5 ±1.5%, inhibited RSV-N gene expression by 97.7 ± 0.1%, and significantly decreased the RSV-G protein level in HEp-2 cells. All other tested compounds did not show these inhibitory effects. To our knowledge, this is the first study to show GD3, the predominant ganglioside in breast milk to have inhibitory effects on RSV. Enrichment and addition of GD3 to infant formula may offer protection to formula fed infants who are unable to consume breast milk. Further work is needed to understand the actual structural motifs that play a role in RSV inhibition.

F.41. Systemic Effects of Acute Lung Infection by Pneumovirus of Mice (PVM)

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Acute lung infections in asthmatic patients are linked to increased disease susceptibility and exacerbations, while in mouse models, pneumovirus of mice (PVM) infection in early-life drives development of spontaneous airway disease. This suggests that in addition to localized, acute inflammation, lung infections may also have systemic, long-term effects on immune cell function. To characterize systemic changes, we used an eight-day PVM disease model to assess factors relevant to hematopoietic cell development in the bone marrow. Intranasal PVM infection results in extensive lung damage, with myeloid cell infiltration and labored breathing. Inflammatory mediators increase in lung tissue and serum, with increased circulating myeloid cells in the blood. In bone marrow, infection results in increased myeloid progenitors and hematopoietic stem cells (HSCs). Infection also results in striking decreases in osteoblast-associated gene expression and osteoblast numbers, in the absence of detectable PVM virus within the bone. Importantly, osteoblasts play a key role in the stem cell niche, maintaining HSC quiescence. Thus, decreased osteoblasts could drive increases in HSC/progenitors and myeloid production. Our findings identify systemic changes following PVM infection, particularly within the bone marrow microenvironment. These changes may underlie the effects of acute lung infection on long-term immunity and disease susceptibility.

F.42. Basis of MAIT Cell Ligand Recognition

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MAIT cells (Mucosal-associated invariant T cells) are an evolutionarily conserved subpopulation of T cells

restricted to the HLA class Ib molecule, MR1 (MHC-related protein 1). MAIT cells were recently discovered to recognize vitamin B metabolites, in contrast with conventional T cells that recognize a diverse range of peptides. Alanine-scan mutagenesis revealed conserved key residues in the invariant a-chain necessary for recognition of the MAIT ligand(s), providing a basis for the highly conserved Va7.2Ja33 region. MAIT TCR usage was characterized in primary human MAIT cells and vitamin B metabolite reactive T cells. These findings provide a striking comparison to the iNKT receptor, in that the MAIT TCR, a component of the adaptive immune system, has evolved similar innate recognition characteristics.

F.43. HMGB-1 is a Critical Factor on the UV-Induced Conjunctival Inflammation
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Pterygium is a common ocular surface disease in person and characterized by inflammatory cell infiltrates, cellular proliferation, angiogenesis and inflammation. Chronic ultraviolet B (UVB) light exposure is a causal factor in the pathogenesis of this disease, but the mechanism is not completely understood. HMGB-1 (high mobility group box 1), nuclear protein that acts as a damage-associated molecular pattern after its releases by necrotic cell. We show that UVB irradiation induce mouse conjunctival inflammation through HMGB-1 which is released by conjunctiva epithelial cell and ameliorated inflammation after blockage effect of HMGB-1 with its specific antagonist Abox and sRAGE. Our data also demonstrate translocation into cytoplasm and secretion into extracellular space of HMGB-1 depends on UVB induced ROS production in conjunctiva epithelial cell and were reduced by scavenger of ROS, NAC and mitotempo *in vitro*. Furthermore, intraperitoneal administration of NAC resulted in amelioration of UVB irradiation induced conjunctival inflammation, but mitomtempo did not inhibit. In human pterygium tissue, epithelial cell also were shown that translocation of HMGB-1 to the cytosol. Movement and secretion of HMGB-1 were increased after UVB irradiation in human normal conjunctiva tissue. Thereby, HMGB-1 is one of the critical factors in UVB induced conjunctival inflammation and human Pterygium. Moreover, it is expected be useful for further understanding of the pathogenesis of UV induced ophthalmic lesion.

F.44. Low Dose Antigen Exposure in Extreme Early Life Promotes Mucosal Immunity in Lambs
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Mucosal tolerance is defined as a state of antigen-specific non-responsiveness to oral antigens to prevent local and peripheral overreaction to oral antigens. In newborn lambs, the gut-wall is semi-permeable for up to 36 hours after birth to allow maternal antibodies from the colostrum to enter the suckling neonate's circulation. We propose antigen introduced in extreme early life can readily access the gut-associated lymphoid tissues (GALT) and circumvent induction of mucosal tolerance. To test this hypothesis, newborn lambs were fed low doses of ovalbumin (OVA) starting immediately birth for either a single day, every day for three days, or until nine days of age. At four weeks of age, lambs were immunized with OVA in Incomplete Freund's Adjuvant via intraperitoneal (i.p.) injection. Lambs gavaged with low dose OVA for nine days developed significant serum anti-OVA IgG titres (prior to i.p. injection), but not IgA titres. These lambs showed significant anti-OVA IgA titres in lung washes indicating induction of mucosal immunity. When splenocytes were re-stimulated with OVA *ex vivo*, the group of newborn lambs administered OVA for three days produced significantly higher IFN-γ expression relative to media-stimulated cells suggesting induction of antigen-specific, Th-1 biased cell-mediated immunity. Thus, perinatal antigen exposure primes local and distal mucosal antibody production as well as cell-mediated immunity in newborn lambs. Current research is focusing on introducing Lawsonia intracellularis antigens orally into piglets to induce mucosal immunity. We will characterize the immune response and establish whether extreme early life oral vaccination protects against disease (i.e. *Porcine Ileitis*).

F.45. Caspase Recruitment Domain-Containing Protein 9 (CARD9) is Dispensable for Immunity to Oropharyngeal Candidiasis (OPC)
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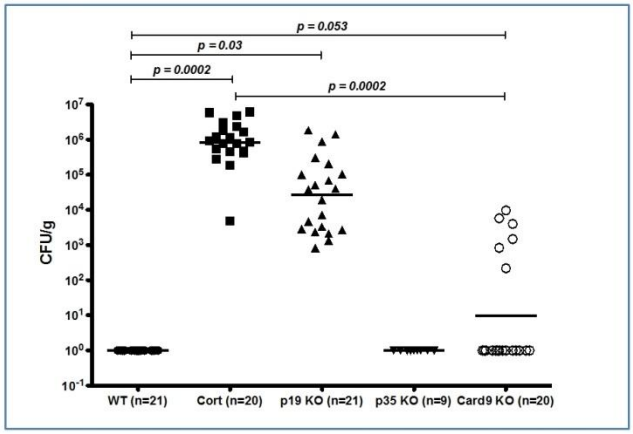
CARD9 is a myeloid restricted adaptor that signals downstream of C-type lectin receptors (CLRs). CLRs such as Dectins are key fungal pathogen recognition receptors, and CARD9 $^{-/-}$ humans are susceptible to mucosal and systemic infections with the commensal yeast *Candida albicans*. CARD9 induces protective Th17 type CD4^{+} T cell responses to systemic Candida albicans, but its role in mucosal candidiasis has not been elucidated. We subjected Card9$^{-/-}$ mice to an acute five-day model of OPC. We previously demonstrated that IL-17 is necessary for immunity in this model, which reflects the human condition, as IL-17RA$^{-/-}$ humans are susceptible to chronic mucosal candidiasis. Unexpectedly, Card9$^{-/-}$ mice were only mildly susceptible to OPC, whereas cortisone treated and IL-23$^{-/-}$ mice were highly susceptible (Fig1). Time course experiments revealed that most CARD9$^{-/-}$ mice, similar to WT mice, cleared yeast burden by day three. While cortisone treated and IL-23$^{-/-}$ mice had systemic candidiasis, CARD9$^{-/-}$ mice exhibited no hematogenous dissemination of *C. albicans* to visceral organs or brain despite evidence of GI tract colonization. Therefore, while CARD9 is necessary for protective immunity to disseminated *C. albicans*, it plays a surprisingly minimal role in innate immunity to oral mucosal infections.

Figure 1: Tongue Fungal Burden



F.46. PI3K Signaling Mediates Protection of Oral Epithelial Cells from *C.Albicans*-Induced Damage

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Mucosal epithelium is important in host defence and surveillance, being the cell layer that initially encounters most microorganisms. *Candida albicans* is the most common fungal pathogen of humans and is commonly found at mucosal surfaces. Although immune cell responses to this pathogen are well documented, epithelial cell (EC) responses are poorly described. Here, we report the gene expression profile for organotypic oral epithelium after early (6 hours) and late (24 hours) infection by *C. albicans*. Infection results in enrichment of several ontology groups associated with infection, immunity and signal transduction, indicating active EC responses to fungal infection. Functional analysis of signal pathway activation, demonstrated MAPK, NF-κB and PI3K pathway activation. Inhibition of PI3K signaling resulted in an increase in lactate dehydrogenase and IL-1α release (damage) but decreased GM-CSF and G-CSF secretion. However, there was no effect on MAPK (MKP1/c-Fos) or NF-κB (IκBα) activation. This data indicates that as well as MAPK and NF-κB signaling, C. albicans infection of ECs leads to PI3K signaling. Further, PI3K signaling is important in EC protective responses to damage, whilst MAPK signaling is solely responsible for 'danger' detection. As such, this pathway represents a potential target for future therapeutic treatments.

F.47. Influence of Metals onto sIgA, IgA1 and IgA2 Production in Saliva and Cytokine Production by Lymphocytes in Patients Undergoing Implantation Therapy in Dentistry

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The purpose of the study was to compare sIgA, IgA1 and IgA2 production in saliva before and after implantation therapy and to compare *in vitro* cytokine production by stimulated lymphocytes in patients undergoing implantation therapy in dentistry. Saliva from patients was collected before and after implantation therapy and sIgA, IgA1 and IgA2 antibody production was established using RID method. Lymphocytes isolated from patients were stimulated by mercury and titanium antigens and production of 39 cytokines was established using Quantibody INF-3 array. Patients which reacted to titanium antigen and therefore zirconia implant was used in these patients, we found non-significant increase in sIgA, IgA1 and IgA2 production in saliva after implant therapy. On the other hand, in patients tolerating titanium, we

did not find any differences in sIgA, IgA1 and IgA2 production before and after implantation. When compared to non-stimulated lymphocytes, lymphocyte culture stimulated by mercury antigen produced significantly increased levels of eotaxin-2, MIP-1a and MIP-1b, lymphocyte culture stimulated by titanium antigen produced significantly increased levels of IL-1ra and significantly decreased levels of IL-10. The study was supported by PRVOUK-P28/LF1/6 (Ministry of Education, Youth and Sports, Czech Republic) and by NT 13087-3 (Ministry of Health, Czech Republic).

F.49. Sublingual Plasmid cDNA Expressing RANTES Induces CCR5-Positive Dendritic Cells for the Induction of Pneumococcal Specific SIgA Antibody Responses

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Sub-lingual (SL) antigen (Ag) delivery is a non-invasive route which avoids possible Ag entry into the central nervous system and avoids degradative enzymes and lower pH of the stomach. Since the SL region contains high numbers of CCR5-expressing dendritic cells (DCs), we examined the adjuvant activity by a cDNA vector expressing RANTES (pCCL5). C57BL/6 mice were SL immunized four times at weekly intervals with pneumococcal surface protein Ag (PspA, 1 µg/mouse) plus pCCL5 cDNA (150 µg/mouse). One week after the last immunization, PspA-specific Ab responses were determined by ELISA. In addition, the frequency and phenotype of DCs were determined by flow cytometry. Mice given PspA plus pCCL5 showed increased levels of PspA-specific SIgA Abs in nasal washes, saliva, fecal extracts and vaginal washes when compared with mice immunized with PspA plus empty plasmid. Interestingly, SL delivery of pCCL5 cDNA as a mucosal adjuvant elicited significantly increased numbers of $CCR5^+$ DCs in the periglandular lymph nodes (PGLNs) but not in cervical lymph nodes when compared with naïve mice which harbor only small numbers of PGLN lymphoid cells. These results clearly suggest that SL immunization activates $CCR5^+$ SL DCs which migrate into the PGLNs and subsequently elicited pneumococcal-specific SIgA Ab responses.

F.50. Autocitrullinated Peptidylarginine Deiminase from *Porphyromonas Gingivalis:* Part of an Infection Model in Rheumatoid Arthritis?

Madleine Sohn, Bianka Marklein, Chritina Grimm, Karl Skriner. Charité, Berlin, Germany

Background: Anti-citrullin peptide auto-antibody (ACPA) are highly specific for rheumatoid arthritis (RA) but their origin is still unknown. Peptidylarginine deiminase from *Porphyromonas gingivalis* (PPAD) a peridontitis inducing bacteria is the only known bacterial enzyme creating citrullinated proteins. Results: PPAD is released in outer membrane vesicles (POMVs) is N-terminaly cleaved rendering it into an active citrullinating and autocitrullinating enzyme. In 30% of 69 early RA patients sera anti citrullinated PPad antibodies can be detected but in none of the controls done 53 periodontitis (PD), 50 Systemic Lupus Erythematosus and 50 healthy human sera. POMVs can interact with histones and citrullinate them to protect bacteria from antibacterial response of histones. Moreover the anti H1 histone antibody responses in sera are be blocked by POMVs. *P.gingivalis* vesicles containing the the PAD induce a mild inflammatory response in the joints of a Collagen Antibody Induced Arthritis (CAIA) model of arthritis.Conclusions: We have identified the first foreign antigen specifically detected in early RA sera that might induce by molecular mimicry the only specific RA autoantibodies (ACPAs) leading to RA. *P. gingivalis* vesicles induce a mild form of arthritis and maybe part of an infection model of RA.

F.51. Novel Aspects on Gut Mucosal IgA Memory B Cell Development

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Previous studies have demonstrated that oral immunization promotes long-lived plasma cells and memory B cells in GALT. However, little detail is known about the distribution, frequency, phenotype or molecular characteristics of these cells. Therefore, we developed an adoptive transfer model with sorted

high affinity B1-8hi NP-specific IgH knock-in GFP$^+$ B cells and NP-cholera toxin (CT) (NP; hapten nitrophenyl) that allowed us to visualize the expansion, distribution and maintenance of GFP$^+$ NP-specific mucosal memory B cells and long-lived plasma cells. Following oral immunization specific gut IgA responses were found to be oligoclonal, affinity matured and synchronized through invasion of pre-existing Peyer's patch (PP) germinal centers (GC). Two years after oral priming with NP-CT oral or parenteral challenge immunizations elicited a very strong memory response in the gut LP. By contrast, parenteral priming failed to stimulate gut IgA memory. Memory B cells had fewer mutations and showed less affinity maturation than long-lived IgA plasma cells. Only few GFP$^+$ memory B cells were found and these resided in B cell follicles in GALT. All NP-memory B cells expressed CD80 and PD-L2, but CD73 appeared to be the best marker. To conclude, gut IgA memory B cell development is effectively stimulated by oral immunization.

F.52. Fcγ Receptor IIB Expressed on B Lymphocytes is Important for Mucosal Antigen-Induced Tolerance and Foxp3$^+$ Regulatory T Cells

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The IgG receptor FcγRIIB, which is the only Fcγ receptor expressed on B cells, plays an important role in the maintenance of immunological tolerance to self-antigens (Ags) and in regulating immune responses to exogenous Ags. In this study, we investigated the role of FcγRIIB in Ag-specific CD4 T cell tolerance induced by mucosally administered Ag (ovalbumin, OVA) coupled to cholera toxin B subunit (Ag/CTB) or given alone. We found that sublingual (s.l.) administration of either OVA/CTB conjugate or a high dose of OVA alone efficiently suppressed parenteral immunization-induced OVA-specific T cell proliferation and delayed type hypersensitivity responses in wild type (WT), but not FcγRIIB-/- mice. These effects were restored in FcγRIIB$^{-/-}$ mice that received WT B cells. Furthermore, B cell deficient (μMT$^{-/-}$) mice that received B cells transgenically overexpressing FcγRIIB demonstrated even stronger tolerization than μMT$^{-/-}$ mice receiving WT B cells. These results suggest that FcγRIIB expression on B cells provides a critical checkpoint for the development of Ag/CTB-induced Foxp3$^+$ Treg cells and oral tolerance.

F.53. Feeding Mice Uniquely Engineered Plants Protects Against Collagen-Induced Arthritis: A Future for Salads Against Rheumatoid Arthritis?

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Whereas oral tolerance is an attractive approach to induce protection against autoimmune diseases, it has largely been unsuccessful due to the large antigen requirements. This is especially true for oral treatments against Collagen-induced Arthritis (CIA). We have successfully developed a plant-based expression system for our patented tolerogenic fusion protein, CTA1R7K-COL-DD. Although plant-expressed vectors seldom have proven effective at stimulating CD4 T cell tolerance we asked if this vector could protect against CIA. From previous studies we know that nasal, but not oral, delivery of the CTA1R7K-COL-DD fusion protein induces tolerance and prevents CIA-disease. To advance the therapeutic potential of our tolerogenic vector, we tested if feeding mice Arabidopsis plants expressing the fusion protein could impact on CIA-disease development. To our surprise, mice fed transgenic plant were significantly protected against disease. Hence, for the first time we could show that a plant expressed tolerogenic vector was effective at protecting against CIA-disease with decreased disease incidence and arthritic score. This finding opens up for oral treatment protocols against rheumatoid arthritis and suggests the possibility that salads may be effective at preventing disease. The present project will further explore the protective potential of feeding transgenic plants and assess the underlying mechanism of action.

F.54. Bystander Oral Tolerance Promotes Reduction of Inflammatory Lesions Through Treg Cells in Mice Infected with Leishmania Amazonensis

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The Oral Tolerance is a physiological mechanism of systemic hyporeactivity to an immunogen previously ingested and bystander suppression is a inhibited response to a second immunogen when it is presented along with the immunogen for which it was established oral tolerance. Previous studies with mouse strains, extreme phenotypes to susceptibility (TS strain) and resistance (TR strain) to Oral Tolerance, showed that when infected with Leishmania amazonensis, TR strain develops an exacerbate lesion while a regulatory activity of the TS strain depresses the inflammation and avoid acute lethal response. The bystander suppression reduced the inflammatory lesion in TR strain, similarly to the swelling of infected TS strain. Mice previously tolerized and immunized showed an increase of $CD4^+$ $CD25^+$ $Foxp3^+$ cells, increase of IL-10 and decrease of IFN-γ in draining lymph nodes of the bystander site when compared with naïve or immunized mice. The transfer of regulatory T cells ($CD4^+$ $CD25^+$), from tolerized to infected mice, resulted in reduction of inflammatory lesion. These results suggest the involvement of Treg cells in the Bystander Suppression mechanism and demonstrate their importance in the reduction of inflammatory lesions in animals infected with Leishmania amazonensis.

F.55. Oral Tolerance Susceptibility Promotes Morphological Changes in Schistosoma Mansoni

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The Schistosoma mansoni infection was studied in two strains of mice genetically selected for extreme phenotypes of susceptibility (TS) and resistance (TR) to oral tolerance. The objective was to analyze by Transmission Electron Microscopy the influence of the host immune regulatory profile on the worm morphology. Parasites recovered from TR mice showed no morphological changes. However, specimens collected from TS mice, exhibited tubercle swelling with blunted and shortened spines in lower density. These tegument alterations were similar to those described with artemether or praziquantel treatment, supporting observations that the host immune system influences the tegument development and function of worms harbored in non anti-helminthic treated TS mice. The ileum oogram from TS mice showed a higher percentage of dead eggs and a lower percentage of immature eggs than TR mice, but had similar quantities of collected eggs. This suggests that in TS mice the alterations in adult worm tegument prevented egg development, as a consequence of decreased glycogen granules and extensive lysis of internal structures, but not egg production reduction. These results corroborate our previous Scanning Electron Microscopy study indicating the influence of the host immune regulatory profile on the development and function of the worm reproductive system and tegument.

F.56. Oral Tolerance Susceptibility Promotes Production of IL-4 that is Related to Granuloma Development in Schistosomiasis

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The Schistosoma mansoni infection was studied in two strains of mice genetically selected for extreme phenotypes of susceptibility (TS) and resistance (TR) to oral tolerance. TR strain presents good inflammatory responses and a non-tolerogenic profile while TS mice are non-inflammatory but high-tolerogenic, with high percentages of $CD4^+CD25^+Foxp3^+$ T regulatory cells and able to produce high levels of inhibitory cytokines such as IL-10. The aim of this study is to correlate the cytokines production to the pathology caused by infection. TS strain has higher weakness, apathy, prostration and mortality due to more intense hepatosplenomegaly caused by the larger size of hepatic granulomas and extensive fibrosis of these granulomas. Both strains produced IFN-γ, but TS mice produced IL-4 and IL-10 in a larger quantity, however IL-10 was not able to regulate the growth of exacerbated hepatic granulomas in this lineage. High levels of IL-4 in TS mice are consistent with the exacerbation of granulomas, since IL-4, as well as IL-13, induces collagen synthesis and is related to the development of fibrosis in schistosomal granuloma. Additional studies are needed to confirm our proposals and to understand the mechanisms underlying the difference between the immune responses of these strains in the schistosomal-host relationship.

F.57. *Schistosoma Mansoni* Infection Promotes Reduction of B Lymphocytes in the Bone Marrow of Mice Genetically Selected for Susceptibility to Oral Tolerance

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The *Schistosoma mansoni* infection was studied in two strains of mice genetically selected for extreme phenotypes of susceptibility (TS) and resistance (TR) to oral tolerance. TR strain present good inflammatory responses and a non-tolerogenic profile, while TS mice are non-inflammatory, but high-tolerogenic profile. The aim of this work is the study of the production of B lymphocytes in mice selected for humoral response to oral tolerance infected with *S. mansoni*. TS strain has higher weakness, apathy, prostration and mortality due to anemia and hepatosplenomegaly more intense due to the larger size of hepatic granulomas. We observed a reduction of percentage of T $CD4^+$ lymphocytes in the liver of infected mice in both strains and a reduction in all subpopulations of B lymphocytes in bone marrow (precursors, immature, mature and plasma cells) more pronounced on TS strain than TR strain, possibly due to extensive mobilization of immature B cells induced by inflammation and hematopoiesis deviation for synthesis of granulocytes in TS mice. TR strain did not show changes in their subpopulations of B lymphocytes. Additional studies are needed to confirm our proposals and to understand the mechanisms underlying the difference in immune response of these strains in the *schistosoma*-host relationship.

F.58. Recent Progress in Understanding Host Immunity to Avian Coccidiosis: Role of IL-17 Family Cytokines

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Host-pathogen interaction leading to protection against coccidiosis is complex, involving many aspects of innate and adaptive immunity to intracellular parasites. Recent application of global gene expression microarray analysis to investigate gut innate immune response to Eimeria infections led to the discovery of many novel host genes modulated by different life cycle stages of coccidian parasites. Furthermore, these new findings illustrated the uniqueness of the innate immune response to Eimeria and the role of many innate immune cells and their secreted proinflammatory cytokines which influence the local host-parasite interactions in avian coccidiosis. Lately, a new type of T lymphocytes which secretes IL-17 family cytokines has been shown to influence local inflammation and mediates host defense against gut pathogens on the mucosal surface. In avian coccidiosis, IL-17 secretion was induced in the intestinal intraepithelium where Eimeria parasites undergo intracellular development during the early phase of host response to coccidiosis. Furthermore, the level of IL-17 response correlates with genetically determined coccidiosis disease resistance. Further studies on the role of IL-17 family cytokines in avians and their potential role in host defense against intestinal parasitism will be important in developing a new strategy against avian coccidiosis.(This project was supported by the Next-Generation BioGreen 21 No. PJ008084, RDA, Korea).

F.59. The Role of the Mucosal Immune System in the Induction of Th2 Responses Against the Helminth Parasite *Schistosoma Mansoni*

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The adult worms of the medically important parasite, *Schistosoma mansoni* reside within the hepatic venous system, feeding on the host blood supply. However, they fail to provoke a strong immune response until the commencement of egg deposition by the female worms, when there is rapid induction of a Th2 response. Many eggs are flushed in the direction of blood flow into the liver. However, in order to perpetuate the parasite lifecycle, some eggs must successfully transit across the intervening tissue and into the intestinal lumen to exit the host. The eruption of eggs through the intestinal wall can lead to severe pathology in the intestine. In order to understand what role the intestinal immune system plays in the induction and regulation of the Th2 response against this pathogen, we have developed a surgical

model of egg challenge by injection into the intestinal serosa. Injection of freeze/thawed schistosome eggs generated a potent egg-specific Th2 response in the lamina propria and mesenteric lymph nodes of recipient mice. Ongoing work is characterising the phenotype of immune cells following egg deposition. The role of antigen-presenting cells in the induction of this response is also being addressed using CD11c-DOG mice.

F.60. Giardia-Induced Alterations in Duodenal Mucosa Lymphocyte Populations May Persist for One Year After Treatment

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Giardia infection may cause mucosal inflammation in humans. Little is known about longer term changes. This study describes intraepithelial lymphocytes (IEL) and lamina propria (lp) T and B cells in duodenal villi and crypts during and after Giardia infection. Formalin fixed duodenal biopsies from 36 referred patients with chronic giardiasis (CG), 19 recovered volunteers one year after treatment of uncomplicated giardiasis (G1Y) and in 18 healthy volunteers (HC) were classified according to levels of inflammatory changes. Also IEL and lp crypt and villus T cells (CD3, CD4, CD8) and B cells (CD20) were quantified. Microscopic inflammation was present in 89% of CG patients, in 16% of G1Y, but not in the HC. Intraepithelial T cell levels were similar, however the IEL CD4/CD8 ratio was increased in GC ($p<0.001$) and G1Y ($p=0.004$) compared to HC. Villus lp T cell levels were also similar, but the villus/crypt ratios of both CD4 and CD8 T cells were elevated in CG ($p<0.001$). Findings correlated with grades of inflammatory changes. B cell levels were increased in lp in both CG ($p<0.001$) and G1Y ($p=0.003$) groups compared to HC. Conclusion: Giardiasis may cause severe duodenal inflammation, with persisting changes in mucosal T and B lymphocyte populations, also after normalization of routine microscopic findings.

F.61. Suppression of Allergic Airway Inflammation by Extracts of the Nematode Parasite Oesophagostomum Dentatum

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Certain helminths establish long-term chronic infections resulting in attenuated responsiveness to "bystander" antigens such as allergens or vaccines. In this study we investigated whether parasite-derived products suppress the development of allergic inflammation in a mouse model. We show that extract derived from adult male Oesophagostomum dentatum (eMOD) induced Th2 and regulatory responses in BALB/c mice. Stimulation of bone marrow-derived dendritic cells induced production of regulatory cytokines IL-10 and TGF-β. In a mouse model of birch pollen allergy, co-administration of eMOD with sensitizing allergen Bet v 1 markedly reduced the production of allergen-specific antibodies in serum. Furthermore, eMOD prevented the development of airway inflammation, as demonstrated by attenuation of bronchoalveolar lavages eosinophil influx, peribronchial inflammatory infiltrate, and mucus secretion in lungs and IL-4 and IL-5 levels in lung cell cultures. Reduced secretion of Th2-related cytokines by birch pollen-re-stimulated splenocytes and mesenteric lymph node cells was observed in eMOD-treated/sensitized and challenged mice in comparison to sensitized and challenged controls. The suppressive effects of eMOD were heat-stable. Finally, immunization with model antigens in the presence of eMOD reduced production of antibodies to thymus-dependent but not to thymus-independent antigen, suggesting that suppression of the immune responses by eMOD is mediated by interference with antigen presenting cell or T helper cell function but did not directly suppress B cell function In conclusion, the identification and characterization of parasite-derived immune-modulating molecules might have potential for designing novel prophylactic/therapeutic strategies for immune-mediated diseases.

F.62. Phenotype of Antigen Presenting-Cells in Payer's Patches of Mice with Colitis Affects Intestinal Nematode Adaptation

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Primary exposure of mice to the intestinal nematode *Heligmosomoides polygyrus* reduces inflammation in an experimental model of colitis; a significant decrease in a clinical disease symptoms and improvement in colonic damage were observed. The protective effect of prior *H. polygyrus* L4 larvae which inhabit the small intestine, at 6 days post-infection on colitis was associated with an increase in the expression of leukocytes including $CD68^+$ macrophages and neutrophils, proinflammatory cytokines IL-1b, IL-6, TNF-a and opioids POMC, MOR1 and b-endorphin expression in the small intestine and inhibition of those in the colon (Parasite Immunology, 2012, 34: 536-546). Interestingly, the infiltration of leukocytes into the small intestinal mucosa in active inflammatory reaction promotes development of the *H. polygyrus* L4; enhanced numbers and length of L4 and different larvae location on the small intestine. We have found differences in the composition and activity of antigen presenting cells APC; dendritic cells, macrophages and B cells of PP considering MHC II, CD80, CD86 receptor expression and differences in the production of pro-inflammatory and regulatory cytokines secreted by the Payer's patches (PP) cells. The changes in the PP milieu is associated with the weaker recognition and better adaptation of the nematode larvae in the small intestine.

F.63. Chitosan Alters Activity of Innate Immune Cells in Mice Infected with Trichinella Spiralis
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Chitosan is proposed as an adjuvant and drug carrier, particularly for mucosal administration. Despite its undeniable chemical advantages and utility, not much is known about chitosan influence on immunity. Aim of the study was to evaluate the effect of chitosan administration on innate immune cells in Trichinella spiralis infectied mice. Male C57Bl6 mice were intraperitonealy injected every day with 500 µg of chitosan (>375 kDa) dissolved in adipic acid 5 days before infection with 400 L-1 T. spiralis larvae. During intestinal phase of infection, peritoneal cells were isolated and phenotyped with flow cytometry. We observed increased cell infiltration into the peritoneum after chitosan treatment; the percentage of different populations was affected. Less CD11bhi $F4/80^+$ cells were present while increased number of CD11blo cells occurred. Together with lower MHC Class 2 expression it suggests that cells were attracted to the place of injection but they were not activated. Levels of cytokines in peritoneal lavage supported this observation; chitosan increased secretion of MCP-1 and TGF-β which promote cell infiltration, induce tissue healing and regulatory responses. These changes effected in higher level of infection what suggests that chitosan enhances immunosuppression evoked by the parasite itself.

F.64. Human Plasma-Derived Polymeric IgA and IgM Antibodies Associate with Secretory Component to Yield Biologically Active Secretory-Like Antibodies
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Immunoglobulin-based immunotherapy leads to important health benefits. Most immunoglobulin products are based on IgG antibodies, whereas to date the use of IgA for therapeutic application has remained anecdotal. In particular, purification or production of large amounts of secretory IgA (SIgA) for potential mucosal application has not been achieved. This study aims at investigating whether polymeric IgA (pIgA) from human plasma is able to associate with secretory component (SC) to provide a source of SIgA. We found that plasma pIgA carried J chain (20%) and that SC could bind to pIgA. As for the natural product, their association was covalent with a 1:1 stoichiometry, and the interaction with SC stabilized pIgA exposed to intestinal proteases. Similar results were obtained with human plasma IgM. These data led us to explore the protective function of secretory-like antibodies in an *in vitro* model of reconstituted intestinal epithelial Caco-2 cell monolayers using Shigella flexneri as the infectious agent. Plasma pIgA or reconstituted SIgA were shown to delay the bacteria-induced damage of monolayers. These data demonstrate that association of human plasma-derived IgA or IgM with SC is feasible and yields SIgA- and SIgM-like molecules with similar biochemical and functional characteristics as mucosa-derived immunoglobulins.

F.65. Bovine Lactoferrin Regulates Intestinal Expression of Immunoglobulin A and Secretory Component in Mice Under Acute or Chronic Stress

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Immunoglobulin A (IgA) and its associated peptide fragment, secretory component (SC), are essential for intestinal homeostasis. Since both bovine lactoferrin (bLf) and stress modulate IgA production, the aim of the present study was to analyze the influence of bLf on IgA and SC expression in mice under acute or chronic stress. Groups of six mice orally treated with 50, 500 or 5000 µg of bLf during seven days were immobilized for one hour on day seven (acute stress) or for all sevem days (chronic stress) of treatment. After day seven, mice were euthanized, epithelial cells were isolated from the proximal and distal small intestine and analyzed by chemiluminescent western blot to detect IgA α chain and SC expression. Compared to the control (saline solution treatment under stress), for acute stress greater α chain (proximal) and SC expression (both regions) were detected with 50 µg of bLf, while for chronic stress greater α chain and SC expression were found in proximal region with 50 µg and 500 µg of bLf, and greater SC expression in distal region with 5000 µg. These preliminary results suggest that under stress conditions bLf regulates IgA and SC expression differentially in each intestinal region.

F.67. Changes in Mucosal Histology and Immune Pattern from Fermented or Unfermented Bifido Milk Intake

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The benefits attributed to Bifidobacteria are their ability to physically interfere with the adhesion of pathogenic species to intestinal cells and their ability to enhance the host immune function. Functional foods are the mainly delivery form of probiotics, but the differences between fermented or unfermented product in bifido benefits are forbidden. The probiotic activity is changed through many factors not just for the strain specificity but also by the technological process used, like fermentation and moreover by the matrix that is delivery. The main action proposed to probiotic function is the pathogens control through interaction with indigenous microbiota and immune system modulation. Despite of that, which cellular and molecular mechanisms exert beneficial or dangerous actions are not clear understood yet. Differences in mucosal morphology and immunity promoted by different food technological treatment using the same matrix and the same probiotic strain - fermented or unfermented bifido milk - in health BALB/c mice, suggesting that changes in functionality of bifidobacteria and/ or the metabolites produced by fermentation process, is the key to improve beneficial effect in the host gut mucosa throughout increase in mucus and cellularity production, changes in immune pattern and preservation of mucosal epithelia in health Balb/c mice .

F.68. Effect of a Probiotic Fermented Milk in an Over-Exposition of the Antigen Ovoalbumin in a Mouse Allergy Model

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Previously we found that probiotic fermented milk (PFM) containing Lc-DN114001 administration regulates IgE production. We analyzed the immune response in lungs when mice are re-stimulated with ovoalbumin (OVA). Groups: Normal-Control (NC), Basal (B-5 days-PFM); OVA-Sensitization-Control (SC), Previous (P-5d-PFM$^+$OVA$^+$H2O) and Continuous-(C-5d-PFM$^+$OVA$^+$PFM) treatment. At 7- and 15-day post-sensitization (dPS) and 2-day post-re-stimulus (dPR) we studied in BAL-fluid: specific-IgE, IgG, total-S-IgA, IL-10, IL-4, INFγ; in lungs tissue: IL-4$^+$, IL-10$^+$ cells, and in serum: IL4, specific-IgG1 and IgG2a. Specific-IgE was significantly reduced in P and C treatment at 7dPS but not after OVA-re-stimulation, total-specific-IgG was increased in P and C at 2-day PR. IgG2a (Th1-balance) was

significantly higher in P and C at 7dPS compared to SC and to IgG1. IFNγ increased only in C at 7- and 15-day PS. IL-10 increased in C at 7-day PS. Total-S-IgA increased in C at 7-day PS. IL-4$^+$cells were augmented in SC. PFM was only effective in the primary response to IgE by IL10 production favoring Th1-response, without suppressing S-IgA and with regulated levels of IFNγ until 15-day PS in C group

F.69. Effect of a Probiotic Strain and a Probiotic Fermented Milk on the Gut Immune Response in a Stress Model in Mouse

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Stress negatively influences the immune system and gastrointestinal physiology. In a mouse stress model, we evaluated the effect of the probiotic strains Lactobacillus casei (Lc) CRL-431 and a probiotic fermented milk (PFM) containing Lc DN-114001 in immune recovery such us the number of IgA$^+$ cells in small intestine and cytokines levels released from in intestinal epithelial cells (IEC), spleen (SM) and peritoneal macrophages (PM) supernatant culture. Mice were divided into four groups: normal-control (NC), stress-control (SC) and stressed mice fed with Lc (S$^+$Lc) or with PFM (S$^+$PFM). INF-γ levels decreased in IEC supernatant in S$^+$Lc group compared to the NC. The IL-6 levels increased significantly in stressed mice compared to NC, while probiotic or PFM administration maintained normal IL-6 levels. The PFM administration induced an important IL-10 production in the supernatant of SM culture with lower levels of IL-6 compared to the control groups, while the opposite effect was observed in the culture of PM. Probiotic administration restored the number of IgA$^+$ cells in stressed mice. Stress induces an inflammatory state in the intestine with increases of IL-6 and IFN-γ and decreased IgA$^+$ cells. Probiotic or PFM administration normalized IgA levels and regulates the intestinal immune response with IL-10 increases.

F.70. Effect of *Lactobacillus Casei* CRL 431 and its Fermented Milk on the Intestinal and Systemic Immune Response in an Experimental Model of Obesity in BALB/c Mouse

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Obesity is a chronic inflammatory state with adipose tissue hypertrophy. We evaluated the effect of L. casei CRL431 (Lc) and its fermented milk (FM) in an experimental model of obesity. Mice received standard diet (G1) or high fat diet (G2) and were supplemented with milk, FM, Lc or water during two months. Liver steatosis observed in mice from G2 that received water or milk was improved in mice given FM or Lc. The small intestine, from G2 showed shorter villi and diminution of IgA$^+$ and F4/80$^+$ cells compared to mice from G1 or G2 given FM or Lc. NKT cells in liver were also increased in mice from G2 given FM. These improvements had not effect in the protection against *Salmonella* infection. The administration of FM or Lc in G2 increased the phagocytic activity of spleen and peritoneal macrophages prior to the OVA-immunization and maintained these increases in peritoneal macrophages post-immunization, compared to mice from G2 given water. No modifications in the anti-OVA-IgG concentrations were observed in the serum of mice from G2 compared to G1. Results show the potentiality of probiotic supplementation to the diet of obese hosts to improve the gut response altered by obesity.

F.71. Recombinant Probiotic *Escherichia Coli Nissle* 1917 Promotes Intestinal Epithelial Wound Healing Response via Human Epidermal Growth Factor Receptor

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The gastrointestinal mucosa can trigger defensive restitution in response to ulcerative injuries by support of epidermal growth factor (EGF) since EGF mediates epithelial migration and proliferative re-epithelialization. For full recovery of mucosal injuries, many researchers focus on development of efficient methods for localized delivery of muco-active biotherapeutics. The study designed the use of safe commensal E. coli Nissle 1917 which can deliver human EGF in conjunction with lipase ABC transporter

recognition domain (LARD). Using the *in vitro* physically wounded monolayer model, ABC transporter-mediated EGF secretion by commensal *E. coli Nissle* 1917 was demonstrated to promote wound healing migration. Furthermore, the epithelial wound closure was dependent upon the EGF receptor-linked activation, which exclusively involved subsequent signaling pathway of the mitogen-activated protein kinase kinase (MEK)-extracellular-related kinase (ERK) 1/2. Especially, the migrating frontier of the wounded edge displayed the strongest EGF receptor-linked signaling activation in the presence of the recombinant probiotic. The present study implicates a potent intervention using probiotics via efficient delivery system of therapeutic biologics (This work was supported by the Basic Science Research Program through the National Research Foundation of Korea, funded by Ministry of Education, Science, and Technology Grant 2012R1A1A2005837)

F.73. Anti-Inflammatory *Lactobacillus Rhamnosus* CNCM I-3690 Strain Protects Against Oxidative Stress and Increases Lifespan in *Caenorhabditis Elegans*

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Nutritional interventions using antioxidant food-grade compounds are currently an option to help improve health and quality of life in the elderly. Live lactic acid bacteria (LAB) administered in food, such as probiotics, may be good antioxidant candidates. To identify and characterize new potential antioxidant probiotic strains, we have developed a functional screening method using *Caenorhabditis elegans* as host. *C. elegans* were fed on different LAB strains and nematode viability was assessed after oxidative stress. One strain, identified as *Lactobacillus* rhamnosus CNCM I-3690, protected worms by increasing their viability by 30% and average worm lifespan by 20%. Moreover, transcriptomic analysis of *C. elegans* fed with this strain showed that increased lifespan is correlated with differential expression of the DAF-16/insulin-like pathway, highly conserved in humans. This strain also had a clear anti-inflammatory profile when co-cultured with HT-29 cells, stimulated by pro-inflammatory cytokines, and co-culture systems with HT-29 cells and DC in the presence of LPS. Finally, this Lactobacillus strain reduced inflammation in a murine model of colitis. This work suggests that *C. elegans* is a fast, predictive and convenient screening tool to identify new potential antioxidant probiotic strains for subsequent use in humans.

F.74. Dual-Colonization of *Lactobacillus Rhamnosus* Strain GG and *Bifidobacterium Animalis subsp. Lactis* Bb12 Probiotics Enhances Mucosal B Cell Responses to an Oral Human Rotavirus Vaccine in a Neonatal Gnotobiotic Pig Model

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Probiotic colonization is a potential strategy to modulate oral rotavirus (RV) vaccine responses and reduce the severity of RV infection. However, the effect of probiotics on RV vaccines and virulent RV infection is poorly understood. We evaluated the impact of co-colonization by two dominant bacterial species in the breastfed-infant gut, *Lactobacillus rhamnosus* strain GG and *Bifidobacterium animalis lactis* Bb12, on B cell responses to an attenuated human RV (AttHRV, Wa strain) vaccine and protection post-virulent homologous HRV challenge in a neonatal gnotobiotic pig model. Following HRV challenge, probiotic-colonized, vaccinated piglets had reduced diarrhea duration, significantly lower fecal scores and reduced peak RV shedding titers compared to vaccinated, uncolonized pigs. Also, intestinal IgA HRV antibody titers and duodenal HRV IgA antibody secreting cell numbers were significantly higher in probiotic-colonized, vaccinated pigs compared to vaccinated, uncolonized pigs post-challenge. However, serum IgG RV antibody titers were significantly lower in probiotic-colonized, vaccinated pigs compared to vaccinated, uncolonized pigs, both pre- and post-HRV challenge. Thus colonization with selected probiotics may augment vaccine-primed intestinal IgA antibody responses to HRV vaccines post-challenge, resulting in reduced severity of HRV infection. These findings have important implications for improving oral RV vaccine efficacy in children in developing countries.

F.75. Lactobacilli Gavage Reduces Intestinal Inflammation upon Intestinal Perturbations
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Recent data demonstrate that intestinal symbiotic bacteria can promote or prevent autoimmunity in organs distant to the intestine, such as the pancreas and the brain. This has triggered a global interest in the effect of intestinal symbionts on host immunity and physiology. As a consequence, therapeutic or prophylactic strategies based on the oral administration of probiotics witness a vigorous renaissance. Administration of probiotic strains has been shown to reduce inflammation in mice and it seems plausible that Lactobacilli could affect intestinal as well as systemic immunity. To study such effects, we have determined by qPCR the type of proximal and distal immune responses induced by oral intake of Lactobacilli in SPF mice. We have shown that, given daily after intestinal perturbations caused by antibiotics, DSS or Citrobacter rodentium infection, Lactobacilli could modulate the amount of pro-inflammatory cytokine mRNA produced in the ileum, the colon, as well as in more distal organs such as the spleen.

F.76. Colonization of Conventional Mice with Lactobacillus Rhamnosus GG (LGG) Promotes Development of Protective Immune Responses
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LGG has been studied in clinical trials for treating and/or preventing several disorders. However, there is concern regarding probiotic viability in the intestinal tract delivered via the oral route. We sought to define the immune-protective mechanism in adult mice by evaluating conventional mice colonized with LGG at birth. We gavaged pregnant mice with LGG for three days before delivery day and fed pups with LGG immediately after birth until postnatal day five to develop life-long colonization. Paraformaldehyde-fixed LGG was used for mock colonization. The colonization rate determined by DNA fingerprint method was between 50-70%. LGG colonized mice showed 15% increase of bodyweight at 1 to 5-week-old and no difference at age older than five weeks, and increased the fecal sIgA level, as compared to un-colonized mice and fixed LGG-treated mice. The percentage of B lymphocytes ($B220^+$ cells) in spleen was 25% increased in LGG colonized mice. Fecal bacterial culture showed increased Lactobacillus colonies in LGG colonized mice. DSS-induced colitis in uncolonized mice was significantly ameliorated by LGG colonization. Thus, neonatal colonization of mice with a probiotic bacterium provides health benefits to the adult animal, in part by modulating B cell immune function. These findings suggest life-long immune-mediated protection to the probiotic-colonized host.

F.77. Alveolar Responses to RSV Infection is Altered Differentially by Exogenous Osteopontin Administration in Different Age Groups
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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, we intended to examine the alveolar 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. Lymphocytes, macrophages and neutrophils were enumerated in cytospin slides prepared of bronchoalevolar lavage fluid (BALF) obtained from RSV-infected mice with or without OPN treatment. Control groups received only phosphate buffer solutions. Pulmonary damage was assessed by measuring the Lactic Dehydrogenase (LDH) levels in the BALF. Four-day- old pups as compared to 14- and 20-day-old mice had a decreased lymphocyte response; but developed an enhanced neutrophil response, which was associated with increased LDH levels. Administration of OPN had downregulatory effects on the lymphocyte responses in all age groups with proportional upregulation of macrophage responses. This effect was less pronounced in the older age groups. Understanding the underlying mechanisms of OPN-mediated differential immunomodulation in different age groups may help develop newer therapeutic strategies.

F.78. CD4$^+$CD25^{hi}CD127^{lo}Foxp3$^+$ Regulatory T Cells Generated *in vitro* From Blood CD45RA$^+$ Precursors Suppress Mucosal Effector T Cells in Crohn's Disease Patients

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Thymically derived regulatory T cells (Tregs) prevent inappropriate activation of multiple immune cell types. Defective Tregs are present in the gut of Crohn's (CD) patients, potentially contributing to chronic mucosal inflammation. Cell-based therapy with Tregs is conceptually attractive but the optimal method to generate phenotypically stable, functionally suppressive Tregs from CD patients *in vitro* is unknown. Tregs were isolated from CD peripheral blood (PB) either by GMP-compatible MACS enrichment (CD8-depletion followed by CD25-enrichment), or FACS-sorting on the basis of CD4$^+$CD25^{hi}CD127^{lo}CD45RA$^+$ or CD45RA$^-$ and expanded *in vitro* for 24 days (n=10 each). MACS-Tregs had substantial CD8$^+$ contamination and produced significantly more IL-17 and IFN-γ than CD45RA$^+$ and CD45RA$^-$ Tregs ($p<0.01$ each). CD45RA$^+$ Tregs exhibited the highest CD25 and FOXP3 expression, were more likely to maintain a stable phenotype (even when exposed to inflammatory stimuli) and were also significantly better at suppressing T responder cell proliferation than other Treg subsets ($p<0.01$). CD45RA$^+$ Tregs also suppressed proliferation and activation (CD154 expression) of MLN and LP T cells from inflamed CD mucosa. In conclusion, FACS-sorted CD45RA$^+$ Tregs generate the most homogenous, phenotypically stable and suppressive Treg cell product from CD patients, including suppression of mucosal T cells, strengthening the rationale for using this population as a novel therapy.

F.79. Commensal Microbiota Shapes the Gut Immune System Through Epigenetic Modifications

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Colonization of gut-indigenous bacteria to germ-free mice induces differentiation and proliferation of colonic regulatory T cells (Treg); however, the underlying mechanism remains largely unknown. We found that colonization of bacteria to germ-free mice upregulated the expression levels of an adaptor protein for DNA methyl transferase (Dnap) in colonic CD4$^+$ T cells. Dnap-deficient mice were defective in colonic Treg expansion and spontaneously developed severe colitis due to activation of TH1 and TH17 responses. Genome-wide gene expression profiling followed by molecular network analysis indicated that cell cycle-dependent kinase inhibitors, Cdkn1a, was de-repressed in Dnap-deficient T cells. This change was associated with hypomethylation of the promoter region of this gene. These data suggest that Dnap-dependent epigenetic silencing of Cdkn1a is required for vigorous expansion of colonic Treg cells in response to bacterial colonization. This mechanism is essential for the maintenance of gut immune homeostasis.

F.80. Treg Cells Mediate Suppression of IL-1β Production

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Regulatory T cells (Tregs) are indispensable for regulating adaptive immunity as well as inflammation mediated by the innate immune system. Dysregulation of innate immunity can lead to uncontrolled activation of the inflammasome and subsequent maturation and release of pro-inflammatory IL-1 family cytokines, resulting in excess inflammation and tissue destruction. Delivery of Tregs can dampen mucosal inflammation and cure colitis in experimental models, but whether or not they can suppress inflammasome activation is unknown. To test this, we co-cultured human Tregs or conventional T cells (Tconv) with allogeneic macrophages and stimulated the inflammasome by addition of LPS and ATP. We found that Tregs but not Tconvs potently suppressed the transcription and release of mature IL-1β. Similar experiments confirmed this finding in mice. To investigate the mechanism(s) by which Tregs suppress IL-1β production, mouse Treg-conditioned media was added to macrophages stimulated with

LPS and ATP, and found to similarly block IL-1β in a dose-dependent manner. Preliminary data suggest that IL-10, but not TGFβ, in the Treg-conditioned media mediates this effect. These data represent the first evidence for Treg-control of IL-1β production and have implications for the application of Treg cell therapy in inflammatory bowel disease.

F.81. Activation of Heat Shock Factor 1 Stimulates Treg Function and IL-10 Secretion

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Genetic deficiency of HSF1 has been shown to result in a more severe chemically-induced colitis, yet efforts to enhance HSF1 activation in models of inflammatory bowel disease have not been attempted. HSF1 has been shown previously to translocate to the nucleus and drive expression of a number of anti-inflammatory genes such as IL-10. We hypothesize that activation of HSF1 will enhance Treg action and attenuate murine colitis. Heat shock of naïve $CD4^+$ T cells results in an increase in Treg induction in the presence of TGFβ. Conversion from naïve cell to Treg coincided with shuttling of HSF1 into the nucleus within 1 hr of stimulation with anti-CD3. Genetic deletion of HSF1 resulted in a decrease in intestinal $Foxp3^+$ Treg frequency along with impaired suppressive function *in vitro*. Using Celastrol, an HSF1 activator, we enhanced Treg suppressive function and drove translocation of HSF1 from the cytoplasm into the nucleus *in vitro* and attenuated established inflammation in the T cell driven CD45RBHi colitis model, increasing the frequency of $Foxp3^+$ IL-10 producing Tregs in the colonic lamina propria. Targeting the heat shock pathway to activate Tregs for therapeutic benefit represents an exciting and novel strategy for the treatment of inflammatory diseases.

F.82. Inflammatory Monocytes Regulate Pathologic Responses to Commensals During Acute Gastrointestinal Infection

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Commensal flora can promote both immunity to pathogens and mucosal inflammation. How commensal driven inflammation is regulated in the context of infection remains poorly understood. Here, we show that during acute mucosal infection, Ly6Chi inflammatory monocytes acquire a tissue specific regulatory phenotype associated with production of both IL-10 and the lipid mediator prostaglandin E2 (PGE2). Notably, in response to commensals, Ly6Chi monocytes can directly inhibit multiple aspects of neutrophil activation, including reactive oxygen species production, in a PGE2-dependent manner. Further, in the absence of inflammatory monocytes, mice develop severe neutrophil-mediated pathology that can be controlled by PGE2 analog treatment. Complementing these findings, modulating PGE2 production with non-steroidal anti-inflammatory drugs led to alterations in neutrophil activation and host mortality. These data demonstrate a previously unappreciated dual action of inflammatory monocytes in controlling pathogen expansion while limiting commensal mediated damage to the gut. Collectively, our results place inflammatory monocyte derived PGE2 at the center of a commensal driven regulatory loop required to control host-commensal dialogue during inflammation.

F.83. Pleurocidin, a Novel Anti-microbial Peptide, Induces Human Mast Cell Activation Through the FPRL1 Receptor

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Pleurocidins are a novel family of α-helical antimicrobial peptides structurally and functionally similar to cathelicidins, one of the major antimicrobial peptides families. Since cathelicidins stimulate mast cell chemotaxis and mediator release, we postulated that pleurocidins similarly activate human mast cells. A screen of 20 pleurocidin peptides revealed that some were capable of degranulating human mast cells.

Pleurocidin NRC-04 caused mast cells to adhere, migrate, degranulate and release eicosanoids. Moreover, pleurocidin increased [$Ca2^{+}$]i mobilization, and induced the production of proinflammatory chemokines such as CCL2 and CCL4. Our evaluation of possible cellular mechanisms suggested that G proteins, phosphoinositol-3 kinase, phospholipase C and phosphokinase C are involved in pleurocidin-induced mast cell activation as evidenced by the inhibitory effects of pertussis toxin (G protein inhibitor), wortmannin (phosphoinositol-3 kinase inhibitor), U-73122 (phospholipase C inhibitor) and Ro-31-8220 (phosphokinase C inhibitor), respectively. We also found that human mast cells express the FPRL1 receptor at both the mRNA and protein levels. Given that FPRL1 inhibitor affected pleurocidin-mediated mast cell activation, we concluded that this receptor is likely to be functional in human mast cell stimulation by pleurocidin. Our finding that pleurocidin activates human mast cells through GPCR signaling pathway suggests that this novel peptide might have immunomodulatory functions.

F.84. Implications of Nasopharynx-Associated Lymphoid Tissue (NALT) in the Development of Allergic Responses

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Background: Nasopharynx-associated lymphoid tissue (NALT) serves as an important inductive site for mucosal immunity in the upper respiratory tract. Despite its importance in the mucosal immune system, little is known regarding the role of NALT in airway allergic immune responses. We aimed to elucidate the role of NALT in the induction of upper airway allergic responses in a mouse model. Methods: Inhibitor of DNA binding/differentiation 2 $(Id2)^{-/-}$ and $Id2^{+/-}$ mice were exposed to the ovalbumin (OVA)-induced allergic rhinitis model. The allergic parameters, such as allergic symptoms, serum OVA-specific IgE levels, eosinophil infiltration, and cytokine profiles in the nasal mucosa were compared between $Id2^{-/-}$ and $Id2^{+/-}$ groups. Results: NALT-null, $Id2^{-/-}$ mice displayed significantly lower allergic responses compared with $Id2^{+/-}$ mice. Lethally irradiated $Id2^{-/-}$ mice were engrafted with C57BL/6 wild-type bone marrow cells and showed still significantly lower allergic immune responses compared with equally treated $Id2^{+/-}$ mice. In addition, IgE class switch recombination (CSR)-associated molecules, such as εGLT, ε mRNA and AID mRNA, were detected in NALT from OVA-sensitized wild type mice. Conclusion: These results show the critical role of NALT for the induction of allergic responses in the upper airway at least in part by means of class switching to IgE in situ.

F.85. Cellular and Molecular Immune Mechanisms in Pathogenesis of Nasal Polyposis

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Nasal polyposis (NP) is a chronic and recurrent inflammatory disease affecting up to 4% of population in developed countries. Innate immune mechanisms together with various subsets of APCs (Antigen Presenting Cells) and T cells may play an important role in pathogenesis of NP. Using immunohistochemistry, we first documented significantly increased number of APCs according to the increased proportion of MHC II, DC-SIGN and CD1d-positive cells in NP vs. nasal mucosa. Next, we investigated a potential involvement of regulatory T cells, but found no substantial differences in number of $CD3^{+}Foxp3^{+}$ cells within NP compared to nasal mucosa. In our previous study, we have documented substantially increased expression of TLRs 3,4,5,7,8 and 9 in NPs. However, no substantial differences in expression of selected intracellular Nod-like receptors (Nod1, Nod2, Nalp1 and Nalp3) was found in this study. On the other hand, we found increased expression of iNOS in both epithelial and lamina propria compartments of NP. These data suggest that APCs and innate immune mechanisms may play important role in formation of NP. Better understanding of the molecular immune mechanisms in NP may shed more light on pathogenesis as well as possible environmental triggers of this frequent but not fully understood disease.

F.86. Intranasal Immunization of a Flagellin-Adjuvanted Peptide Vaccine Enhances Tumor Protection in a Mouse Model

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Human papillomavirus (HPV), a significant cause for cancer-related deaths in women worldwide, is a sexually transmitted mucosal pathogen. Therefore mucosal immune responses constitute an essential feature for vaccination strategies against HPV-associated lesions. However, mucosally administered vaccines generally evoke low immunogenicity, which is partially due to the lack of an effective mucosal adjuvant. In our previous studies, Vibrio vulnificus FlaB, a bacterial flagellin, has exhibited to be a strong mucosal adjuvant activity by stimulating the Toll-like receptor 5 (TLR5). In this study, we tested whether the FlaB protein could serve as an effective mucosal adjuvant for HPV vaccine based on mixture of E6 and E7 peptides, which play a major role in the induction and maintenance of cellular transformation for cancer. As a result, intranasal co-administration of the peptide mixture with FlaB primed a significant level of IFN-γ production by both draining lymph node cells and splenocytes cells. Furthermore, the FlaB mucosal adjuvant conferred excellent protection against TC-1 tumor challenge with a high survival rate. These results support that FlaB can be used as a promising mucosal adjuvant for nasal HPV vaccine development.

F.87. Classic Plasmablasts and CD27 Plasmablasts Infiltrate Peripheral Blood Following CVD 1208S (Shigella) Vaccination

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There are no licensed Shigella vaccines; however, various candidates have emerged, including the live attenuated CVD 1208S strain (ΔguaBA, Δset, Δsen), which was safe and immunogenic in Phase 1 trials but appeared reactogenic in several subjects in a Phase 2 study in which subjects received a freshly harvested vaccine formulation grown in an animal product-free medium different from that previously used. Immune responses to Shigella were evaluated, including vaccine-induced plasmablasts (PBs) and their expression of gut mucosa and other homing markers (integrin α4β7, CD62L and CXCR3). PBs were detected by ASCs-ELISPOT in peripheral blood on day seven post-immunization. Anti-LPS ASCs were more abundant than anti-IpaB ASCs, and the IgA isotype was more common than IgG. Flow cytometry confirmed the presence of classic PBs (CPBs) (CD19dim CD20- CD27high CD38high) as well as a distinct population lacking CD27 (CD27-). Single and simultaneous expression of homing markers demonstrated that CPBs up-regulated gut mucosa (integrin α4β7) and inflammation sites (CXCR3) homing markers. Even though CD27- PB also somewhat up-regulated integrin α4β7 and CXCR3, the homing site remains undefined (presumably bone marrow). Sorting experiments confirmed that PBs expressing integrin α4β7 alone or in combination with CD62L were responsible for antibody production (anti-LPS and anti-IpaB).

F.88. Recombinant IL-7 as a Mucosal Vaccine Adjuvant

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Approaches to elicit efficient mucosal immunity, a pre-requisite to effective protection against mucosal infection, are largely unsatisfactory. We recently showed that systemic injection of IL-7 induces mucosal production of chemokines, thereby provoking massive circulating T cells recruitment into the mucosa (Blood 2009, 114(4):816-25). We here demonstrated that application of 5 to 15µg of simian IL-7 to rhesus macaques vaginal mucosa increases significantly CXCL12, CCL5, CCL20 and CCL28 local transcription, as quantified by qRT-PCR, thus provoking massive mDC, macrophages, B and T cells infiltration in the mucosa, as quantified by immunohistochemistry and image analysis. Further, macaques immunized with a cocktail of antigens (DT, TT, HBsAg) directly applied on the vaginal mucosa two days after vaginal IL-7 administration developed a stronger mucosal specific immune response than animals receiving PBS before the antigenic cocktail. Indeed, IL-7 pre-treatment allowed for a robust production of antigen-

specific IgA and IgG in vaginal secretions, as detected by ELISA. By attracting immune cells, local IL-7 administration prepares the mucosal immune system, gathering conditions that result in enhanced antigen-specific vaginal immune responses. Hence, IL-7 appears as a mucosal adjuvant to increase vaginal antibody response, the most promising way to confer protection to numerous infections.

F.89. Immunogenicity and Safety of a Live Attenuated Cold-Adapted Influenza Vaccine Administered by the Nasal and Sublingual Route in Healthy Adult Volunteers

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FluMist® is a live attenuated influenza vaccine. Clinical trials have demonstrated that it was superior to conventional vaccines in protecting against flu. However, rare but serious safety issues have been reported with nasal immunization of the vaccine and attributed to retrograde uptake of vaccine components into the central nervous system. In animal studies, sublingual administration of live influenza virus has been shown to avoid this untoward effect while eliciting strong mucosal and systemic immunity. To examine the immunogenicity and safety in humans, Flumist® was administered to 20 healthy adults sublingually and nasally, respectively. Antibody-secreting cells (ASCs) were determined in the pre- and post-vaccination blood samples by Elispot assay using magnetic beads coated with antibodies to HLA-DR and CD19 for total ASCs or antibodies to α4β7 for mucosal ASCs Highly comparable ASC responses against influenza virus were observed after nasal (44%, 8/18) and sublingual (42%, 8/19) vaccination. Seventeen and twenty-six percent of vaccinees showed demonstrable mucosal ASC responses in nasal and sublingual group, respectively. Given the fact that sublingual immunization of live influenza vaccine elicited similar ASC responses compared with nasal vaccination, the sublingual route could provide a potentially safer and programmatically more acceptable alternative than the nasal route especially in infants and the elderly.

F.90. Protection Against Intestinal Taeniosis is Elicited by Oral Immunization with Recombinant *Taenia Solium Calreticulin*

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Human neurocysticercosis is a parasitic disease of the central nervous system caused by the larval stage of *Taenia solium*. This infection still represents a public health problem in many developing tropical and subtropical countries affecting human beings and livestock. Epidemiological studies show that proximity to the human tapeworm carrier is a major risk factor for acquiring cysticercosis; therefore its prevention through vaccination is an option to interfere with egg production and thus, interrupt transmission of the disease to humans and pigs. Hamsters were orally immunized with recombinant *T.solium* calreticulin (rTsCRT) using cholera toxin (CT) as adjuvant, weekly for four weeks. Fifteen days after the last boost animals were challenged with four *T.solium cysticerci*; 33-44% reduction in tapeworm establishment and in worm size was found. A second experiment further showed increased transcription of mRNA for IL-4 and IFN-γ, goblet cell hyperplasia and mucin production in the mucosa surrounding the implantation site of the parasite. Specific IgG and IgA antibodies in serum and fecal supernatants were detected after the second immunization, being more pronounced after challenge. These data suggest that oral vaccination with rTsCRT^{+}CT results in an unfavorable environment for *T. solium*, promoting an impaired tapeworm development and prompt expulsion.

F.91. Application of Cathelicidin LL-37 as Mucosal Adjuvant Promotes Antigen-Specific Systemic and Mucosal Immunity via Modulation of Peyer's Patch Microenvironment

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Oral mucosal antigen administration is an easy, economical, and efficient strategy to induce immune response not only in systemic but also in mucosal compartments. However, successful oral immunization requires the adjuvant for efficient antigen delivery and breaking the tolerogenic environment. We, in this study, suggest the cathelicidin LL-37 as a mucosal adjuvant. We have shown that oral priming with LL-37-conjugated antigen evoked antigen-specific antibody response in systemic and mucosal compartments. The adjuvant activity is resulted from the enhanced germinal center formation and activation of follicular dendritic cells (FDCs) expressing the formyl peptide receptor-2 (FPR-2), one of LL-37 receptors, by chemotactic activity of LL-37 in Peyer's patch. Immune-modulatory effect of LL-37 is closely related with the Th17-skewed adaptive immune responses through the induction of $CD11c^{+}CD70^{+}$ mature DCs and inflammatory cytokines. Expression of FPR-2 on M cells, which is reported first in this study, is notable in immune induction in mucosa since the molecule can be exploited for antigen delivery. Collectively, we conclude that LL-37 is potential oral mucosal adjuvant acting through chemotactic effect, DC maturation, and antigen-targeting into the M cells.

F.92. A Novel Mucosal Therapeutic Vaccine Candidate Against Chronic Hepatitis B: From the Laboratory to the Clinical Trials

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Introduction: Chronic hepatitis B is a major health problem, with more than 350 million people infected worldwide. Available therapies have limited efficacy and require long-term treatments. A mucosal approach for chronic hepatitis B immunotherapy has been tested in preclinical and clinical studies using a novel vaccine formulation comprising the surface and core virus like particles. Materials and Methods: The therapeutic vaccine candidate comprised two recombinant HBV antigens: HBsAg and HBcAg. Several preclinical studies including immuno-toxicological evaluations using HBsAg transgenic mice have been completed. Until now, four clinical trials concluded including a Phase 3 trial to be finished in the first part of 2013. Results: The vaccine candidate was very immunogenic in mice after intranasal or combined administrations inducing higher antibody titers, potent proliferative responses and the secretion of gamma interferon by spleen cells. The formulation also demonstrated its safety in toxicological studies. Several clinical trials Phase 1-3, conducted in two countries, evidenced the safety and efficacy of the vaccine candidate. Conclusions: The obtained results support the future introduction of this product in the clinical practice. To our knowledge, this is the first therapeutic approach exploiting mucosal route against a chronic infectious disease to reach Phase 3 clinical trial.

F.93. Cholera Vaccine-Induced Anti-Bacterial and Anti-Toxic Immunity Protects Against *Vibrio Cholerae* Infection in a Murine Pneumonia Model

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Although oral vaccines against cholera have been licensed for human use, the assessment of protective immunity has been hindered due to the lack of appropriate animal models. In this study, we demonstrated a murine pneumonia model which was induced by intranasal administration with live *Vibrio cholerae*. Bacterial components of *V. cholerae*, but not cholera toxin, induced lethal and acute pneumonia by enhancing massive inflammation. Intranasal immunization with Dukoral™, a commercial cholera vaccine comprised of killed whole bacteria and recombinant cholera B subunit (rCTB), increased both mucosal and systemic antibody responses as well as protection against *V. cholerae* infection. Although rCTB-free Dukoral and rCTB alone partially protected against the infection, reconstitution of rCTB-free Dukoral with rCTB restored full protection. Parenteral immunization with Dukoral™ provoked strong systemic antibody responses, but failed to induce mucosal antibody responses as well as protection against the infection. Taken together, both anti-bacterial and anti-toxic immunities are required to promote protection against *V.*

cholerae-induced pneumonia and this murine pulmonary model is useful for pre-clinical assessment of candidate cholera vaccines.

F.94. Optimizing the Assessment of HIV-Specific Genital Antibodies in Exposed Uninfected Women Participating in Microbicide Trials

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Vaginal microbicides may lead to mucosal immunity against HIV in users by exposing them to drug-inactivated virus. We first conducted a pilot study in a small cohort of HIV-positive and negative women in Seattle to compare sampling devices for vaginal antibody measurements. We used Luminex bead arrays to measure IgG and IgA antibodies binding to a panel of nine HIV-1 envelope antigens in eluents from three different vaginal sampling devices: Dacron swabs, flocked nylon swabs, and Merocel sponges. IgG antibodies from HIV-positive women reacted broadly across the full panel of HIV-1 Env antigens, whereas IgA antibodies showed narrow reactivity only to Env gp41. Best results were obtained from the Merocel sponge eluents. We then tested vaginal swabs obtained from 57 HIV-seronegative African women who participated in a clinical microbicide trial. We detected vaginal HIV-1 Env-specific IgA but not IgG antibodies in ten of these women. The multiplexed Luminex assay is well suited to measuring HIV-specific antibodies in mucosal secretions, with Merocel sponges being a superior sampling device compared to swabs. Whereas HIV-seropositive women always demonstrate broadly Env-binding IgG and narrowly Env-binding IgA antibodies in their vaginal secretions, vaginal Env-binding antibodies in HIV-exposed seronegative women are restricted to the IgA subtype.

F.95. A Novel Concept for Tolerization of Diabetogenic $CD4^+$ T Cells by Intranasal Treatment with CTA1R7K-peptide-DD

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Type I-diabetes (T1D) results from $CD4^+$ T cell dependent immune destruction of insulin producing β-cells in the pancreas islets. Tolerization of diabetogenic $CD4^+$ T cells would therefore be an important advancement in the development T1D treatments. We previously described that a mutated (R7K), enzyme killed, cholera toxin A1 subunit based adjuvant CTA1R7K-DD induces specific tolerance rather than enhancement of immunity. Intranasal (i n) treatment with CTA1R7K-DD containing a type II collagen peptide reduced recall responses to the peptide, and moreover, ameliorated murine collagen induced arthritis. Here, we investigate tolerization of diabetogenic T cells of the non-obese diabetic (NOD) mouse, exploring diabetogenic TCR transgenic BDC2.5 $CD4^+$ T cells. Treatment of BDC2.5 NOD mice i n with CTA1R7K-PS3-DD fusion protein (PS3 = BDC2.5 TCR specific peptide) reduced proliferation and IFN-γ production to PS3 peptide restimulation. Transferred $CD4^+$ BDC2.5 T cells induced T1D in 80-100% of recipient NOD.scid mice. In contrast, 95% of recipients of cells from BDC2.5 NOD mice treated with CTA1R7K-PS3-DD remained healthy. The i n treatment resulted in systemic increase in $Foxp3^+$ $CD4^+$ BDC2.5 T cells most enhanced in draining mediastinal lymph nodes. The data suggest that CTA1R7K-peptide-DD may prevent T1D development by the induction of peptide-specific $CD4^+$ Treg cells.

F.96. Intranasal Delivery of Recombinant HIV-1 Fowl Poxvirus Vaccines Can Induce Excellent High Avidity CD8 T Immunity by Recruiting Unique Antigen Presenting Cell Subsets to the Lung Mucosae

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We have investigated the mechanism of rFPV uptake via mucosae, safety as a mucosal vector and how some cytokines (i.e. IL-4/IL-13) modulate CD8 T cell avidity at the vaccination site. Following intranasal (i.n.) immunization with vaccine vectors co-expressing HIV antigens and GFP, we have shown that (i) rFPV peak expression occurs at 12 hour-24 hour post vaccination (p.v.) and no virus is detected 96h p.v.

(ii) FPV-HIV-GFP only disseminates to the initial vaccination site (lung), and not to spleen, gut, nor has it crossed the blood-brain barrier, indicating that rFPV is a safe mucosal delivery vector. To understand how different priming vectors could induce, low or high avidity CD8 T cells, 24-hour post i.n. vaccination antigen presenting cell subsets (APC's) in lung were analyzed using flow cytometry. Results indicated that (i) in contrast to i.n. recombinant vaccinia virus delivery, rFPV recruited uniquely different dendritic cell and macrophage subsets to the lung mucosae. (ii) transient inhibition of IL-13 at the vaccination site enabled the recruitment of elevated numbers of uniquely different APC's to the lung mucosae, which most likely were responsible for the induction of high-avidity HIV-specific CD8 T cells. Our data clearly indicate that avidity is determined at the priming vaccination site/cell milieu (not booster), and the APC's they induce.

F.97. Eyedrop Vaccination Induce Systemic and Mucosal Immune Against Influenza Virus in Ferret
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Our previous study demonstrated that eyedrop vaccination can protect mice from lethal influenza virus challenge. In this study, we further investigated if protective immunity can be equipped by eyedrop vaccination in other animal models such as ferrets or beagles. We vaccinated ferrets with 10^5 TCID50 of live-attenuated A/California/7/09 x PR8 H1N1, Sw/Korea/PZ4/06 H1N2 and A/Uruguay/716/07 x PR8 H3N2 vaccine strain-influenza viruses two times with two- week intervals by eyedrop. Two weeks after the last vaccination, production of serum IgG and nasal IgA anti-influenza virus Abs was significantly increased. When ferrets were challenged with 10^5 or 10^6 X TCID50 of influenza viruses, all eyedrop vaccinated ferrets showed significantly less body-weight loss, lower body-temperature increase, earlier virus clearance and intact lung-tissue morphologies compared to these of PBS group. Furthermore, eyedrop contrast medium were not detected in olfactory bulb (OB) while intranasally treated medium contaminated OB area in ferret's brain. As shown in ferrets, eyedrop or subcutaneously vaccinated commercial canine parvo virus (CPV) vaccines induced significantly similar levels of anti-CPV serum IgG production. Thus, we first propose that eyedrop vaccination against pathogenic viruses is a safe and effective for both ferrets and beagles.

F.98. Vitamin A Deficiency Impairs B Cell Responses to Rotavirus Vaccination and Challenge in a Gnotobiotic Piglet Model
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Vitamin A deficiency (VAD) is associated with reduced efficacy of vaccines and higher incidence of diarrheal infections in children. We established a VAD gnotobiotic piglet model to study the effects of VAD on an oral human rotavirus (HRV) vaccine and virulent HRV challenge. Piglets derived from VAD and VA sufficient (VAS) sows were orally vaccinated with attenuated HRV (3X) and/or supplemented with VA and/or challenged with virulent HRV. VAD piglets had significantly lower hepatic VA levels which coincided with higher magnitude and longer duration of diarrhea and HRV shedding in vaccinated and control VAD piglets as compared to vaccinated and control VAS piglets, respectively. VAD vaccinated pigs had lower serum and intestinal IgA HRV antibody titers and lower intestinal IgA antibody secreting cells post-challenge as compared to VAS vaccinated piglets. Serum IgG HRV antibody titers were similar in VAD and VAS vaccinated piglets. However, VAD vaccinated piglets had higher IgG2 HRV antibody titers post-challenge, suggesting imbalanced Th1/Th2 responses. We conclude that VAD piglets are more susceptible to HRV diarrhea and have lower IgA and imbalanced antibody responses to HRV vaccine, suggesting that VAD in children in developing countries may result in more severe rotavirus infection and lower vaccine efficacy.

F.99. Lymphocytes Activated in the Peyer's Patches, but not in Other Lymphoid Tissues, are Essential for Intestinal IgA Responses Against *Recombinant Salmonella*
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A previous study showed that Peyer's patches (PP) are indispensable for inducing intestinal IgA responses to orally administered *recombinant Salmonella* expressing fragment C of tetanus toxin (*rSalmonella*-Tox C). In this study, we focused on the PP leukocytes involved in the induction of antigen (Ag)-specific intestinal IgA immunity to oral *rSalmonella*-Tox C. Our results showed that FTY720-treated mice had reduced tetanus toxoid (TT)-specific intestinal IgA responses, whereas the numbers of TT-specific IgA antibody (Ab)-forming cells in PP were increased. In contrast, the frequencies of PP-derived dendritic cells (DCs) and bacterial colonies in mesenteric lymph nodes and spleen were not altered in the FTY720-treated mice. Furthermore, $CCR7^{-/-}$ mice elicited TT-specific IgA responses. These results indicate that the migration of activated DCs or bacterial Ag is not engaged in the induction of intestinal IgA immunity. In addition, when PP-null mice were given PP cells and immunized with *rSalmonella*-Tox C, no IgA anti-TT responses were induced; interestingly, however, irradiated mice reconstituted with PP cells had restored TT-specific intestinal IgA Abs. These results suggest that lymphocytes need to be activated in the PP, but not in other lymphoid tissues, for the induction of intestinal IgA immunity against *rSalmonella*.

F.101. Protective Immunity by Mucosal Vaccination Against *Orientia Tsutsugamushi*

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Scrub typhus is caused by the bite of an *Orientia tsutsugamushi*-infected trombiculid mite on skin surface in human. A number of studies have attempted to develop vaccine against *Orientia tsutsugamushi* via systemic route with limited success. Recently, it was found that pneumonia was frequently induced in patients suffering from Scrub typhus, leading to a hypothesis that intranasal vaccination would enhance protective immunity against *O. tsutsugamushi*. The current study demonstrates for the first time, at the best of our knowledge, that 1) When mice were challenged through intraperitoneal route, bacteria disseminated mostly into lung, eventually causing pneumonia, 2) Vaccination with four outer-membrane proteins, 22, 47, 56, 110 kDa, induced protective immunity when administrated via intranasal route, but not systemic route, 3) Antigen-specific serum IgG were increased in mice vaccinated via either systemic or intranasal route, but antigen-specific serum IgA responses were induced only in mice vaccinated via intranasal route. Interestingly, however, serum antigen-specific IgA induced by intranasal vaccination did not well respond to *O. tsutsugamushi*, leading to a conclusion that serum IgA could not provide protective immunity. 4) Vaccination via intranasal route induced higher T cell response in spleen than that via systemic route when the same dose was administrated, and 5) Vaccination via intranasal route, not systemic, provided long-term protective immunity. In summary, the current study provided a useful information for assessing scrub typhus in a mouse model that developed pneumonia after being infected with *O. tsutsugamushi*, and elicited protective immunity against scrub typhus when vaccinated through intranasal route.

F.102. Enhanced Systemic and Mucosal Immune Response of Eye-Drop Immunization Against Influenza Virus with Adjuvants

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The eye-drop route has been evaluated as an alternative to mucosal routes for vaccine administration, especially against the influenza virus. To induce increased systemic and mucosal antibody production with inactivated-virus vaccine administration, however, other safe and efficient adjuvants are needed to be tested beside cholera toxin (CT). Here, we assessed various types of adjuvants as an anti-influenza serum and mucosal antibody production-enhancer. Among many adjuvants, poly (I:C) showed as much enhancement of antigen specific serum IgG and mucosal IgA antibody production as CT after vaccination of OVA protein with or trivalent Has-subunit or H1N1 virus split-vaccine antigens. With the inactivated-influenza vaccine antigens, poly(I:C) as an eye-drop vaccine adjuvant showed significantly similar antibody production efficacy compared to that of intra-nasal vaccination, and the induced immunity

protected mice from lethal (15X LD50) influenza virus challenge. In addition, inoculation of poly (I:C) plus vaccine antigens in eyes showed almost no increase in inflammatory cytokine mRNA expression or changes in ratio of cell population within 24 hours unlike CT, which recruited $CD11c^+CD8$- cells in conjunctiva after 24 hours of vaccine inoculation in conjunctiva. On the basis of these findings, we firstly propose that eyedrop poly (I:C) is an effective and safe mucosal adjuvant against influenza infection.

F.103. The Adjuvant Function of Cholera Toxin Critically Depends on the Specific Expression of Gsα in $CD11c^+$ Dendritic Cells, Activating the $CD11b^+$ Subset to Promote a Balanced Th1/Th2 Response Independently of IL-12 and $CD8\alpha^+$ Dendritic Cells

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Cholera toxin (CT) is an excellent mucosal adjuvant, often reported to induce a skewed Th2 response, however several studies have found that CT also augments Th1 differentiation. It was previously shown that CT supresses the development of $CD8\alpha^+$ dendritic cells (DC) and down-regulates IL-12 expression, thus inhibiting IL-12-dependent Th1 responses. This effect was caused by the induction of increased intracellular cAMP levels, a process initiated by the ADP-ribosylation of the Gsα subunit by CT. However, the importance of Gsα and cAMP induction for the adjuvant effect of CT has not been demonstrated *in vivo*. Here we investigate the requirements for Th1 induction by CT; we found that CT effectively induced both Th1- and Th2 responses independently of IL-12 and $CD8\alpha^+$ DCs. Furthermore, $CD11b^+$ DCs were strongly activated to prime T cells following immunization with CT. The specific requirement for Gsα in $CD11c^+$ DCs for their activation and the consequential induction of adaptive immune responses was demonstrated using transgenic mice where the floxed Gsa gene was targeted for deletion with a CD11c-cre transgene. Strikingly, in these mice, CT failed to induce the maturation of DCs, and as a consequence T cell responses and antibody production were completely abrogated.

F.104. Dose Responses Indicate Regulatory Mechanisms may Limit Efficacy of Adenoviral Vector-Induced Immunity after Oral Vaccination in Mice

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Because oral vaccines are easy to administer and have the potential to elicit protective immune responses locally and systemically; the development of a safe, efficacious oral vaccine platform could significantly decrease susceptibility to infectious disease worldwide. We are therefore investigating induction of mucosal immunity following oral vaccination with replication-incompetent adenovirus Type 5 vectors (rAd5). In a dose-response study in mice comparing intra-muscular and oral vaccination, intra-muscular rAd5-Ova vaccination produced strong transgene-specific antibody titres in serum and mild responses in faecal extracts at both high and low doses. Unexpectedly, oral vaccination with low-dose rAd5-Ova induced trangene-specific antibody in the mucosa of 4/5 mice and 1/5 had measurable titres in the serum, whereas no Ova-specific antibody was detectable in the high-dose group. Moreover, boosting increased titres in the low-dose oral and intra-muscular groups, but had no effect in either high-dose groups. Our results suggest the ability of rAd5 to resist degradation and transduce cells of the gastrointestinal tract is not the primary factor limiting efficacy of orally-delivered rAd5 vectors. Ongoing analyses of cellular responses and future experiments will examine whether negative regulatory mechanisms triggered by rAd5 in the gut blunt its ability to induce protective immunity following oral vaccination.

F.105. Influence of Maternal Murine Immunization with *Neisseria Meningitidis* Outer Membrane Antigens on the Immune Response in Offspring

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One of the most critical stages in immune system development occurs in neonatal life. During the first days and months following birth, newborns are exposed to countless new antigens to which they need to mount appropriate immune responses. We investigated the ability of native outer membrane vesicles (NOMV) of *N. meningitidis B*, nasally as a model in neonatal mice. Experimental groups contained two

litters (average of six pups per litter). NOMV doses ranging from 10µg were administered intranasally (i.n.) in a 5-µl volume that was gradually introduced into the pup's nare with a micropipette. The first dose was given on day seven after birth, and a second dose was given in an identical manner on day 22 after birth. An enzyme-linked immunosorbent assay was performed with polystyrene maxisorb microtiter plate. Electrophoresis and Immunoblotting were performed on a 13% polyacrylamide gel. We demonstrate for the first time using Neisseria antigens that intranasal immunization of newborn mice with NOMV on days seven and 22 after birth elicits high titers of bactericidal antibodies, previously found to protect against *N. meningitidis*. Mice immunized as neonates induced NOMV-specific mucosal and systemic immunoglobulin A (IgA), IgG, and gamma interferon secretion. A mixed Th1- and Th2-type response to NOMV was established after the boost and was maintained thereafter. NOMV induces specific antibodies and cell-mediated immunity in the presence of high levels of maternal antibodies.

F.106. Novel Self-Assembling Peptide Nanofiber Adjuvants for Mucosal Vaccines

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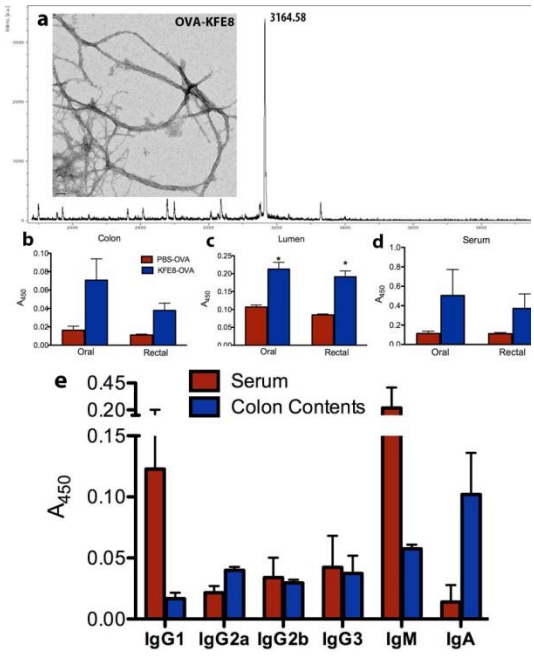

Mucosal surfaces cover an immense surface area and are the major entrance site for pathogens. Despite this, development of mucosa-targeting vaccines lags far behind its systemic counterparts partly due to lack of effective mucosal vaccine adjuvants. We have previously developed a self-assembling peptide nanofiber adjuvant platform that elicits strong and persistent antibody responses in mice when injected systemically. Using a mouse malaria model it was shown that the nanofiber adjuvants elicit protective serum IgG. To determine whether self-assembling peptide nanofibers are capable of eliciting mucosal immune responses, mice were immunized orally or rectally with peptide nanofibers bearing the model antigen OVA and antibody isotypes were investigated in the colon contents, luminal washes, or the sera after four weeks. ELISA data indicated the presence of anti-OVA antibodies in the colon contents, luminal washes of the small intestine, and sera in mice vaccinated via the oral or rectal route. Isotyping data indicated anti-OVA IgG1 and IgM in the sera and secreted IgA in the colon contents. These data suggest that self-assembling peptide nanofibers can act as effective mucosal immune adjuvants and our future studies will investigate the protective ability of peptide nanofiber-based mucosal vaccines against opportunistic pathogens.

F.107. Next-Generation HPV Prophylactic Vaccines: Optimizing Construct Design and Evaluating Mucosal Immunization Routes

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Around 5% of human cancers are caused by mucosal epithelial infection by any one of at least 15 different high-risk oncogenic strains of human papillomaviruses (HPV). Currently licensed vaccines primarily induce type specific neutralizing antibodies. Conversely, vaccine antigens based on the minor capsid protein (L2) induce broadly cross-neutralizing antibodies. Our goal is to develop a mucosally deliverable next-generation HPV vaccine based on L2 antigens. We hypothesized that fusing the conserved L2 epitope, RG-1, to Cholera Toxin B subunit (CTB), would enhance the mucosal immune response to the L2 peptide. Mice (BALB/c) were immunized with CTB-L2 antigens via oral or sublingual mucosal delivery routes, or subcutaneously. Production of virus neutralizing IgG in serum correlates with

protective efficacy of HPV vaccines; consequently IgG titers against CTB and L2, and HPV pseudovirus neutralizing titers were measured in blood serum. All routes of immunization induced cross-neutralizing antibodies at levels predicted to be adequate for protection against HPV-16 and HPV-18, with sublingual delivery eliciting superior cross-neutralizing activities. Sera from mice immunized with CTB fused with HPV-16 L2 epitopes at the amino terminus had higher cross-neutralizing antibody titers against HPV-18 compared with sera from mice immunized with carboxy-terminal CTB-L2 fusions.

F.108. Mucosal Immunization Protects both Respiratory and Systemic Lethal Infections with a Multi-drug Resistant, Hypervirulent Acinetobacter Baumannii Strain

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Acinetobacter baumannii (Ab) is a major cause of hospital- and community-acquired infection in many parts of the world and causes severe pneumonia, bacteraemia and sepsis. The infection is often difficult to treat because of its rapid development of multi- or pan-drug resistance. Vaccination is an effective strategy for the prevention and treatment of multi-drug resistant pathogens such as *A. baumannii*. Intranasal immunization of mice with an inactivated whole cell *A. baumannii* prototype vaccine or purified native OmpA, in the presence of mucosal adjuvant (AMVAD and c-di-GMP), induced antigen-specific mucosal IgA and systemic IgG responses of similar magnitudes. Moreover, intranasal vaccination with the whole cell vaccine, but not the OmpA, protected mice against both lethal respiratory and systemic *A. baumannii* challenge. The immunization significantly prevented the extrapulmonary dissemination of the pathogen and reduced the magnitude of the bacteraemia. Depletion of neutrophils completely abolished the vaccine-induced protection. On the hand, passive transfer of hyperimmune sera provided no protection against the mortality but slightly delayed the time to death of the challenged mice. Moreover, mice that have been recovered from a previous infection remain susceptible to the reinfection. Collectively, these results suggest that mucosal immunity in addition to antibodies may play a crucial role in vaccination against *A. baumannii* -associated pneumonia and sepsis.

F.109. Prevention of Type 1 Diabetes by Intranasal Administration of Gliadin in NOD Mice

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Induction of long-term tolerance to beta-cell autoantigens has been investigated both in animal models and in human Type 1 Diabetes (T1D) in order to prevent the disease. As regards external compounds, the dietary plant protein fraction has been associated with high penetrance of the disease, whereas gluten-free diets prevent T1D in animal models. Herewith, we investigated whether i.n. administration of gliadin or gluten may arrest the diabetogenic process. I.n. administration of gliadin to 4-week-old NOD mice significantly reduced the diabetes incidence. Similarly, the insulitis was lowered. In addition, i.n. gliadin also rescued a significant fraction of prediabetic 13-week-old NOD mice from progressing to clinical onset of diabetes compared to OVA-treated controls. Vaccination with i.n. gliadin led to an induction of $CD4^+Foxp3^+$ Tregs and even more significant induction of gamma/delta T cells in mucosal, but not in non-mucosal lymphoid compartments. This prevention strategy was characterized by an increased proportion of IL-10 and a decreased proportion of IL-2, IL-4 and IFN-gamma-positive $CD4^+Foxp3^+$ Tregs, and IFN-gamma-positive gamma/delta T cells, preferentially in mucosal lymphoid organs. In conclusion, i.n. vaccination with gliadin, an environmental antigen with possible ethiological influence in T1D, may represent a novel, safer strategy for prevention or even early cure of T1D.

F.110. SmPill®: A Novel Oral Vaccine Delivery System that Enhances Intestinal Mucosal Immune Responses

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Enteric infections place a great strain on health services worldwide, especially in developing countries. It is widely accepted that vaccination against these infections is a highly effective public health measure, with the most attractive route being oral. However, developing oral vaccines against enteric infections has not been without difficulty. Challenges include the degradation of the vaccine in the acidic environment of the stomach and the proteolytic action of the duodenum, the poor immunogenicity of many vaccines in the gut and the lack of effective mucosal adjuvants. We have addressed these challenges in an integrated manner using a unique approach to oral (intragastric) vaccination: the Single Multiple Pill (SmPill®). This novel enterically coated capsule, preserves vaccine stability by protecting the antigen from the low pH environment of the stomach and also allows for controlled vaccine release in the small intestine. We have identified α-galactosylceramide as an effective oral adjuvant within SmPill® formulations, which promotes both mucosal and systemic antibody responses. A formulation containing α-galactosylceramide and cholera toxin B subunit (CTB) induced protection against cholera toxin challenge. Furthermore, encapsulation of novel candidate vaccine whole cell killed Vibrio cholerae and enterotoxigenic Escherichia coli (ETEC) into SmPill® microspheres significantly increased their capacity to generate vaccine-specific intestinal-mucosal IgA antibody responses *in vivo*. These results indicate that SmPill® technology has potential in the development of novel non-living oral vaccines for enteric diseases.

F.111. Gender Differences in Intestinal Immune Cell Frequencies

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Although gender biases in systemic immune responses are described, to our knowledge, it is unknown whether immune differences are also present in the intestinal immune system. This is relevant for gender dependent functional foods. Therefore we studied the basal intestinal immune cell populations in males and females. We used male and female Balb/c (Th2-skewed immune response) and B6 mice (Th1-skewed immune response) (3-5 months). Immune cells of the spleen and Peyer's patches (PP) were isolated and stained with antibodies against CD3, CD8, Tbet, GATA3, RORgt, Foxp3 and CD25. We measured percentages of Th1, Th2, Th17 and Treg positive cells using flow cytometry. Results were tested using 2-way ANOVA. Females of both strains showed significantly decreased Th1 cell frequencies in both PP ($p=0.003$) and spleen ($p=0.012$). Moreover, strain differences were observed in the Th17 population in the PP, the percentage of $Foxp3^+$/CD25- cells in both PP and spleen, and in the percentage of $Foxp3^+/CD25^+$ cells in the spleen. Our results shown that gender differences in immune populations can be detected in the intestinal immune system (PP). This suggests that functional food should be gender specifically designed. Studies into gender differences of other immune cell populations in the intestine are in progress.

F.112. Cationic Poly-D, L-lactide-co-glycolide (PLG) Microparticles Enhance the T Cell Response to DNA Vaccine and Protect Mice from Tumor Challenge

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Cationic poly-D, L-lactide-co-glycolide (PLG) microparticles have shown to target to antigen presenting cells efficiently and become an attractive vaccine delivery system. In this study, a DNA vaccine plasmid expressing ovalbumin (pOVA) in the presence of the cationic surfactant CTAB could bind onto the surface of PLG microparticles and form PLG/pOVA DNA vaccine. To evaluate the potential of PLG formulated DNA vaccine, we tested the immunogenicity and protection from OVA-expressing EG-7 tumor cell challenge. An increase of CD86 expression in a RAW.264 cell was observed as well as a higher IFN-gamma production in PLG/pOVA immunized mice. The response of OT-1 specific polyfunctional T cells was also more strong in PLG/pOVA immunized mice than pOVA alone. Correspondingly, the PLG/pOVA immunization provided the better protection in the inhibition of EG-7 tumor cells than pOVA alone. In summary, cationic PLG formulated DNA vaccine could be a powerful too to enhance T cell response and protect from tumor challenge.

F.113. Vaccine-Induced Intestinal and Salivary IgA Correlate with Reduced SIV Viremia in Orally Challenged Neonatal Macaques

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Vertical transmission of HIV in breast milk remains a primary route of pediatric infections. While serum antibodies are critical for elimination of HIV within the host, the importance of mucosal IgA in preventing oral transmission is not known. Neonates produce less IgA than adults and are thus more susceptible to oral pathogens. Here we tested whether an oral pediatric SIV vaccine could induce SIV envelope (Env)-specific salivary and intestinal IgA, and protect against oral SIV acquisition. Rhesus macaques were immunized orally at birth with a live, attenuated Mycobacterium tuberculosis strain engineered to express SIVgag/env and boosted with rMVAgag/pol/env. Nine weeks after birth, the infants were orally challenged using a weekly regimen of low-dose SIVmac251 (5000 $TCID_{50}$). Although vaccination did not prevent infection, vaccinated infants with lower viremia were those that had the highest prechallenge SIV Env-specific IgA in fecal extracts (p=0.0019) and saliva (p=0.034). Serum IgG and IgA antibodies to SIV Env were also induced by vaccination, but these were not associated with viremia. Thus, mucosal, but not systemic, antibodies may have contributed to control of virus replication. Vaccine strategies that promote the development of mucosal IgA antibodies may improve the efficacy of pediatric vaccines to prevent oral HIV acquisition. R01 DE019064, R01 DE022287

F.114. 'Natural Th17' Cells Act as Innate Sentinels in the Oral Mucosa to Prevent Oral Candidiasis

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A harbinger of immunodeficiency, especially HIV, is opportunistic infection by Candida albicans, a commensal fungus found in the oral mucosa of most healthy humans. Numerous studies indicate that immunity to oropharyngeal candidiasis (OPC, thrush) is mediated by IL-17, the signature cytokine of Th17 cells. It has long been presumed that during OPC IL-17 is produced by conventional Th17 cells. However, innate cell types have emerged as sources of IL-17, though their role in OPC is largely unknown. *C. albicans* is not a commensal organism in mice, providing an ideal system in which to study innate immunity to this fungus in a naïve setting. Here we show that conventional Th17 cells are dispensable for acute innate responses to OPC, as mice lacking STAT3 in the CD4 compartment were fully resistant to infection. NK and NKT cells were also dispensable. However, immunity to OPC required RORγt and Act1. Moreover, cells with a rearranged antigen receptor are involved, as $Rag1^{-/-}$, SCID, IL-$7R\alpha^{-/-}$ mice or mice administered Cyclosporine A were highly susceptible. Analysis of oral tissue by flow cytometry revealed a $CD3^{+}CD4^{+}CD44hiTCR\beta^{+}$ resident population in the tongue, which produced IL-17 within 48 hours after Candida infection. This population was absent in susceptible $Rag1^{-/-}$ mice. Collectively, these data implicated 'natural Th17' cells, $CD3^{+}CD4^{+}CD44^{hi}$ IL-17-expressing lymphocytes that develop independently of IL-6 and STAT3 but are dependent on IL-23 and RORγt. These cells are thus ideally positioned to serve as sentinels in the oral mucosa where they can be rapidly mobilized to induce IL-17 and prevent development of candidiasis.

F.115. Comparative Evaluation of Intranasal and Subcutaneous Route of Immunization Against Experimental *Neisseria Meningitidis* B Using DDA-BF as Adjuvant

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Colonization of the nasopharynx by *N. meningitidis* is a prerequisite for invasive meningococcal disease. This present study compares the efficacy of *N. meningitidis* outer membrane vesicles (OMVs) antigens and the (DDA-BF) adjuvant, for the cellular and humoral immune response. Complexes of 2 µg of OMVs in 0.1 mM of DDA-BF were colloidally stable, exhibiting a mean diameter and charge optimal for antigen presentation. Immunogenicity tests for these complexes were performed in outbred mice. OMVs/DDA-BF of this antigen preparation (5µl) was sufficient to induce a (DTH) response after intranasally (i.n) compared with 40X greater amount in 200µL subcutaneously immunization for IgG antibodies production. In our studies 4 doses i.n was necessary and IgG were prodiced in a prime/boost immunization

schedules. The use of this novel cationic adjuvant for the first time with a *N.meningitidis* OMV by intranasally immunization revealed good potential for future nasal vaccine design. Vaccination via intranasal and subcutaneous routes triggered immune activation in the spleen and cervical lymph node, while the former route of vaccination lead to higher antigen-specific lymphocyte proliferation. A single dose of OMV/DDA-BF was sufficient to induce a (DTH) response. The immunization assays showed that OMV /DDA-BF induced IgG /IgG2a antibodies suggesting a mixed Th1/Th2 profile.

F.116. Protection of Hemolytic Uremic Syndrome from Enterohemorrhagic *E. Coli* by Eyedrop Vaccination Using Modified Outer Membrane Vesicles

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To investigate whether eyedrop vaccination of modified outer membrane vesicles (mOMVs) and mOMV-waaJ was effective to protect hemolytic uremic syndrome from Enterohemorrhagic *E. coli* O157:H7 infection, BALB/c mice were vaccinated with mOMVs and mOMV-waaJ through ocular route. MOMVs and mOMV-waaJ were prepared from cultured MsbB- and STxA-deficient mutants of *E. coli* O157:H7 and additional waaJ mutation. After two weeks of the last immunization, the vaccinated group with mOMVs elicited more humoral and mucosal immune responses than mOMV-waaJ and PBS groups. In the challenge test, the vaccinated mice with mOMVs were protected from the lethal dose of wild-type OMV administered intraperitoneally. The PBS group were not protected from wtOMVs challenge, and exhibited more elevated levels of BUN and Cr than vaccinated mice with mOMVs. The unvaccinated mice also showed significant pathological changes in renal tissues. Eyedrop vaccination of mOMVs plus polymyxin B elicited less humoral and mucosal immune responses. There were no body weight changes and histopathologic abnormalities in the ocular tissue after eyedrop instillation of mOMVs. Thus, we suggest that eyedrop vaccination of mOMVs would be an efficient and safe vaccine strategy for development of preventive means against *E.coli* O157:H7 infection and its sequela HUS.

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