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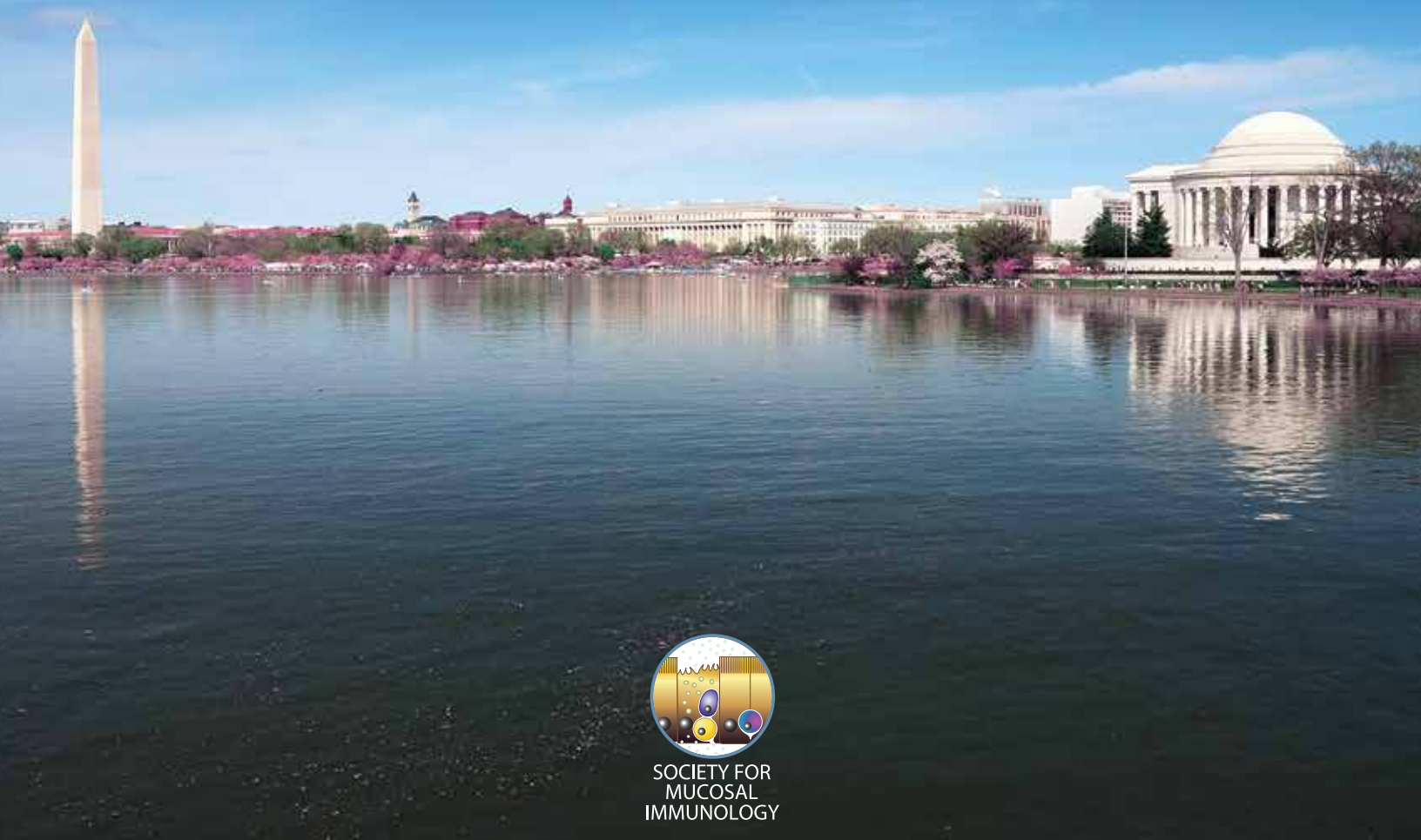
ABSTRACT SUPPLEMENT



SOCIETY FOR MUCOSAL IMMUNOLOGY

Table of Contents

Categories

Index

Aging and Mucosal Immunology

W01. Analysis of Immunization in Outbred Mice with Diferent Adjuvants: Interference in Production and Maternal-Fetal Transfer of Antibodies

Adriana Freitas de Almeida and Elizabeth De Gaspari. Adolfo Lutz Institute, São Paulo, Brazil

Neisseria meningitidis is a gram-negative diplococcus. Its incidence is bigger in mainly children under two years. Newborns and children are particularly susceptible to this type of infection, so further studies on the use of the maternal immunization are needed. Our objective was to analyze the interference of adjuvants in the production of antibodies produced and transferred on maternal-fetal immunization. The outer membrane vesicles (OMVs) of *N. meningitidis* were used for the production of the antigenic preparations, with alum (HA) or DODAB-BF. Outbred mice females were immunized with these preparations by the prime-boost and then the offspring was bled with 3, 6, 9 and 12 weeks of life and the antibodies were analyzed by ELISA. Offspring of mothers immunized with OMV^{+}DODAB-BF by subcutaneous administration presents significant antibodies, as OMV^{+}HA immunization by the subcutaneous route, therefore, the DODAB-BF can be an alternative. The antibodies from offspring seem to fall from the sixth week of life of these animals. IgG1 seems to be the best isotype that crosses the placental barrier. Intranasal route of immunization seems to present no significant antibody placental transfer. The use of adjuvant DODAB-BF is being analysed for the first time in an antigenic preparation with OMVs of *N. meningitidis* compared to OMV^{+}HA on immunization by subcutaneous administration. When the female mice are immunized by the intranasal route, it has been seen that the antibodies appear not be transferred properly to the offspring. So, further studies are needed to conclude whether immunization with DODAB-BF is effective by intranasal route.

W04. Gram-Positive Bacteria Regulates Analgesic Tolerance to Morphine

Li Zhang, Jingjing Meng, Santanu Banerjee, and Sabita Roy. University of Miami, Miami, FL

Morphine, as a naturally occurring opioid, is an integral component of pain relievers. Clinical use of morphine is limited by undesired side effects including analgesic tolerance, withdrawal, and addiction. Previous data in our lab showed that chronic morphine treatment induced bacterial translocation from gut, which led to chronic systemic inflammation. Body of literature showed that chronic inflammation was correlated with tolerance to analgesic effect of morphine. Microbiome analysis demonstrated that morphine treatment induced an expansion of gram-positive bacteria and the translocated bacteria in the systemic system belonged to *Firmicutes* phyla. The purpose of this study was to investigate the role of gut gram-positive bacteria in the analgesic tolerance of morphine. Morphine induced bacteria translocation and systemic inflammation were significantly attenuated in the TLR2KO mice. However, depletion of gram-positive bacteria by vancomycin disrupted the gut homeostasis and exacerbated analgesic tolerance to morphine. Moreover, VSL#3 probiotics, which consisted of eight beneficial gram-positive bacteria, alleviated the analgesic tolerance to morphine and chronic inflammation induced by repeated morphine administration. Our study indicated the function of gram-positive bacteria in analgesic tolerance to morphine. Future studies will elucidate and characterize the gram-positive bacterial communities that are critical to maintain gut homeostasis and contribute to analgesic tolerance to morphine.

Antigen Uptake

W05. Goblet Cell Associated Antigen Passages Form via Bulk Endocytosis Following Compound Exocytosis

Jenny Gustafsson[1], Keely G. McDonald[1], Kathryn Knoop[1], Matthew Jones[1], James Fitzpatrick[1], Wayne Lencer[2] and Rodney Newberry[2]. [1]Washington University School of Medicine, St. Louis, MO; [2] Harvard Medical School, Boston, MA

Small intestinal goblet cells (GCs) form goblet cell associated antigen passages (GAPs) to deliver luminal substances to lamina propria dendritic cells. The process by which GCs form GAPs has been enigmatic. GAP formation is linked to GC secretion by compound exocytosis (CE), an event resulting in rapid expulsion of multiple mucin granules and resultant membrane tears, suggesting that GAP formation results from uptake of luminal contents via tears and basolateral secretion. Using super-resolution microscopy, we observed that luminal fluorescent antigens taken up by GCs localized in a network of vesicular appearing structures at the periphery and base of the GCs. Focused ion beam scanning electron microscopy (FIB-SEM) was used to construct a 3D ultrastructural model of GAP formation. FIB-SEM revealed that luminal antigen was located in endosomes, multi vesicular bodies (MVBs), the transgolgi network, newly formed mucin granules, and vesicles at the basolateral surface of the GC. Disruption of endocytosis, microtubules, actin filaments, and microtubule motor proteins, blocked GAP formation without affecting the ability of GCs to secrete by CE. Vesicles containing luminal antigens did not have markers of early endosomes, but did stain positive for Rab3d, a marker also found in mucin granules. In summary, we show that GAP formation is a bulk endocytic process occurring after secretion by CE. This function may have evolved from the need for this highly secretory cell type to rapidly recover cell membranes after secretion for recycling into new secretory vesicles and recycling to the plasma membrane for delivery to dendritic cells.

W06. Increased Abundance of M Cells in the Gut Epithelium Dramatically Enhances Oral Prion Disease Susceptibility

David S. Donaldson[1], Anuj Sehgal[1], Daniel Rios[2], Ifor Williams[3] and Neil Mabbott[1]. [1]Roslin Institute, University of Edinburgh, Edinburgh, Scotland, United Kingdom; [2]Broad Institute of MIT and Harvard, Cambridge, MA; [3] Emory University School of Medicine, Atlanta, GA

Antigen-sampling M cells, present in the follicle associated epithelium of gut associated lymphoid tissues can be exploited by pathogens to gain entry to the host. Many natural prion diseases of humans and animals are acquired through the consumption of contaminated food or pasture. It has been suggested that oral prion infectivity may depend on M cell-mediated transcytosis of prions across the gut epithelium. Therefore, alterations to the M cell density, for example during aging or as a consequence of pathogen co-infection, could affect M cell uptake of prions and alter disease susceptibility. To confirm that oral prion infection depends on M cells, we utilised a conditional model of M cell deficiency in which RANK, a receptor necessary for M cell development, is only absent on intestinal epithelium cells. In the specific absence of M cells, the accumulation of prions within Peyer's patches and the spread of disease to the brain was blocked, demonstrating a critical role for M cells in the initial transfer of prions across the gut epithelium. Mice were also treated with RANKL to enhance the M cell numbers prior to oral infection with prions. Prion uptake from the gut lumen was enhanced in RANKL-treated mice, resulting in reduced survival times and a dramatically increased disease susceptibility, equivalent to a 10-fold higher infectious titre of prions. These data demonstrate M cells are the critical gatekeepers of oral prion infection, whose density in the gut epithelium directly limits or enhances disease susceptibility. Therefore, factors which alter M cell-density in the gut epithelium may be important risk factors which influence host susceptibility to orally acquired prion diseases.

Celiac Disease

OR.56, T04. IL-10 Signaling is Essential to Prevent Gluten-Dependent Intraepithelial CD4 CTL Infiltration and Epithelial Damage in the Small Intestine

Lea MM Costes[1], Dicky Lindenbergh-Kortleve[1], Lisette van Berkel[1], Sharon Veenbergen[1], Ytje Simons-Oosterhuis[1], Julien J. Karrich[1], Björn E. Clausen[3], Tom Cupedo[1] and Janneke Samsom[1]. [1]Erasmus Medical Center, Zuid-Holland, Netherlands; [2] University Medical Center of the Johannes Gutenberg University, Rheinland-Pfalz, Germany

Celiac disease (CeD) is a gluten-dependent, chronic, small intestinal inflammatory disease where infiltration of activated cytolytic intraepithelial lymphocytes (IEL) causes epithelial damage leading to crypt hyperplasia and villous atrophy. The initial events underlying the infiltration of cytolytic IEL in CeD patients remain elusive. We recently showed that in mice, interleukin-10 (IL-10) is essential to maintain gluten tolerance. Moreover, IL-10 acts by regulating $CD11c^+$ myeloid cells. Thus, we hypothesized that disrupting IL-10 regulation of $CD11c^+$ myeloid cells would lead to a gluten-dependent infiltration of cytotoxic IEL and epithelial damage in the small intestine. Indeed, in mice lacking IL-10 receptor alpha signaling in $CD11c^+$ myeloid cells (*Cd11c*^{cre}*Il10ra*$^{fl/fl}$ mice), dietary gluten drove the infiltration of inflammatory IEL associated with crypt hyperplasia in the small intestine. Two functionally distinct inflammatory IEL populations were detected: a $CD4^{pos}CD8\alpha\alpha^{pos}$ population predominantly expressing *Ifng*, a main driver of CeD, and a $CD4^{pos}CD8\alpha^{neg}$ population predominantly expressing *Il17.* Moreover, the $CD4^{pos}CD8\alpha\alpha^{pos}$ IEL had a cytotoxic CD4 lymphocyte (CD4 CTL) phenotype characterized by Thpok negativity and positivity for Runx3, 2B4, granzyme B and CD103. Crucially, accumulation of the CD4 CTL population depended on dietary gluten. Epithelial cell activation is a prerequisite to allow interactions with cytolytic IEL. Accordingly, small intestinal epithelial cells of *Cd11c*^{cre}*Il10ra*$^{fl/fl}$ mice exhibited MHCII upregulation and increased expression of *Il15* and unconventional MHCI molecules which disappeared on a gluten free diet. Altogether, our data indicate that IL-10 regulation of $CD11c^+$ myeloid cells is pivotal to prevent a gluten dependent small intestinal infiltration of cytotoxic CD4 CTL and epithelial damage.

OR.57, T03. Selecting the Repertoire and Functional Niche of V Delta 1 Gamma Delta T Cells in Health and Disease

Toufic Mayassi[1], Kristin Ladell[2], Herman Gudjonson[1], James McLaren[2], Jagoda Rokicka[1], Ian Lawrence[1], Kathryn Lesko[1], Hoda Sayegh[1], Cezary Ciszewski[1], Carol Semrad[1], Sonia Kupfer[1], Stefano Guandalini[1], Aaron Dinner[1], David Price[1] and Bana Jabri[1]. [1]University of Chicago, Chicago, IL; [2] Cardiff University, Cardiff, Wales, United Kingdom

Gamma delta (γδ) T cells are innate-like lymphocytes hypothesized to play a role in lymphoid stress surveillance. The specificity and functional niche of Vδ1 (TRDV1) γδ T cells in human immune responses is still poorly understood. We gained insight into both by comparing the T cell receptor (TCR) repertoire and functional capacity of Vδ1 T cells in small intestinal intraepithelial lymphocytes (IEL) and peripheral blood lymphocytes (PBL) in healthy individuals and patients with celiac disease (CeD). Using a systems approach, we identified in healthy individuals γδ T cells with TRDV1 and TRGV4/9 biased TCR usage that lack the ability to produce cytokines, but rather exhibit an NK-like phenotype characterized by unique expression of activating natural killer receptors (NKRs), providing them a unique functional niche. Infiltrating lymphocytes coupled with tissue alterations in the celiac lesion lead to a reshaping of the Vδ1 IEL T cell compartment, characterized by a CDR3 driven expansion of IFN-γ producing Vδ1 IEL that lack expression of activating NKRs found in healthy individuals; alterations that persist even after treatment with a gluten free diet, implying a lasting alteration to the Vδ1 IEL T cell compartment in CeD. These data highlight the plasticity of the intestinal γδ T cell compartment and how varying microenvironments can alter the repertoire and functional niche of these cells.

OR.58, T07. Type 1 IFN Could Promote Loss of Oral Tolerance Towards Gluten: Relevance for CD

Valentina Discepolo[1], Romain Bouziat[2], Reinhard Hinterleitner[2], Sangman Michael Kim[2], Valerie Abadie[3], Luis B. Barreiro[3] and Bana Jabri[2]. [1]University Federico II, Naples, Italy; [2] University of Chicago, Chicago, IL; [3]University of Montreal, Montreal, PQ, Canada

Celiac Disease (CD) is an immune-mediated systemic disorder occurring upon gluten ingestion in genetically susceptible (HLA-DQ2 and/or HLA-DQ8) individuals. How those subjects lose oral tolerance to gluten remains unclear. Interleukin (IL)-15 is known to play a pivotal role, however not all CD subjects overexpress IL-15. Several studies suggest that type-1 interferons (IFN) and double-stranded-RNA (dsRNA) viruses may trigger CD. Whether either type-1-IFN or IL-15 or both are over-expressed in the gut of CD patients is unknown. Moreover, the impact of type-1-IFNs and dsRNA analog poly(I:C) on promoting inducible Foxp3$^+$ regulatory *T cell* (iTreg) and inflammatory T-helper1 (Th1) cell responses to dietary antigens remains unclear. Immunohistochemistry and gene expression analysis by microarray revealed that CD patients could be sub-divided based on IL-15 and type-1-IFN-inducible gene expression. Particularly, 25% of patients displayed a type-1-IFN signature in their intestinal mucosa in absence of IL-15 overexpression, suggesting that in these subjects, type-1 IFNs, but not IL-15, may be involved in CD pathogenesis. Next, we demonstrated that type-1-IFN and poly(I:C) could block iTreg conversion but not induce Th1 immunity to dietary antigens. An impairment of Treg conversion by type-1-IFN was associated with loss of oral tolerance to gliadin in a CD-relevant HLA-DQ8 transgenic mouse model. Finally, both type-1 IFN and poly(I:C) promoted activation of tissu-tranglutaminase2. Our data demonstrate that CD is a heterogeneous disorder and that in a subset of CD patients, viral infections and type-1 IFN may be involved in triggering loss of oral tolerance and inflammatory T cell responses to gluten.

PR.04, T05. From Human to Mouse and Back: Advances in the Development of a Mouse Model for Celiac Disease

Valerie Abadie[1], Sangman M. Kim[2], Thomas Lejeune[1], Jordan Ernst[2], Eric V. Marietta[3], Cezary Ciszewski[2], Anne Dumaine[4], Matthew Zurenski[2], Joseph A. Murray[3], Chaitan Khosla[5], Luis B. Barreiro[1] and Bana Jabri[2]. [1]University of Montreal, Montreal, Canada; [2] University of Chicago, Chicago, IL; [3] Mayo Clinic, Rochester, MN; [4]CHU Sainte-Justine Research Center, Montreal, Canada; [5] Stanford University, Stanford, CA

The development of new therapies for celiac disease (CD) has proven challenging because of our incomplete understanding of the mechanisms underlying the pathogenesis of the disease and the lack of a suitable mouse model. CD patients have increased expression of the cytokine IL-15 and IL-15 over-expression in the lamina propria is involved in the loss of oral tolerance to gluten, the development of a gluten-specific T_H1 immune response, intraepithelial lymphocytosis, and anti-gluten and anti-transglutaminase 2 (TG2) antibodies. Because the adaptive immunity to gluten and epithelial stress are both required for licensing intraepithelial lymphocytes (IE-CTLs) to kill epithelial cells and subsequently induce villous atrophy, we engineered a humanized HLA-DQ8 mouse that over-express IL-15 both in the lamina propria and the intestinal epithelium. Following dietary gluten challenge, this mouse presents the main features of CD including an expansion in IE-CTLs, antibodies against gluten and TG2 and villous atrophy. CD4$^+$ T cells are required for disease development, and identically to the human situation, our mice do not develop intestinal tissue damage when CD4$^+$ T cells are depleted. A gluten free diet (GFD) in CD patients normalizes the celiac-specific antibodies and histological alterations. Importantly, following a GFD, antibodies titers and tissue damage decline in our mouse model. Thus, our mouse model represents the first physiological animal model of active CD where the administration of gluten alone triggers tissue alteration and an invaluable premedical model that enables us to assess the efficacy of new therapeutic options such as TG2 inhibitors as a treatment for CD.

T01. The Composition of Lineage-Negative Intraepithelial Lymphocyte Cells is Shifted Towards an IFN-γ Producing Subset in Patients with Celiac Disease

Melanie Uhde, Anna Bunin, Suzanne K Lewis, Benjamin Lebwohl, Boris Reizis, Peter HR Green and Govind Bhagat. Columbia University, New York, NY

The human intestinal epithelium harbors a heterogeneous lymphocyte population that regulates epithelial integrity and serves as a first line of defense against microbial pathogens. In celiac disease (CD), infiltration of $CD3^+$ intraepithelial lymphocytes (IELs) results in the destruction of the small intestinal epithelium and the development of villous atrophy. Here, we describe the phenotype and composition of innate lymphoid cell type 1 (ILC1)-like IEL subsets based on the expression of CD103 and NKp44 in the duodenum of healthy adults and CD patients. In patients with active CD, the frequency of $NKp44^+$ $CD103^+$ cells (double positive, DP) was significantly decreased, while the frequency of $NKp44^-$ $CD103^+$ cells (single positive, SP) was increased compared with non-celiac controls. The increase of SP cells correlated with the severity of villous atrophy in CD patients. Furthermore, SP cells isolated from CD patients expressed higher levels of IFN-γ, a hallmark cytokine of CD, than SP cells isolated from non-celiac controls. The composition of SP and DP cells in patients adhering to a gluten-free diet and in patients who developed refractory CD was similar to the composition observed in patients with newly diagnosed celiac disease. Together, our data confirm the heterogeneity within and among ILC populations described by previous studies and indicate a potential role of an IFN-γ producing subset in CD pathology and the development of refractory CD.

T02. A New CXCR3 Expressing Human Intestinal Primary Epithelial Cell System: Potential *in vitro* Tool for Studying Pathophysiological Mechanisms of Celiac Disease

Asit Panja, Prerona Panja, Bhatt Rachana and Joseph Ansamma. AlfaGene Bioscience Inc., Fords, NJ

Expression of chemokine receptor CXCR3 in intestinal epithelium has been implicated in celiac disease by means of its potential role in gliadin peptide uptake and triggering immune responses in the small intestine. Therefore, having an CXCR3 expressing primary epithelial cell system in long-term culture would be a valuable tool for discovery and development of new treatments for celiac and/or other inflammatory diseases. Earlier, we have developed a method for growing human intestinal primary epithelial cells (HIPECs) in long-term culture. Now we examined two small intestinal HIPEC lines for CXCR3 expression by immunocytochemical staining and subsequent fluorescence microscopic analysis. Studies for gliadin peptide uptake and production of proinflammatory cytokine production by these new CXCR3 expressing primary epithelial cells are in progress. Results from these studies with this new cell system may help better understand the mechanisms gliadin triggered inflammation via CXCR3 aiding in the development of new treatment for celiac disease.

T06. Protective Effects of Aryl Hydrocarbon Receptor Signalling in Celiac Disease Mucosa and in Poly I:C-Induced Small Intestinal Atrophy Mouse Model

Ivan Monteleone, Roberta Izzo, Davide Di Fusco, Vincenzo Dinallo, Irene Marafini, Federica Laudisi and Giovanni Monteleone. University of Rome Tor Vergata, Rome, Lazio, Italy

Aryl hydrocarbon receptor(AhR), a transcription factor activated by a large variety of natural and synthetic ligands, represents an important link between environment and immune-mediated pathologies. In this context, the aim of this study was to characterize the mucosal expression and role of AhR in celiac disease(CD). AhR expression was analyzed lamina propria mononuclear cells(LPMC) isolated from duodenal biopsies of CD patients and healthy controls(CTR) by flow cytometry. To assess how AhR expression is controlled in CD gut mucosa, a neutralizing IL-21, IL-15 or IFN-g specific abs was added to *ex vivo* organ cultures of biopsies taken from untreated CD biopsies or peptic-tryptic digest of gliadin(PT)-stimulated treated CD biopsies. Inflammatory cytokines were also analyzed in CD mucosal explants either left untreated or treated with 6-formylindolo[3,2-b]carbazole(Ficz), a natural ligand of AhR. The role of AhR was evaluated in a mouse model of poly I:C-driven small intestine atrophy. Intestine tissue from patients with CD expressed significantly less AhR than controls. The addition of PT to *ex vivo* duodenal organ cultures of inactive CD patients reduced AhR expression while the presence of neutralizing IL-21 or IL-15 specific antibody increased the expression of AhR. Moreover, Ficz reduced the expression of IFN-g and TNF-a in *ex vivo* duodenal CD organ cultures. Mice given Ficz are protected from poly I:C-driven intestinal atrophy and shown reduced levels of inflammatory cytokines in small intestine mucosa. Data show that AhR-driven signals inhibit pathogenic responses in the gut mucosa and suggest that AhR-related compounds may be therapeutically useful in CD.

Dendritic Cells

OR.21, W14. Regulation of Steady-State Dendritic Cell Migration

Vuk Cerovic[1], Joël Guillaume[1], Girmay Desalegn[1,2], Georgios-Leandros Moschovakis[2], Reinhold Förster[2], Simon Milling[3] and Oliver Pabst[1]. [1]RWTH Aachen University, Aachen, Nordrhein-Westfalen, Germany; [2]Hannover Medical School, Hannover, Niedersachsen, Germany; [3]Univerisity of Glasgow, Glasgow, Scotland, United Kingdom

The intestinal immune system maintains a delicate balance between the induction of immunity to pathogens and tolerance to harmless antigens from food and intestinal microbiota. A crucial step in the induction of both immunogenic and tolerogenic responses is the CCR7-dependent migration of dendritic cells (DCs) from the intestine to the mesenteric lymph nodes (MLNs). However, while inflammation-induced DC migration has been extensively studied, comparatively little is known about the magnitude and control of steady state intestinal DC migration. Notably, it is unclear if individual DCs in the steady state migrate in response to specific signals or if this continual migration represents a stochastic process. Here we use a range of techniques, including isolation of lymph DCs and *in vivo* cell tracking, to reliably quantify and characterise the phenotype of migrating DCs from the intestine, lymph, and MLN. Steady state migration of intestinal DCs is highly dynamic, with between 30,000-50,000 DCs migrating from the mouse small intestine to the MLN each day, replacing the entire MLN migrating DC pool within 48h. We show that CCR7-expressing DCs represent a minor proportion of total intestinal DCs and have a phenotype reminiscent of lymph DCs, characterised by high expression of surface MHCII, upregulation of costimulatory molecules, partial downregulation of CD11c and minimal BrdU incorporation. Therefore, even before leaving the intestine, CCR7-expressing DCs adopt a distinct phenotype, strongly suggesting that steady state DC migration is not a purely stochastic process, but that the DCs make a 'decision to migrate' in response to specific environmental signals.

OR.22, W08. TIM4 Expression on Colonic Dendritic Cells Correlates with a Migratory Signature

Hannah Wessel, Elizabeth Mann, Verena Kaestele, Johannes Mayer, Daniel Wall and Simon Milling. University of Glasgow, Glasgow, Scotland, United Kingdom

Apoptotic cell antigen uptake by intestinal dendritic cells (DC) and their migration to the mesenteric lymph nodes (MLN) is critical to maintain tolerance. However, the mechanisms and apoptotic cell receptors involved in this process remain elusive. TIM4 is a phosphatidylserine receptor with poorly defined functions on DC. Four colonic lamina propria (cLP) DC subsets can be identified by their expression of CD11b and CD103. Although $CD103^+$ $CD11b^-$ DC had the highest percentage (10%) of $TIM4^+$ cells among cLP DC subsets, *Citrobacter rodentium* infection doubled the frequency of both $CD103^-$ $CD11b^+$ and $CD103^+$ $CD11b^-$ TIM4-expressing DC. We performed thoracic duct cannulation on previously mesenteric lymphadenectomised mice and analysed TIM4 expression on DC from efferent lymph, comparing it to migratory MLN and cLP DC. TIM4 expression on all DC subsets was 2-3x higher in the lymph and the MLN (30% and 20% respectively of migratory DC) than the cLP of naïve mice. Next, using violet light we irreversibly photoconverted the colons of Kaede mice from ubiquitously expressing Kaede-green to Kaede-red, allowing us to track Kaede-red^+ cLP DC. After 24/48h, $TIM4^+$ migratory MLN DC had a higher frequency of Kaede-red^+ cells than $TIM4^-$ DC, indicating a greater propensity of $TIM4^+$ DC to migrate from the cLP. Indeed, CCR7 mRNA was higher in cLP $TIM4^+$ $CD103^+$ $CD11b^-$ DC than their $TIM4^-$ counterparts, both at steady state and during infection with *C. rodentium*. Future experiments will determine the functional role of TIM4 in DC migration and antigen presentation of bacterial and apoptotic cell antigen.

OR.23, W07. The Role of Tgfβ and Retinoic in the Differentiation of CD103$^+$CD11b$^+$ Dendritic Cells from Pre-cDC

Jennifer Montgomery and Allan Mowat. Univeristy of Glasgow, Glasgow, Scotland, United Kingdom

Dendritic cells (DC) play a key role in regulating intestinal immune responses and the intestine contains a unique subset of DCs that expresses both CD103 and CD11b that have distinct functions. However, it is unclear what factors in the local environment might drive the differentiation of such unusual DCs. Although it has been suggested that mediators such as TGFβ, GM-CSF and retinoic acid (RA) may be involved in the generation of CD103$^+$CD11b$^+$ DCs, how these factors might interact with each other is unknown and the *in vivo* implications have not been explored. In recent studies, we have found that the numbers of CD103$^+$CD11b$^+$ DCs are reduced in CD11c-cre-TGFβR$^{fl/fl}$ mice that lack TGFβR mediated signalling in DCs and here we have developed *in vitro* models to study the role of TGFβ and the other mediators in driving the differentiation of conventional DC precursors (pre-cDC). These experiments show that a combination of flt3L, TGFβ, GM-CSF and retinoic acid (RA) is needed to specify the selective development of CD103$^+$CD11b$^+$ DC from pre-cDCs. Interestingly non-epithelial stromal cells from the small intestine can induce the differentiation of CD103$^+$CD11b$^+$ DCs in the presence of flt3L alone. This property is markedly inhibited in mice on a vitamin A deficient diet and is not replicated by stromal cells from the spleen. Together our data indicate that the presence of CD103$^+$CD11b$^+$ DCs in the intestine is determined by tissue-specific stromal cells that drive the local differentiation of pre-cDCs via TGFβR and RA dependent mechanisms.

OR.24, W16. IRF8-Dependent DCs Play a Key Role in the Maintenance of CD8 T Cell Tolerance to Epithelial-Derived Antigen

Thorsten Joeris[1], Cristina Gomez-Casado[2], Petra Holmkvist[2], Katarzyna Müller-Luda[2], Simon Tavernier[3], Vuk Cerovic[4], Bart N. Lambrecht[3] and William W. Agace[2]. [1]Danish Technical University, Copenhagen, Denmark; [2] Lund University, Lund, Sweden; [3] Ghent University, Ghent, Belgium; [4] RWTH Aachen University, Aachen, Germany

In steady state, rapid tolerization of CD8$^+$ T cells reactive towards epithelial-derived self-antigens is crucial to maintain tissue homeostasis. It has been suggested that the maintenance of peripheral CD8$^+$ T cell tolerance is a unique function of lymph node stromal cells (LNSCs) with the ability to express and present intestinal tissue antigens. However, antigens derived from apoptotic intestinal epithelial cells (IECs) are also taken up by dendritic cells (DCs), transported to gut-draining lymph nodes and cross-presented to CD8$^+$ T cells. Since IRF8-dependent DCs are the major cross-presenting DC subset in this context, we aimed to investigate their role in this process. IFABP-tOva mice, expressing the model-antigen Ovalbumin (Ova) in IECs, were used as recipients to set up chimeras using either CD11c-cre.Irf8$^{fl/fl}$ bone marrow, which cannot generate IRF8-DCs, or cre-negative Irf8$^{fl/fl}$ control bone marrow. Transfer of Ova-specific CD8$^+$ T cells (OT-I cells) to control chimeras resulted in their rapid tolerization; including their conversion to IL-10-secreting and FoxP3-expressing CD8$^+$ regulatory T cells (CD8$^+$ Tregs), which required induction of the gut-homing chemokine receptor 9 (CCR9) for optimal expansion in the gut mucosa. Upon transfer to CD11c-cre.Irf8$^{fl/fl}$ chimeras this tolerization and conversion of OT-I cells to gut-homing CD8$^+$ Tregs was severely impaired. Instead, OT-I cells developed into cytotoxic effector T cells, causing epithelial destruction and intestinal inflammation. This demonstrates that maintenance of peripheral tolerance is not a unique function of LNSCs, but that in steady state IRF8-dependent DCs are crucial to assure the rapid tolerization of CD8$^+$ T cells reactive to epithelial-derived self-antigens.

OR.25, W12. Lung Migratory CD11b$^+$ Conventional Dendritic Cells are Necessary and Sufficient For T Follicular Helper Cell Induction

Biyan Zhang[1], Kumar Krishnaswamy[2], Gowthaman Uthaman[1], Ulf Yrlid[3], Adam Williams[4] and Stephanie Eisenbarth[1]. [1]Yale University, New Haven, CT; [2] AstraZeneca, Molndal, Vastra Gotaland, Sweden; [3] University of Gothenburg, Gothenburg, Sweden; [4] Jackson Labs, Farmington, CT

T follicular helper (Tfh) cells are a subset of CD4$^+$ T cells that promote antibody production during vaccination. Although dendritic cells (DCs) initiate Tfh cell priming, data regarding which DC instructs Tfh cell differentiation differ. We found that these discrepancies might exist, in part, due to the unusual sites used for immunization in murine models, which differentially bias which DC subsets can access antigen. We used intranasal immunization as a physiologically relevant route of exposure that delivers antigen to all tissue DC subsets. Using a combination of mice in which the function of individual DC subsets is impaired, we determined that CD11b$^+$ migratory type 2 conventional DCs (cDC2s) are necessary and sufficient for Tfh induction. DC-specific deletion of the guanine nucleotide exchange factor DOCK8 resulted in an isolated loss of CD11b$^+$ cDC2 but not CD103$^+$ cDC1 migration to lung-draining lymph nodes. Impairment in cDC2 migration or development in DC-specific Dock8 or Irf4 knockout mice, respectively, led to reduced Tfh cell and antibody development, whereas loss of CD103$^+$ cDC1s in Batf3$^{-/-}$ mice did not. We show that migratory CD11b$^+$ cDC2s uniquely carry antigen into the subanatomic regions of the lymph node where Tfh cell priming occurs and express molecules known to promote Tfh cell differentiation, such as CD25. This is the first work to define the nature of the pulmonary DC that regulates T-dependent antibody responses and thereby has implications on our understanding of mucosal immune responses to infection and potential vaccination.

PR.03, W18. MyD88-Dependent Dendritic and Epithelial Cell Crosstalk Orchestrates Immune Responses to Allergens

Seddon Y. Thomas[1], Gregory S. Whitehead[1], Motoki Takaku[1], James Ward[1], Xiaojiang Xu[1], Keiko Nakano[1], Miranda Lyons-Cohen[1], Hideki Nakano[1], Kymberly Gowdy[2], Paul Wade[1] and Donald Cook[1]. [1]National Institute of Environmental Health Sciences, Research Triangle Park, NC; [2] Brody School of Medicine at East Carolina University, Greenville, NC

Sensitization to inhaled allergens is dependent on activation of conventional dendritic cells (cDCs) and on the adaptor molecule, MyD88. However, many cell types in the lung express *Myd88*, and it is unclear how signaling in these different cell types reprograms cDCs and leads to allergic inflammation of the airway. By combining ATAC-seq with RNA profiling, we found that MyD88 signaling in cDCs maintained open chromatin at select loci even at steady state, allowing genes to be rapidly induced during allergic sensitization. A distinct set of genes related to metabolism was indirectly controlled in cDCs through MyD88 signaling in airway epithelial cells (AECs). In mouse models of asthma, *Myd88* expression in AECs was critical for eosinophilic inflammation, whereas *Myd88* expression in cDCs was required for Th17 cell differentiation and consequent airway neutrophilia. Thus, both cell-intrinsic and cell-extrinsic MyD88 signaling controls gene expression in cDCs and orchestrates immune responses to inhaled allergens.

W09. Identification of Salivary Gland Dendritic Cells with Antigen Cross-Presenting Capacity and Memory Resident T Cells

Lu Lu, Yukinori Tanaka, Naoto Ishii, Takashi Sasano and Shunji Sugawara. Tohoku University Graduate School of Dentistry, Sendai, Miyagi, Japan

Immune cells such as dendritic cells (DCs), professional antigen-presenting cells, macrophages and lymphocytes are that are essential for effective immunity and tolerance. However, the characteristics of immune cells in steady-state salivary glands (SGs) currently remain unknown. We herein identified CD64$^-$CD11c$^+$ classical DCs (cDCs) as well as CD64$^+$ macrophages among CD45$^+$MHC class II$^+$ antigen-presenting cells in steady-state murine SGs. SG cDCs

were divided into $CD103^+CD11b^-$ cDC1s and $CD103^-CD11b^+$ cDC2s. Both cDC subsets markedly expanded in response to the Flt3 ligand (Flt3L), were differentiated from common DC precursors. Furthermore, the SG $CD103^+CD11b^-$ cDC2s possess antigen cross-presenting capacity. We also detected resident memory CD4 and CD8 T cells, which express homing receptors, such as CCR4, CCR6, CC10 and a4b7, in addition to natural killer cells. These results suggest that cDCs (cDC1s and cDC2s), macrophages, resident memory T cells and natural killer cells in SGs contribute to immune surveillances in the tissues.

W10. Delayed Onset of T Cell Transfer Colitis in the Absence of *IRF4* Dependent Dendritic Cells

Lieneke Pool[1], Aymeric Rivollier[1], Katarzyna Müller-Luda[2] and William W. Agace[2]. [1]Danish Technical University, Frederiksberg C, Hovedstaden, Denmark; [2]Lund University, Lund, Skane Lan, Sweden

Inflammatory Bowel Disease (IBD) is a chronic incurable inflammatory disease that is believed to result from an abnormal immune response to bacterial components of the commensal microflora in genetically susceptible individuals. Pathogenic $CD4^+$ T cells, which accumulate in the inflamed mucosa, are believed to be key drivers of the disease. While dendritic cells (DCs) are important in the priming of intestinal adaptive immunity and tolerance, their role in the initiation and perpetuation of chronic intestinal inflammation remains unclear. Here we used the $CD45RB^{hi}$ T cell transfer model of colitis to assess the role of *Irf4* dependent DCs in colitis development. Four weeks after adoptive transfer of naïve T cells, CD11c-Cre.$IRF4^{fl/fl}$.$RAG^{-/-}$ mice displayed reduced numbers of monocytes and $CD4^+$ T cell in the colon and intestinal draining mesenteric lymph nodes, and reduced levels of inflammatory cytokines in the serum and intestinal tissues compared with Cre^- littermates. Such differences were not observed 7 weeks after T cell transfer. Collectively these results suggest a role for *Irf4* dependent DCs in the initiation of T cell driven colitis but not long-term disease progression.

W11. Intestinal Dendritic Cell Subsets Migrate Similarly in Response to Poly(I:C) and R848 in a Type I IFN-Dependent Manner

Agnès Garcias López[1], Katarzyna Müller-Luda[2], Julia Hütter[3], Vasileios Bekiaris[1], William W. Agace[2] and Katharina Lahl[1].[1]Technical University of Denmark, Copenhagen, Hovedstaden, Denmark; [2]Lund University, Lund, Sweden; [3] Bayer Pharma AG, Pharmaceuticals Division, Berlin, Germany

Intestinal dendritic cells (DC) migration to the mesenteric lymph nodes (mLNs) is essential for optimal priming of gut-associated T cell responses. Subsets of DCs can prime specific T cell subsets. We here investigate whether migration of these subsets to the mLNs differed in response to adjuvants targeting receptors differentially expressed by DC subsets. Poly(I:C) signals through TLR3/TRIF – TLR3 is specifically expressed on $CD103^+$CD11b- DCs. R848 signals through TLR7/MyD88 and may primarily target $CD103^+CD11b^+$ DCs. Interestingly however, our results reveal similar migration of both subsets in response to either stimulus. We therefore decided to analyze the molecular requirements for DC migration. MyD88 in DCs was required and sufficient for optimal migration to R848, as revealed by using CD11c-Cre.$MyD88^{fl/fl}$ and CD11c-Cre.$MyD88^{LSL}$ mice (lacking and expressing MyD88 only in DCs, respectively). As expected, poly(I:C)-induced migration fully depended on TLR3/TRIF, as mice deficient for either molecule showed no DC migration. Nevertheless, mixed BM-chimeras showed that migration did not strictly depend on TLR3-signalling in the migrating DCs themselves. Type I IFN was required for the migration of both subsets to both stimuli as mice lacking the type I IFN receptor only in DCs showed significantly reduced numbers of migratory DC subsets in the mLNs after stimulation regardless of the adjuvant used. We are currently investigating the source of type I IFN in our settings. Our data suggests that IFNAR signaling plays a key, DC-intrinsic, role in DC migration and that such indirect signals are sufficient to drive migration.

W13. Functions of the 'Alarmin' Cytokine IL-25 in House Dust Mites-Induced Allergic Asthma-Like Chronic Pulmonary Inflammation

Estefania Claudio[1], Hongshan Wang[1,2], Wanhu Tang[1,2], Hye-lin Ha[1,2] and Ulrich Siebenlist[1]. [1]National Institutes of Health, Bethesda, MD; [2] National Institute of Allergy and Infectious Diseases, Bethesda, MD

Allergic inflammatory lung disease is a complex immune response orchestrated by many cytokines, chemokines, and other factors. One of these is the alarmin IL-25, an epithelial cell-derived cytokine secreted mucosal sites. IL-25 activities overlap with those of IL-33 and TSLP; all three activate innate lymphoid cells type 2 (ILC2s). Repeated *in vivo* administration of IL-25 into lungs is sufficient to induce acute type 2 allergic asthma-like conditions, although underlying mechanisms even in this simple model remain to be fully understood. We set out to elucidate unique/significant roles of IL-25 in the chronic asthma-like lung inflammation model induced by house dust mites (HDM). If unique/significant contributions could be identified, IL-25 could be a potential therapeutic target in allergic asthma. To carry out these studies we generated mice with global or conditional deletion of IL-17RB (proprietary chain of the IL-25 receptor) and CIKS (a.k.a. Act-1, Traf3ip2, the adaptor for IL-17 cytokines). Surprisingly we found that blocking IL-25 signaling in lung $CD11c^+$ cells reduced expression of IL-9 and IL-13, cytokines that derive primarily from Th9 and Th2 cells, respectively, during the chronic stages. Thus, ILC2s are not the only relevant target cell of IL-25. We will present investigations of potential mechanisms underlying the effects of IL-25 signaling in $CD11c^+$ lung cells. Our data demonstrate that IL-25 signaling in $CD11c^+$ cells makes physiologically important contributions in the chronic HDM-induced model of allergic asthma, findings that should encourage future studies of this cytokine as a therapeutic target.

W15. Study of Mucosal Immunity in Dock8 Deficiency

Biyan Zhang and Stephanie Eisenbarth. Yale University, New Haven, CT

Dedicator of cytokinesis 8(Dock8) is a guanine-nucleotide exchange factor which has been implicated in a primary immunodeficiency in humans that results in recurrent respiratory tract, skin, and bowel infections. Previous studies in Dock8 knockout (Dock8 KO) mice demonstrated impaired CD8 T cell migration in the skin. In corroboration with a role of Dock8 in cell migration, our lab has identified a subset-specific migration defect in $CD11b^+$ dendritic cell(DC) from the skin and lungs to the draining lymph nodes after immunization through the subcutaneous or intranasal route respectively. As Dock8 patients have impaired mucosal immunity, we seek to investigate if intestinal DC migration is defective in Dock8 KO mice. An examination of DC frequencies in the mesenteric lymph modes revealed a significant loss of $CD103^+$ $CD11b^+$ DCs. Interestingly, 16S sequencing of microbes in fecal pellets of wildtype and Dock8 KO mice revealed similar alpha and beta diversity, suggesting no major disruption in the microbiome. However, analysis of changes in the microbiome at the species level using Lefse revealed an aberrant expansion of segmented filamentous bacteria(SFB). As SFB is a potent inducer of Th17 differentiation and $CD103^+$ $CD11b^+$ might play a role in this response, we seek to understand if Th17 compartment is altered in Dock8 KO mice. Dock8 conditional KO mice in DCs and T cells have also been generated to elucidate the cell-intrinsic role of Dock8 in regulating the gut microbiota and Th17 responses. We hypothesize that Dock8 mutation impairs intestinal $CD103^+$ $CD11b^+$ DC migration, leading to defective Th17 responses and poor mucosal immunity.

W17. Investigating the Role of FAM21 in Innate Immune Dendritic Cells

Rakesh Kulkarni, Siti Khadijah and Wen Chang. Institute of Molecular Biology, Academia Sinica, Taipei, Taipei, Taiwan

Vaccinia virus (VACV) belonging to *Poxviridae* family, is a large DNA virus with a wide host range to infect mammalian cells. Our previous studies with HeLa cells showed that *Vaccinia* mature virus is endocytosed into host cells and a cellular protein Fam21 is required for virus penetration. Fam21 is a component of WASP and Scar homolog (WASH) protein complex that mediates actin polymerization at the endosomal membranes to facilitate cargo-containing vesicles to be sorted out of endosomes. To study the *in vivo* function of Fam21 we generated a conditional Fam21 knockout mice in C57BL/6 background in which Fam21 was specifically knocked out in CD11c-positive dendritic cells (DC) population. Preliminary results indicated the role of Fam21 in antiviral functions of DC as Fam21 conditional knockout mice (CKO) were more susceptible to VACV infection through an intranasal infection route. We generated bone marrow derived dendritic cells (BMDC) from WT and Fam21 CKO mice and the data revealed a decreased phagocytic activity and antigen presentation function of DC isolated from Fam21 CKO mice, consistent with the endocytic role of Fam21 in HeLa cells. The CKO BMDC displayed cell spreading defects consistent with decreased surface levels of integrin CD11c on CKO BMDC. The detailed mechanism of Fam21 in DC development and function will be investigated in future.

M.07. What shapes dendritic cells into critical gatekeepers of TGF-β-dependent intestinal immune responses

Helena Paidassi, CIRI – Inserm/CNRS, Lyon, France

Dendritic cells (DCs) have the unique ability to instruct the adaptive immune system according to antigens, signals and cues encountered in the periphery. In the gut, a diverse range of DC populations have been described, each having specific functions and properties. However, despite advances in defining gene expression profiles and lineage of these DC subsets, how DCs acquire their individual roles in generating and maintaining different T cell subsets remains unclear. Here, we focus on the ability of DCs to induce TGF-β-dependent T cell responses in the gut. TGF-β is produced on a latent form and its activation by DCs expressing αvβ8 integrin is essential for the generation of intestinal regulatory T cells (Tregs) that in turn promote tolerance to intestinal antigens. We have recently shown that αvβ8 integrin is preferentially expressed by mucosal $CD103^+$ DCs, and confers on these cells their ability to activate TGF-β and thus to generate Tregs. However, how these DCs become specialized for this vital function is unknown. Here I will show that β8 expression is controlled by a combination of factors that include DC lineage, and signals derived from the tissue microenvironment and microbiota, associated with DC subset-specific epigenetic changes in the Itgb8 locus. Together, these data provide a key illustrative example of how microenvironmental factors and cell lineage together shape DCs into critical gatekeepers of TGF-β dependent intestinal immune responses via regulation of β8 expression.

Effector T Cells and Cytokines

F01. Human Antigen-Presenting γδ T Cells Promote Intestinal CD4⁺ T Cell Expression of IL-22 and Mucosal Release of Calprotectin

Neil E. McCarthy[1], Christopher J. Tyler[2], James O. Lindsay[3], Andrew J. Stagg[1], Bernhard Moser[2] and Matthias Eberl[2]. [1]The Blizard Institute, Queen Mary University of London, London, England, United Kingdom; [2]Cardiff University, Cardiff, Wales, United Kingdom; [3]Royal London Hospital, Barts Health NHS Trust, London, England, United Kingdom

The cytokine IL-22 plays a critical role in mucosal barrier defence, but the mechanisms that promote IL-22 expression in the human intestine remain poorly understood. Since human microbe-responsive γδ *T cell*s are abundant in the gut and recognise microbiota-associated metabolites, we assessed their potential to induce IL-22 expression by intestinal CD4⁺ *T cell*s. γδ *T cell*s with characteristics of antigen presenting cells (APCs) were generated from human blood and intestinal organ cultures, then co-cultured with naïve and memory CD4⁺ *T cell*s obtained from human blood or colon. The potency of blood and intestinal γδ T-APCs was compared with that of monocytes and dendritic cells by assessing CD4⁺ *T cell* phenotypes and proliferation as well as cytokine and transcription factor profiles. γδ *T cell*s in human blood, colon and terminal ileum acquired APC functions upon microbial activation in the presence of microenvironmental signals including IL-15 and were capable of polarising blood and colonic CD4⁺ *T cell*s towards distinct effector fates. Unlike monocytes or dendritic cells, gut-homing γδ T-APCs employed an IL-6-independent mechanism to stimulate CD4⁺ *T cell* expression of IL-22 without upregulating IL-17. In human intestinal organ cultures, microbial activation of γδ *T cell*s promoted mucosal secretion of IL-22 and ICOSL/TNFα-dependent release of the IL-22-inducible antimicrobial protein calprotectin without modulating IL-17 expression. In conclusion, human γδ T-APCs stimulate CD4⁺ *T cell* responses distinct from those induced by myeloid APCs to promote local barrier defence via mucosal release of IL-22 and calprotectin. Targeting of γδ T-APC functions may lead to the development of novel gut-directed immunotherapies and vaccines.

F02 PR.1. Expressing Th9 Cells Promote Colitis-Associated Cancer (CAC)

Katharina Gerlach[1], Vanessa Popp[1], Stefan Wirtz[1], Andrew McKenzie[2], Benno Weigmann[1] and Markus F. Neurath[1]. [1]University Clinic Erlangen, Erlangen, Bayern, Germany; [2]University Cambridge, Cambridge, England, United Kingdom

Inflammatory bowel disease (IBD) is linked to an increased risk of developing colitis-associated colorectal cancer (CAC). In IBD especially T cells are critical mediators of inflammation suggesting that these cells may play a leading part in CAC, too. Recently, we have identified PU.1-expressing Th9 cells as IL-9-producing cells inducing colitis. Thus, the proinflammatory cytokine IL-9 is crucial for the formation and maintenance of colitis, but its involvement in developing CAC is not revealed so far. IL-9KO mice were treated with AOM/DSS to induce inflammation-dependent colon cancer. Miniendoscopic analysis was done to monitor tumor growth. For restoration of the IL-9-deficient phenotype an IL-9 expressing minicircle DNA vector was injected into IL-9-deficient mice in the AOM/DSS model. Colonic tumors have been isolated, histological sections were taken out and immunofluorescent analysis was done**.** IL-9 deficiency led to significant less inflammation consequently followed by significant reduction of tumors in the experimental model AOM/DSS. Furthermore, we explored a restoring effect on tumor development by IL-9 overexpression in IL-9-deficient mice during AOM/DSS treatment. Additionally, in cryosections of tumorigenic colon tissue more PU.1⁺ *T cell*s were found, pointing out the important relevance for Th9 cells in colon cancer. Our findings uncover a crucial role of IL-9 in the development of colitis-associated neoplasias as IL-9 led to tumor growth in the model of CAC. The presence of PU.1⁺ CD4⁺ -expressing cells in tumorigenic tissue suggests that these cells belong to the recently described Th9 T cell subset.

F03. Disrupted Enteric Th17 Signaling Exacerbates Autoimmune Inflammation

Patricia Castillo[1], Pawan Kumar[2] and Jay Kolls[1,3]. [1]University of Pittsburgh School of Medicine, Pittsburgh, PA; [2]Stony Brook University, Stony Brook, NY; [3]Children's Hostpital of Pittsburgh, Pittsburgh, PA

Th17 cells and the gut microbiome have been increasingly implicated in autoimmune disease including Multiple Sclerosis (MS) and autoimmune hepatitis (AIH). Human data show elevated IL-17 in MS lesions and AIH patient serum. Moreover, IL-17ra$^{-\backslash-}$ mice are protected from both experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, and concanavalin A (con A) induced hepatitis, a murine model of AIH. With regards to the microbiome, both MS and AIH patients display an altered intestinal microbiota, and antibiotic treatment in mice ameliorate both diseases. Despite these data, few studies have investigated how the relationship between Th17 cells and the microbiome influence autoimmune pathologies. In order to study this, we generated intestinal epithelial specific IL-17RA knockout mice (*Il17ra*$^{fl/fl}$ x *villin cre*$^{+}$ mice) and tested these mice in the EAE model of MS and the Con A model of AIH. Data showed that *Il17ra*$^{fl/fl}$ x *villin cre*$^{+}$ mice exhibited earlier EAE onset and increased EAE incidence and severity as compared to littermate cre^{-} controls. Preliminary data suggested these mice have increased intestinal GM-CSF. Similarly, after Con A challenge, *Il17ra*$^{fl/fl}$ x *villin cre*$^{+}$ mice displayed increased lethality and serum alanine aminotransferase levels as compared to cre^{-} controls. This coincided with elevated serum IFNg four hours post con A injection. Investigating the mechanisms underlying exacerbated disease in both models will elucidate the differential role of enteric Th17 cells and the microbiome in autoimmune inflammation. Moreover, it can provide insight into novel therapeutic strategies that target the gut-brain and gut-liver axes.

F04. RORC Antagonist Inhibits IL-17 Production in Gut Commensal-Specific T Cells and Mucosa from Crohn's Disease Patients

Helena Bassolas-Molina[1], Ernest Raymond[2], Mark Labadia[2], Joseph Wahle[2], Christian Harcken[2], Robert Hughes[2], Michael Turner[2], Dustin Smith[2], Elisabeth Calderón-Gómez[1], Miriam Esteller[1], Anna Carrasco[3], Maria Esteve[3], Isabella Dotti[1], Elena Ferrer-Picón[4], Núria Planell[4], Maria Carme Masamunt[1], Marta Gallego[4], Rebeca Barastegui[4], Clàudia Arajol[5], Jordi Guardiola[5], Gerald Nabozny[2], Julián Panés[4] and Azucena Salas[1]. [1]IDIBAPS, Barcelona, Catalonia, Spain; [2]Boehringer Ingelheim, Ridgefield, CT; [3]Hospital Universitari Mutua Terrassa, Barcelona, Catalonia, Spain; [4]Hospital Clínic, Barcelona, Catalonia, Spain; [5]Hospital de Bellvitge, Barcelona, Catalonia, Spain

Increasing evidence suggests that Crohn's disease (CD) results from an aberrant immune response to commensal microorganisms. Since deregulated pathways include Th17 responses, the inhibition of RORC represents a potential therapeutic strategy. Our aim was to determine the *ex vivo* effect of RORC antagonism on circulating leukocytes and intestinal tissue samples. Peripheral blood mononuclear cells (PBMCs) from CD patients were cultured for 7 days with commensal microbial proteins (FrvX and YidX) in the presence of an RORC antagonist. In addition, intestinal biopsies from active CD patients were cultured with an RORC antagonist for 16h. We also examined the effects of commensal-specific CD4^{+} T cells treated with an RORC inhibitor on healthy intestinal epithelial crypts. The RORC antagonist specifically inhibited transcription of Th17-related genes in bacterial antigen-stimulated PBMCs (n=12). Remarkably, biopsies from CD patients (n=15) treated with the RORC inhibitor significantly decreased transcription of IL17A, IL17F, IL22, IL26 and S100A8, whereas expression of Th1 genes TBX21 and IFNG did not change, supporting the target specificity of the compound. Intestinal crypts cultured with supernatants from commensal-specific CD4^{+} T cells (n=9) previously treated with RORC inhibitor showed decreased expression of CXCL1, CXCL8 and CCL20. In conclusion, we demonstrate that blocking RORC specifically decreases the expression of a subset of IL-17 dependent genes in the context of CD. This effect is observed in both immune and epithelial cells,

indicating that RORC antagonism could represent a therapeutic approach for treating CD.

F05. T-Bet Suppresses the IFN-Gamma Mediated Induction of a T Cell Intrinsic Type I IFN Signature during T Helper 1 Responses

Yohei Mikami[1,2], Fred Davis[1], Shigeru Iwata[1,2], Hong-Wei Sun[1], Stephen Brooks[1,2], Han-Yu Shih[1], Takeshi Kawabe[2,3], Kan Jiang[1], Dragana Jankovic[2,3], Alan Sher[2,3], Yuka Kanno[1,2] and John O'Shea[1,2]. [1]National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD; [2]National Institutes of Health, Bethesda, MD; [3]National Institute of Allergy and Infectious Diseases, Bethesda, MD

Type I and type II interferons (IFN) have critical roles in host defense and immunoregulation, with some aspects of their actions overlapping and others being quite distinct. In this study, we compared the actions of type I IFN (IFN-beta) and type II (IFN-gamma) on the transcriptome and metabolism of CD4 T cells. We found that an important factor influencing the distinctive transcriptomes and metabolic profiles triggered by IFN-beta and IFN-gamma was the transcription factor T-bet. In the absence of T-bet, IFN-gamma aberrantly enhanced STAT2 activity and a type I IFN transcriptomic program while subduing glycolysis. Moroever, during the Th1 response induced *in vivo* by Toxoplasma infection, type I IFN signature genes were enhanced in T-bet deficient CD4 T cells compared with wild type cells. We found that T-bet restrained the type I IFN signature triggered by IFN-gamma by inhibiting autocrine production of type I IFNs as well as constraining STAT1, STAT2 and IRF7 expression. Accordingly blocking IFNAR during IFN-gamma treatment inhibited the type I IFN signature and restored glycolytic capacity in T-bet deficient cells. This inhibitory activity of T-bet on multiple components of the type I IFN signaling loop ensures the development of a polarized type II IFN response and appropriate effector cell expansion in T helper 1 dominated infections. This work was supported by the intramural research programs of NIAMS and the NIAID.

F08. Immunobiology of Oral Barrier Tissues' Injury in the Pathogenesis Oral cGVHD

Ana C. Costa da Silva[1], Joshua Dodge[1], Steven Pavletic[2], Frances Hakim[2] and Jacqueline Mays[1]. [1]National Institutes of Health, National Institute of Dental and Craniofacial Research, Bethesda, MD; [2]National Institute of Health, National Cancer Institute, Bethesda, MD

Chronic graft-versus-host disease (cGVHD) remains the major limitation to successful allogeneic hematopoietic stem cell transplantation (allo-HSCT). Oral barrier tissues, including the oral mucosa and salivary gland, are affected in the majority of cGVHD cases; however, understanding of the underlying molecular and cellular mechanisms of this autoimmune-like disease remains incomplete. This study was designed to identify effector cells and salivary proteins associated with the onset and progression of oral cGVHD pathogenesis. Confocal microscopy revealed the presence of Tc1 ($CD8^+Tbet^+$) and Th17 ($CD4^+/CD161^+/IL\text{-}17^+$) cells in both buccal mucosa (BM) and minor salivary glands (MSG) of oral cGVHD patients but not in unaffected post-transplant controls. The type I interferon pathway was also shown to be active in cGVHD MSG, indicated by the presence of the IFN α/β–inducible protein MxA. Flow cytometric analyses confirmed the presence of CD4 and CD8 *T cell* subsets in cGVHD-affected BM and MSG, and an increased frequency of these cells expressing CXCR3. To further elucidate the possible mechanisms for oral cGVHD induction, whole saliva from individual oral cGVHD patients (n=58) and healthy controls (n=10) was analyzed for a 13-plex panel of immune-related analytes and revealed elevated expression of tissue remodeling factors (MMPs and TIMPs) and IFN- and IL-17-induced chemokines (CXCL10, CXCL9 and CCL20), that correlated with clinical scoring of cGVHD severity in a multivariate analysis. Taken together, these findings implicate activation of IFN pathways and presence and activation of Tc1 and Th17 in oral tissues in the pathogenesis of oral cGVHD.

F10. Site-Specific Regulation of CD8 T Cell Activation in the Oral Mucosa

Worawalun Hirunwidchayarat[1], Tatsukuni Ohno[1], Michio Tomura[2] and Miyuki Azuma[1]. [1]Tokyo Medical and Dental University, Tokyo, Japan; [2]Osaka Ohtani University, Osaka, Japan

The oral mucosa is a type II mucosal surface covered by a stratified squamous epithelium, which shares many features with the skin. Oral mucosal wounds heal faster and with less scar formation as compared with the skin. These mechanisms have been explained by differences in production of extracellular matrix proteins and several cytokines. However, the regulation and its mechanisms in the antigen-specific T cell responses have not been clarified yet. To investigate unique features of oral immune responses, we examined T cell responses at the local tissues and regional lymph nodes (RLNs) using DNFB-induced contact hypersensitivity models at the buccal mucosa (BM) and ear skin (ES). BM-challenge induced much faster inflammatory changes with abundant cell infiltration and epithelium damage during 12 to 24h than those at the ES. In addition, these inflammatory changes recovered quickly at 36h. Although recruitment of $CD8^+$ T cells was dominant 24h after both challenge, however, BM tissues contained much higher ratios of $CD8^+$ T cells with $CD62L^-CD44^{hi}$ memory phenotype. Despite the comparable expression levels of IFN-γ in the RLN cells, BM-recruiting $CD8^+$ T cells expressed less IFN-γ, but co-expressed an immune checkpoint receptor PD-1 and a proliferating marker Ki-67, suggesting a unique activation status with proliferation and exhaustion. Our results demonstrate the unique activation status of BM-recruiting $CD8^+$ T cells and these $CD8^+$ T cells may contribute to the rapid inflammation and quick recovery. Our results suggest that activation of $CD8^+$ T cells is modulated at the local tissue-microenvironment.

F11. Unique Mechanisms of Th17 Cell Priming by DC at the Oral Barrier

Tamsin Zangerle-Murray[1], Tovah Shaw[1], Hayley Bridgeman[1], Kelly Wemyss[1], Sheila Brown[1], Niki M. Moutsopoulos[2], John Grainger[1] and Joanne E. Konkel[1]. [1]University of Manchester, Manchester, England, United Kingdom; [2]National Institutes of Health, National Institue of Dental and Craniofacial Research, Bethesda, MD

An area of intense research focus is to understand how tissue-specific signals balance immunity and regulation at barrier sites. One barrier at which this balance frequently fails is the oral barrier, specifically the gingiva, where loss of immune homeostasis leads to periodontitis (PD), a common chronic inflammatory disease. Th17 cells are key mediators of PD pathogenesis and we recently outlined a novel developmental pathway for gingival Th17 cells. Contrasting the skin and gastrointestinal tract, we demonstrated that gingival Th17 cells were not dependent on commensal colonization. Instead, gingival mechanical damage promoted Th17 cell development, outlining physiological damage as a key local cue tailoring immuno-surveillance at the gingiva. Here we further examined the developmental pathway of gingival Th17 cells probing whether there were common or tissue-specific mechanisms involved in DC priming of Th17 cells. Our data demonstrate that DC populations shown to prime Th17 cells at other barrier sites do not mediate this function in the gingiva. Mice lacking IRF4-dependent DCs or monocytes exhibited unchanged populations of gingival Th17 cells. Instead, we found that Batf3-dependent DCs negatively regulated gingival Th17 cells. Loss of Batf3-dependent DCs led to increased frequencies of Th17 cells in the gingiva and, subsequently, enhanced pathogenesis in mouse models of PD. Together our data define the DC network policing the oral barrier and begin to unravel distinct, tissue-specific functions of these crucial mediators of immune responses in the gingiva compared to other barrier sites. Specifically, our data uncover novel functions for Batf3-dependent DCs in maintaining gingival immune homeostasis.

F13. Unique-Tissue Specific Signals Drive Th17 Accumulation at the Oral Mucosal Barrier

Nicolas Dutzan[1], Loreto Abusleme[1], Hayley Bridgeman[2], Teresa Greenwell-Wild[1], Tamsin Zangerle-Murray[2], Mark Fife[2], Nicolas Bouladoux[1], Holly Linley[2], Laurie Brenchely[1], Kelly Wemyss[2], Giorgio Trinchier[1], Patricia Diaz[3], Yasmine Belkaid[1], Joanne E. Konkel[2] and Niki Moutsopoulos[1]. [1]National Institutes of Health, Bethesda, MD; [2]University of Manchester, Manchester, United Kingdom; [3]University of Connecticut Health Center, Farmington, CT

Th17 cells have emerged as key cellular mediators of barrier immunity, participating in immune surveillance and maintenance of barrier integrity. The critical role of Th17 cells in oral barrier immunity has been shown in patients with genetic defects and experimental models of Th17 deficiency. Conversely, exaggerated Th17 responses at this barrier are detrimental and have been shown to promote inflammatory bone loss and tissue damage in the common inflammatory disease periodontitis. However, little is known regarding the physiologic development of Th17 immunity at the oral barrier. Our data show that Th17 cells increase with age in mice and humans. Exploring this age-associated Th17 accumulation, we find that unlike the skin and gastrointestinal tract, oral Th17 cells arise independently of commensal colonization. Moreover, we demonstrate that ongoing damage which occurs physiologically during mastication in the oral environment is a key tissue specific signal that shapes the function of T cells at the oral mucosa, promoting Th17 differentiation. We find that mastication–induced damage through upregulation of IL-6 drives the proliferation of local Th17 cells promoting IL-17 mediated local immunity. Our data highlight the importance of unique local factors in the regulation of regional immunity and uncover the physiologic function of mastication as a key tissue specific cue in the development of the oral immune system.

F14. Notch Ligand Delta-Like Ligand 4 (Dll4)-Mediated Regulatory T Cells Development is Maintained by SMYD3 During Pulmonary Infection

Hung-An Ting[1], Denise de Almeida Nagata[2], Andrew Rasky[1], Matthew Shaller[1] and Nick Lukacs[1]. [1]University of Michigan, Ann Arbor, MI; [2] Genetech, South San Fransico, CA

Respiratory Syncytial Virus (RSV) infection is the most common and severe infection in pediatric populations. Our recent studies have demonstrated that Notch ligand Delta-like ligand 4 (Dll4) maintains regulatory T cells (T_{reg}) differentiation and attenuates immunopathology in RSV infection. Epigenetic alterations, which include histone modifications, are critical in cell differentiation decisions. Recent genome-wide studies demonstrated that T_{reg} cells have increased tri-methylation on histone H3 at lysine 4 (H3K4me3) around the master transcription factor—*Foxp3*—loci. Here we report that Dll4 further up-regulated SET and MYDN domain-containing protein 3 (SMYD3), which is a H3K4 methyltransferase. Dll4 enriched SMYD3-dependent H3K4me3 around *Foxp3* loci during T_{reg} cell differentiation and stabilizes the Treg cell phenotype. Dll4 inhibition decreased H3K4me3 together with decreased expression of *Smyd3* and *Foxp3* during RSV infection. Furthermore, both SMYD3 depletion and Dll4 inhibition destabilized $Foxp3^{EGFP+}$ T_{reg} cells to increase IL-17A production. Our work demonstrates a novel epigenetic mechanism of Dll4/Notch activation of T_{reg} cells to modulate pulmonary infection through control of T_{reg} cell fate decision

F15. Pathogen-Elicited Mucosal Gamma Delta T Cells: A Memory Population with Broad Pathogen Reactivity

Camille Khairallah[1], Zhijuan Qiu[1], Jessica N. Imperato[1], Yue Zhang[1], Julie A. Bettke[1], Kyungjin Cho[2], Kwang Soon Kim[2], Adrianus WM van der Velden[1], James B. Bliska[1], Charles D. Surh[2] and Brian S. Sheridan[1]. [1]Stony Brook University, Stony Brook, NY; [2] Postech, Pohang, Kyongsang-bukto, Republic of Korea

γδ T cells are generally considered innate-like cells critically involved in tissue surveillance and pathogen control at mucosal surfaces. However, we previously demonstrated that oral infection with a mouse-adapted strain of *Listeria monocytogenes* (*Lm*) induced protective $V\gamma4^{+}$ $CD44^{hi}$ $CD27^{neg}$ memory γδ T

cells that were capable of producing IFNγ and IL-17A and resided in the intestines and mesenteric lymph nodes (MLNs). This surprising adaptive-like γδ T cell response prompted us to more closely examine the induction and specificity of these cells. *Ex vivo* stimulation of *Lm*-immune MLN cells with live, but not heat-killed (HK), *Lm* induced early IL-17A production by memory γδ T cells at 6 hours. However, no IFNγ was detectable at this time. IFNγ production was only detected at 24 hours with both live and HK bacteria suggesting that its production depends on antigen processing and presentation. Moreover, while culture supernatants were sufficient to induce IL-17A production, IFNγ production and IFNγ/IL-17A co-production required cellular contact. Surprisingly, IFNγ production by *Lm*-elicited memory γδ T cells was also induced by several heterologous HK bacteria suggesting broadly conserved recognition of bacterial or host-derived infection-induced ligands. Interestingly, *Lm*-elicited memory γδ T cells also developed in germ-free mice and expanded after certain heterologous bacterial challenges *in vivo*. Collectively, these data suggest that $V\gamma4^{+}$ $CD44^{hi}$ $CD27^{neg}$ γδ T cells are a heterogenous subset of adaptive-like T cells with broad sprectrum reactivity to pathogens. These findings implore further evaluation of their impact in infectious diseases and inflammatory disorders of the gut.

F16. Role of Bcl-3 in T Cells Upon Challenge with Lymphocytic Choriomeningitis Virus

Hemant Jaiswal and Ulrich Siebenlist. [1]National Institutes of Health, National Institutes of Allergy and Infectious Diseases, Bethesda, MD

Bcl-3 is an atypical member of the IkB family. Classical IkBs inhibit trans-activating NF-kB dimers such as p50/RelA largely via cytoplasmic retention; they are degraded in response to many signals, which allows nuclear entry of NF-kB. By contrast, Bcl-3 is not degraded and acts in the nucleus to modulate transcription of NF-kB target genes by associating with p50/NF-kB1 and p52/NF-kB2 homodimers. The latter complexes do not trans-activate by themselves, and association with Bcl-3 may promote or inhibit transcription in a highly gene- and context-dependent manner. Bcl-3 has profound biologic roles. *Bcl-3*$^{-/-}$ mice exhibit notably impaired adaptive and innate immune responses. Previously, we have shown that Bcl-3 regulates plasticity and pathogenicity of autoimmune CD4 T cells in the T cell transfer-induced colitis model as well as in EAE. However, the detailed molecular controls of Bcl-3 in T cell differentiation and its antigenic responses are not well understood. To evaluate the possible role of Bcl-3 in antigen-specific T cell responses, mice lacking Bcl-3 specifically in T cells were challenged with acute lymphocytic choriomeningitis virus (LCMV) infection. Preliminary data revealed that *Bcl-3*$^{-/-}$ CD8 T cells had comparable primary antiviral responses at day 8 post infection. However, loss of Bcl-3 in T cells significantly reduced the proportion of LCMV-specific CD8 T cells in spleens 6 weeks post infection. This suggests a crucial role of Bcl-3 in long-term maintenance of antigen-specific CD8 T cells. We will present initial investigations into mechanisms by which Bcl-3 may help to maintain antiviral T cell responses over time.

F17. Effect of Truncal Unilateral Vagotomy on the T-Dependent Isotype Switching of IgA at Inductor and Effector Sites of Small Intestine from BALB/c Mice

Ivonne M. Arciniega-Martinez, Marisol Salas-Pimentel, Aldo Resendiz-Albor and Rafael Campos-Rodriguez. Escuela Superior de Medicina Instituto Politécnico Nacional, Mexico, Distrito Federal, Mexico

Previous studies have shown that the truncal vagotomy diminishes the percentage of lymphocytes in lamina propria, but sIgA increases. Intestine has a different lymphocyte distribution according to the segment -proximal or distal- and the site, inductor or effector; the aim of this study was to determinate if ACh has a role in IgA secretion through the via T-dependent. Male mice underwent SHAM or truncal unilateral vagotomy (Vx). On 14th postoperative day, samples of intestinal liquid were harvested to determine total SIgA;IgM^{+} and IgA^{+} plasma cells of Peyer's patches and lamina propria and intracellular cytokines TGF-β, IL-4, IL-5, IL-6 e IL-10 related with via T- dependent for isotype switching. sIgA of distal segment had an important increased in Vx group compared with SHAM. IgA^{+} and IgM^{+} plasma cells of Peyer's patches had an increase in proximal and distal segment of the Vx group vs SHAM group; however, in lamina propria, the two population cells of Vx group decreased in proximal segment compared vs SHAM group. IL-4, -5, -10 increased in proximal and distal segment in Peyer's Patches of Vx group. In proximal effector site, all the citokines decreased in Vx group, principally TGF-β; but in distal segment all of them were increased in Vx group respect to SHAM. Vagus nerve has a greater influence principally on proximal

segment and efector site. Unilateral troncular vagotomy removes an important source of acetylcholine in small intestine on synthesis and secretion of sIgA. Supported by SIP and COFFA IPN.

F19. Human Oral Mucosa: Immune Cell Network in Homeostasis and Disease

Nicolas Dutzan[1], Joanne E. Konkel[2], Teresa Greenwell-Wild[1] and Niki M. Moutsopoulos[1]. [1]National Institutes of Health, National Institues of Dental and Craniofacial Research, Bethesda, MD; [2]University of Manchester, Manchester, England, United Kingdom

The characterization of immune cell networks at barrier sites have helped towards the understanding of immune mechanisms involved in tissue homeostasis and in local immunopathologies. At the oral barrier, little is known of the immune cell populations participating in health and how these shift in the context of periodontitis, a highly prevalent inflammatory oral pathology. To this end, we collected human oral/gingival tissues from systemically healthy subjects without evidence of periodontitis as well as from patients with severe untreated periodontitis. Tissues were digested and single-cell suspensions were processed for multicolour flow cytometry, allowing an in-depth evaluation of antigen-presenting cell populations (APC), T cell subpopulations, innate lymphoid cells (ILC) and cytokine-secreting cell populations. In health, we find a predominance of T cells and granulocytes/neutrophils. We characterize the sophisticated network of professional APC and phenotype the local innate lymphoid cells. Our analysis of cellular subtypes reveals that the majority of T cells are $CD4^+$ memory resident T cells, the majority of APC are $CD14^{+,}$ and the predominant ILC belong to the ILC1 subcategory. Notably, in disease we document an increase in neutrophils and an up-regulation of IL-17 responses, particularly within the $CD4^+$ T cell compartment. Collectively, our studies provide a first view of the cellular landscape of oral immunity and serve as a baseline for the understanding of local immunopathology. This research was supported by the NIDCR Combined Technical Research Core. This research was supported by the Intramural Research Program of the NIH, NIDCR.

F21. Investigating the Effect of Integrin αvβ8 on $CD4^+$ Effector Memory T Cells

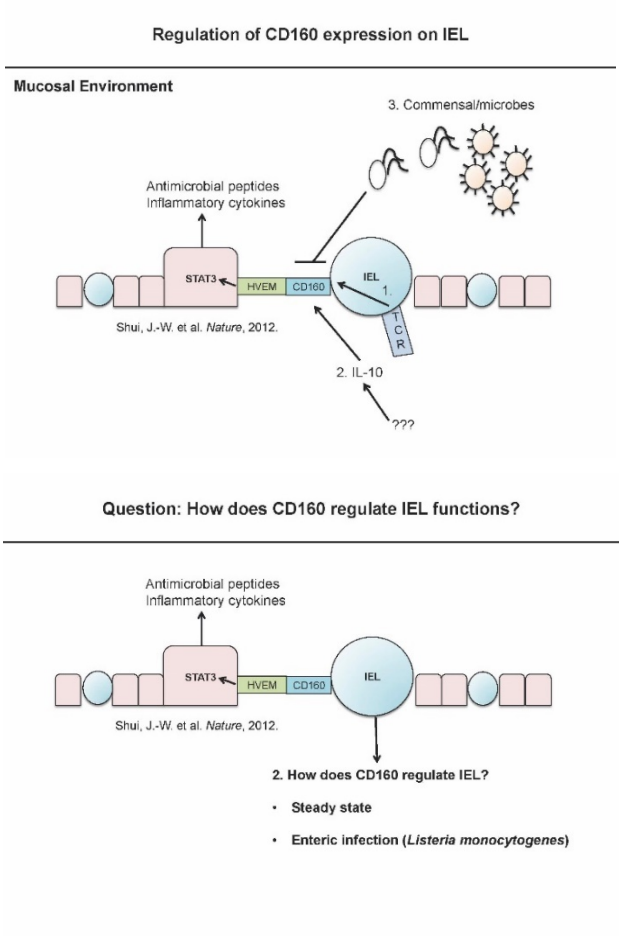
Stephanie Ailsa Houston[1], Joshua Casulli[1], Mark Fife[1], Marion Pepper[2], Tracy Hussell[1] and Mark Travis[1]. [1]University of Manchester, Manchester, England, United Kingdom; [2] University of Washington, Seattle, WA

The adaptive immune response is characterised by the ability of lymphocytes to respond rapidly and effectively to previously encountered antigen. A key cell type in this immunological memory are memory T cell populations, which include effector memory T cells (T_{EM}), central memory T cells (T_{CM}) and resident memory T cells (T_{RM}). Understanding how memory T cell responses are regulated to promote immunological memory is crucial in identifying novel strategies to boost secondary immune responses. A crucial cytokine that regulates a broad range of T cell functions, including memory responses, is TGFβ. TGFβ is made by many cells, but always as a latent complex that requires activation to function. However, how TGFβ responses are controlled to regulate memory T cell responses is completely unknown. Here we show that a population of $CD4^+$ T_{EM}, but not other effector or memory T cell populations, expresses the TGFβ-activating integrin αvβ8, which enables $CD4^+$ T_{EM} to activate TGFβ. Additionally, mice lacking expression of the integrin of T_{EM} showed an expansion of antigen-specific effector $CD8^+$ T cells after secondary infection with influenza, which was accompanied by an increase in granzyme B production. Thus, $CD4^+$ T_{EM}-mediated TGFβ activation via integrin αvβ8 appears to limit the expansion and function of antigen specific $CD8^+$ T cells during a memory response. Such work has important implications in potential therapeutic strategies aimed at boosting secondary immune responses to infection.

F22. CD160 Stimulates CD8⁺ T Cell Responses and Protective Immunity to *Listeria monocytogenes*

Catherine Tan[1], Xiaohui He[1], Shannon Grande[2], Keturah Brown[3], Dennis Kasper[1], Gordon Freeman[4] and Arlene Sharpe[1]. [1]Harvard Medical School, Boston, MA; [2] Bluebird Bio, Boston, MA; [3] Oxford PharmaGenesis, Newtown, PA; [4] Dana Farber Cancer Institute, Boston, MA

CD160 is an immunoglobulin (Ig)-like glycosylphosphatidylinositol (GPI)-anchored cell membrane receptor expressed on NK cells, NKT cells, activated and exhausted CD8⁺ T cells. CD160 can stimulate NK cytotoxicity, IFN-γ production and enhance T cell activity. CD160 expression on exhausted cells correlates with T cell dysfunction, suggesting an inhibitory role in T cell exhaustion. In the intestine CD160 is expressed on both small and large intestinal CD8⁺ intraepithelial lymphocytes (IEL). Interaction between CD160 on IEL and its ligand Herpes Virus Entry Mediator (HVEM) on IEC can lead to HVEM signaling within IEC and protection against *Citrobacter rodentium* infection. However, little is known about the regulation of CD160 expression and its role in controlling CD8⁺ T cell responses. We find that CD160 expression is induced by TCR engagement and further augmented by IL-10. Interestingly, microbiota negatively regulate CD160 expression on IEL. We used CD160 deficient mice ($^{-/-}$) to investigate CD8⁺ T cell functions. CD160$^{-/-}$ mice are healthy at steady state with a modest reduction in IEL numbers in both the large and small intestine. Using an oral infection of *Listeria monocytogenes*, we show that CD160$^{-/-}$ mice cannot clear *Listeria monocytogenes* as efficiently as WT mice. CD160$^{-/-}$ CD8+ T cells have reduced Granzyme B, TNF-α and IFN-γ production. Thus, CD160 has an important for promoting CD8+ T cell effector functions and bacterial clearance during *Listeria monocytogenes* infection.



OR.86, F12. Oral Wild-Type *Salmonella* Typhi Challenge in Human Volunteers: Assessment of CD8⁺ T Cell Multifunctionality and Cross-Reactivity Against Typhoidal and Invasive Non-Typhoidal *Salmonella* serovars

Rekha R. Rapaka[1], Rezwanul Wahid[1], Stephanie Fresnay[1], Thomas C. Darton[2], Claire Jones[2], Claire S. Waddington[2], Christoph J. Blohmke[2], Brian Angus[2], Myron M. Levine[1], Andrew J. Pollard[2] and Marcelo B. Sztein[1]. [1]University of Maryland School of Medicine, Baltimore, MD; [2]University of Oxford and the NIHR Oxford Biomedical Research Centre, Oxford, United Kingdom

Human adaptive cellular immunologic responses to mucosally acquired invasive *Salmonella* infection are not fully characterized. We evaluated CD8 T cell responses elicited in volunteers who developed typhoid disease after an oral challenge with wild-type (wt) *Salmonella* Typhi (ST), against other typhoidal and invasive non-typhoidal *Salmonella* serovars. PBMC from volunteers obtained prior and after challenge with wt

ST were co-cultured with autologous EBV-transformed B cells infected with various invasive *Salmonella* serovars and evaluated by multichromatic flow cytometry. We confirmed previous results that high frequencies of CD8 T effector memory (T_{EM}) cells are elicited against ST in most volunteers, peaking by 4 to 8 weeks in the blood after challenge. Multifunctional CD8 T_{EM} with the CD107a$^+$/IFN-γ$^+$/TNF-α$^+$ phenotype that are induced following oral ST challenge exhibit cross-reactivity to the other clinically relevant typhoid producing serovars *S. Paratyphi* A (PA) and *S. Paratyphi* B (PB), but demonstrated reduced cross-reactivity to the invasive non-typhoidal *S. Typhimurimum* strain D65 (iSTM). We also observed that most CD8 T_{EM} elicited by oral ST challenge that produce high quantities of IFN-γ against ST, PA, and PB, also produce high quantities of TNF-α, while large proportions of CD8 T_{EM} that express high levels of CD107a are predominantly IFN-γ negative. This suggests a hierarchy of systemic, multifunctional cross-reactive CD8 T_{EM} responses elicited by oral ST challenge. These data provide insight into the specificity of CD8 T cells elicited by oral wt ST challenge, and demonstrate the induction of cross-reactive responses to other invasive *Salmonella* serovars.

OR.89, F20. Local Tissue Alarmins and Foxp3$^+$ Treg Cells Control the Induction of Type-2 Immunity from Commensal-Specific Type-17 T cells

Oliver J. Harrison[1], Jonathan L. Linehan[1], Shurjo K. Sen[1], Allyson L. Byrd[1], Seong-ji Han[1], Han-Yu Shih[1], Alejandro Villarino[1], John O'Shea[1,2] and Yasmine Belkaid[1]. [1]National Institutes of Health, Bethesda, MD; [2]National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD

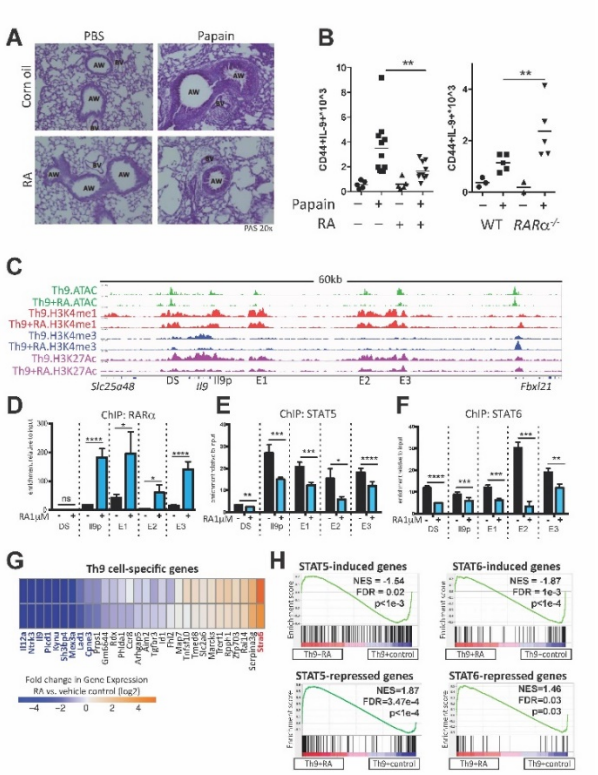
A dynamic dialogue between host and microbiota ensures that commensal colonization occurs as a state of mutualism, the breakdown of which is associated with inflammatory disorders. We recently demonstrated that distinct commensal species drive defined cutaneous T cell responses. Commensal-specific T cell responses were mounted in the absence of canonical inflammation, and promoted local immunity to dermal pathogens. We sought to understand how local tissue signals mould commensal-specific T cell function. Phenotypic and transcriptomic analysis of cutaneous T cells induced by commensals reveal that these cells are distinct from those responding to skin pathogens. Notably, amongst commensal-specific effector T cells, we observed a proclivity towards IL-17A-producing CD8$^+$ T cell differentiation, compared to a type-1 phenotype of pathogen-specific effector T cells. Strikingly, commensal-induced IL-17A-producing CD8$^+$ T cells also demonstrated significant expression of type-2 gene transcripts, including *Gata3*, *Il5* and *Il13*. As such, commensal-specific CD8$^+$ T cells maintain a poised/hybrid type-17/type-2 state of differentiation during steady state. Rapid production of type-2 cytokines by CD8$^+$ T cells was elicited by local tissue alarmins, including IL-18. Furthermore, this poised/hybrid differentiation state was maintained by local Foxp3$^+$ Treg cells, as selective attenuation of dermal Foxp3$^+$ Treg cell function resulted in accumulation of committed Tc2 cell populations following commensal colonization. Thus, commensal-specific CD8$^+$ T cells that accumulate in the skin can rapidly respond to local cues to produce type-2 cytokines. Investigating the epigenetic and transcriptional events underlying generation of commensal-specific T cell responses will aid our understanding of targets for treatment of chronic inflammatory disorders.

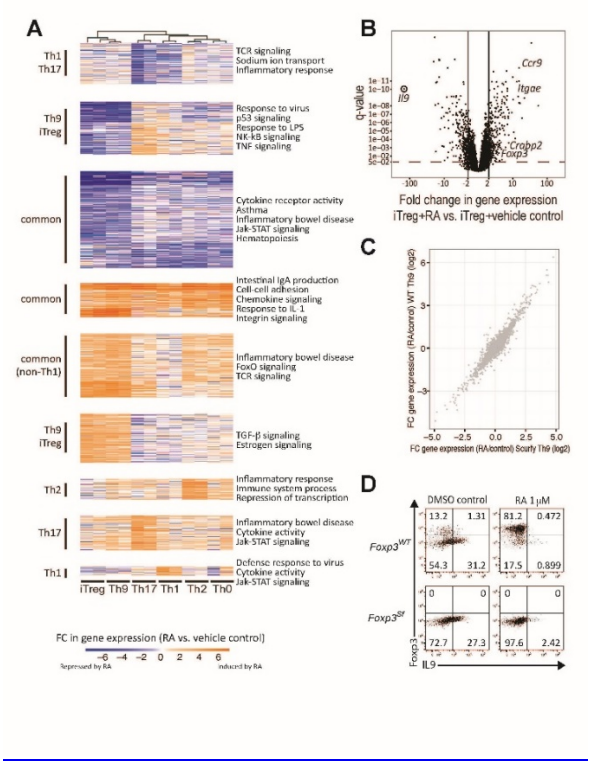
OR.90, F07. Retinoic Acid Represses a Th9 Transcriptional Program

Daniella M. Schwartz[1], Taylor Farley[1], Richoz Nathan[1], Hong-Wei Sun[1], Kan Jiang[1], Han-Yu Shih[1], Franziska Petermann[1], Mikami Yohei[1], Fred Davis[1], Richard Siegel[1], Laurence Arian[2], Francoise Meylan[1] and John O'Shea[1,3]. [1]National Institutes of Health, Bethesda, MD; [2]University of Oxford, Oxford, England, United Kingdom; [3]National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD

Retinoic acid (RA) is an important mucosal factor that modulates CD4$^+$ T cell function by serving as the ligand for a transcription factor (TF) family of receptors (RARs). RA is an essential positive regulator of T effector responses, yet also dampens immune responses by inhibiting effector cytokine production, promoting regulatory T cell (iTreg) differentiation, and upregulating the master TF FoxP3. We aimed to dissect these multifaceted effects of RA and gain a comprehensive view of its action. By comparing the transcriptomes of RA-treated and untreated T helper lineages, we identified subset-specific RA-regulated pathways and a dominant RA effect common to all

CD4$^+$ types. Subset-restricted RA effects were most apparent in iTreg and Th9 cells: the most RA-repressed gene encoded the Th9 cytokine IL(interleukin)-9. To separate RA effects secondary to FoxP3 induction, we used FoxP3-mutant (Scurfy) T cells and demonstrated that immunomodulatory effects, including IL-9 inhibition, are FoxP3-independent. We confirmed that RA inhibits IL-9 production *in vitro*, and an *in vivo* model of allergic lung disease. To elucidate regulatory elements in the *Il9* locus, we mapped chromatin accessibility (ATAC-seq) and enhancer marks (ChIP-seq). We found that RAR-alpha binds directly to the locus and promotes chromatin remodeling to inhibit the binding of Th9 TFs. Finally, we determined that RA represses transcription of Th9-specific genes and antagonizes key Th9 TFs. Our observations suggest that RA-mediated chromatin remodeling prevents transcriptional activation by major Th9 TFs, repressing a Th9 transcriptional program.





OR.91, F18. IL-36γ Signaling Controls the Induced Regulatory T Cell – TH9 Cell Balance via Nfκb Activation and STAT Transcription Factors

Akihito Harusato[1], Hirohito Abo[1], Vu L. Ngo[1], Samuel Won-zu Yi[1], Kazunori Mitsutake[1], Satoru Osuka[2], Jacob E. Kohlmeier[2], Jian-Dong Li[1], Andrew T. Gewirtz[1], Asma Nusrat[3] and Timothy Denning[1]. [1]Georgia State University, Atlanta, GA; [2] Emory University, Atlanta, GA; [3] University of Michigan, Ann Arbor, MI

Regulatory and effector T helper (T_H) cells are abundant at mucosal surfaces, especially in the intestine, where they control the critical balance between tolerance and inflammation. However, the key factors that reciprocally dictate differentiation along these specific lineages remain incompletely understood. Here, we report that the interleukin (IL)-1 family member IL-36g signals through IL-36 receptor, MyD88, and NFkBp50 in CD4$^+$ T cells to potently inhibit Foxp3-expressing induced regulatory T cell (T_{reg}) development by modifying histone acetylation status at Foxp3 locus, while concomitantly promoting the differentiation of T helper 9 (T_H9) cells via a IL-2-STAT5 and IL-4-STAT6 dependent pathway. Consistent with these findings, mice deficient in IL-36g and IL-36R were protected from T_H cell-driven intestinal

inflammation and exhibited increased colonic T_{reg} cells and diminished T_H9 cells. Further we observed significant correlations between human IL-9 and IL-36 cytokines in ulcerative colitis, which were not observed in Crohn's disease. Our findings thus reveal a fundamental contribution for the IL-36/IL-36R axis in regulating the T_{reg}-$T_H9$cell balance with broad implications for T_H cell-mediated disorders such as inflammatory bowel diseases, and particularly ulcerative colitis.

OR.92, F06. Nuclear Receptor REV-ERBα Limits TH17 Cell Inflammatory Capacity

Enric Esplugues[1] and Victor Bornstein[2]. [1]Yale School of Medicine, Guilford, CT; [2] Icahn School of Medicine at Mount Sinai, New York, NY

Interleukin (IL)-17-producing T cells (T_H17) have been identified as a subpopulation of antigen specific $CD4^+$ T cells that orchestrates the development and progression of many autoimmune disorders, including inflammatory bowel disease (IBD). The process by which T_H17 cells are generated has been well established in mouse and in human, however, more research is needed in order to better understand molecular mechanisms controlling pathogenic T_H17 cells. Here, we show that T_H17 cells express the nuclear receptor REV-ERBa, which can suppress their pro-inflammatory capacity. We observed that the master regulator of T_H17 cells, RORgt, binds to the *Rev-erba* locus, which in turn acts as a transcriptional repressor that directly targets RORγt, RORα, and IL-23R, controlling the pathogenic T_H17 cell program at different key levels. Furthermore, we were able to reduce the pathogenicity of mouse and human T_H17 cells by targeting this negative feedback loop with a synthetic REV-ERB agonist. Our observations are the first evidence of a cell intrinsic feedback loop in T_H17 cells that is able to control their pathogenicity. These results indicate that targeting REV-ERBα might be beneficial in the treatment of inflammatory processes and autoimmune diseases.

OR.93, F09. IL-33R Signaling in $CD4^+$ T cells Regulates Intratumoral Myeloid Recruitment

Ernesto Perez-Chanona, Carolyne K. Smith and Giorgio Trinchieri. National Cancer Institute, National Institues of Health, Bethesda, MD

The biological functions governed by IL-33 include the activation of TH1, TH2, $CD8^+$ T cell and NK cell responses, however the contribution of IL-33 to tumor biology remains controversial. By eliminating the lL-33 receptor (IL-33R) in specific immune compartments, including T cells and myeloid cells, we have begun to unravel the complexity of the intratumoral biology of IL-33. Our evidence shows that deletion of *Il33r* in T cells using $Il33r^{fl/fl}$ mice crossed with CD4-Cre expressing mice significantly impaired tumor growth of subcutaneous MC38 colorectal compared with the floxed controls. After three weeks, $CD4^+$ $Gata3^+$ cells and neutrophils were notably more abundant in the tumors of $Il33r^{\Delta\ T\ cells}$ mice, while the $CD8^+$ cell populations remained unaffected. Myeloid cell populations, such as $CD11b^+$ $Ly6C^{hi}$ SSC^{lo} $MHCII^+$ monocyte/macrophages, and $CD11b^+$ $Ly6C^+$ $Ly6G^+$ granulocytes infiltrated in larger numbers into the tumors growing in $Il33r^{\Delta\ T\ cells}$ mice than those in control mice. The contribution of IL-33 towards the anti-tumor activity or tumor-promoting capacity of these cells is under investigation. In addition, our ongoing studies suggest the possibility that not only IL-33R signaling mediates the extent of tumor clearance, but it also contributes to cancer-induced morbidities such as cachexia and the therapeutic efficacy of chemotherapies. Thus, our results suggest that IL-33 signaling may be a target for cancer immunotherapy.

M.01. Environmental Factors and Regulation of the Cytokine IL-22

Lauren A. Zenewicz. University of Oklahoma Health Sciences Center, Oklahoma City, OK

Inflammatory bowel disease (IBD) affects approximately 1-1.5 million people in the United States. Caused by a dysregulated immune response, understanding the interactions between the environment and immune cells is critical to long-term treatment. Chronic inflammation can lead to the low oxygen environment of hypoxia. Cells sense and adapt

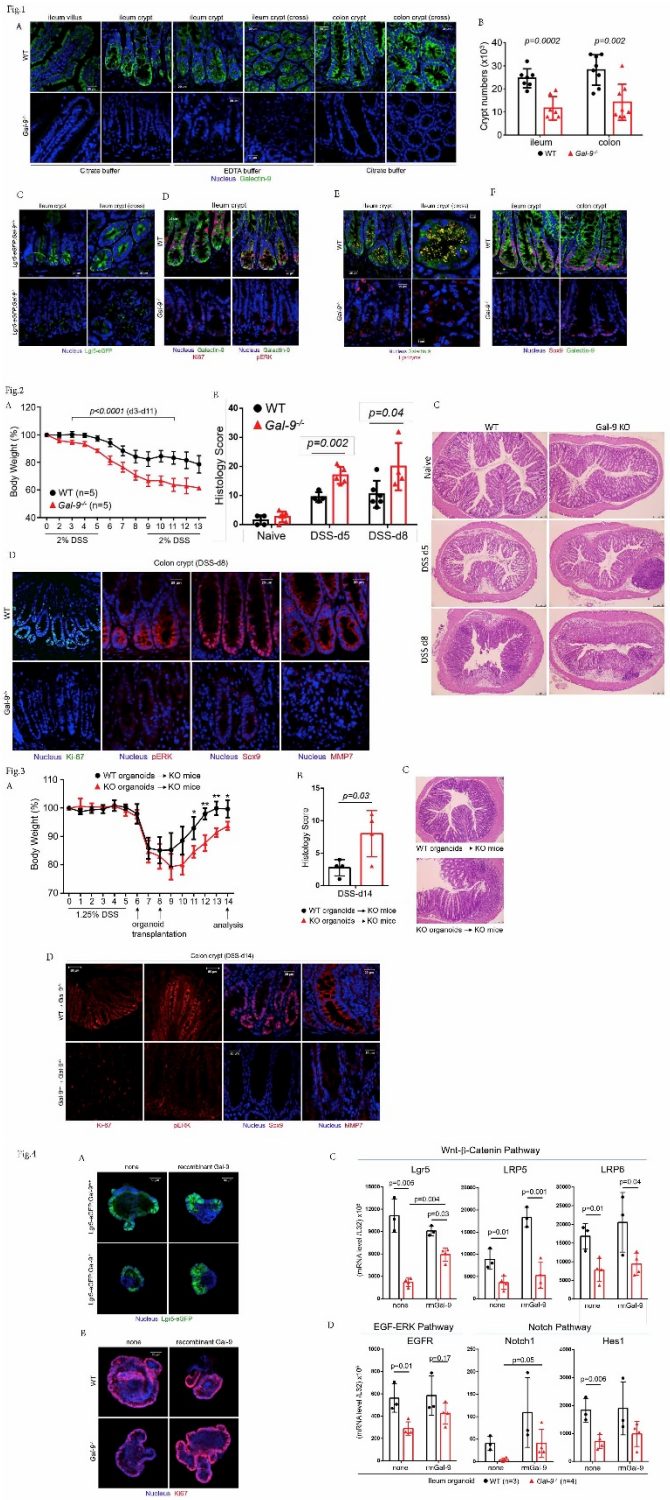
to hypoxia through the hypoxia-inducible transcription factor (HIF) system. During inflammation, IL-22 is a central cytokine in the modulation of tissue responses and is upregulated in IBD. IL-22 induces proliferative and anti-apoptotic pathways and has both protective and inflammatory roles in the inflamed colon. As such, understanding IL-22 expression is critical to rational design of IL-22-related therapeutics for IBD treatment. Although the function of IL-22 is well studied, less is known regarding its regulation in lymphocytes. As IL-22 can be dual-natured, we hypothesized that its biological activity should be tightly regulated in order to limit IL-22 to inflammatory sites. One such environmental cue could be low oxygen. We show that in CD4 T cells IL-22 expression is upregulated in hypoxia, and this is dependent on the transcription factor HIF-1α. This finding has implications on the regulation of *Il22* gene expression and the cytokine's presence in different inflammatory environments. Although there are established links between hypoxia and IBD and between IL-22 and IBD, there has been no systematic examination of links between hypoxia and IL- 22. This is a new direction for IBD research and will lead to new drug development as well as potential coopting of HIF-targeting drugs in cancer therapy pipelines.

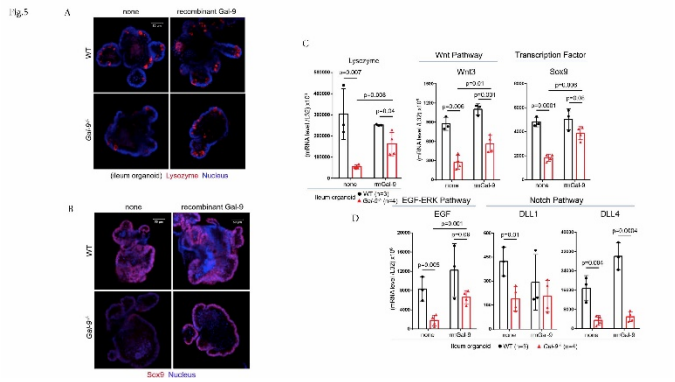
Epithelial Cells and Innate Immunity

OR.45, T38. Crohn's Disease Risk Gene *Galectin-9* Regulates Lgr5^{+} Stem Cell-Paneth Cell Homeostatic Niche

Janaki Narasimha Sudhakar, Hsueh-Han Lu, Ming-Che Liu, Fu-Tong Liu and Jr-Wen Shui. Academia Sinica, Taipei, Taipei, Taiwan

Intestinal epithelial barrier homeostasis results from neutral competition between self-renewing Lgr5^{+} stem cells, which are small cycling cells located at crypt bottoms, interspersed between terminally differentiated Paneth cells, known to produce secretory bactericidal products such as lysozyme and cryptdins. Lgr5^{+} stem cells intimately adhere to Paneth cells, which provide niche factor signals for stem cell maintenance, therefore any defect in Paneth cell homeostasis would ultimately affect the stem cells and causes inflammatory bowel disease (IBD). Intriguingly, many IBD risk genes, including *Nod2*, *Xbp1*, and *Atg16l1*, are associated with dysregulation of Paneth cell homeostasis. Here, we provide evidence that *galectin-9 (Lgals9)*, a glycan-binding lectin and a recently reported CD risk gene, is predominantly expressed in the cytosol of crypt cells and *Gal-9* deficiency in mice leads to decreased crypt numbers, compromised stem cell regeneration, impaired secretory granules formation in Paneth cells, decreased transcription factor Sox9 expression, and increased susceptibility to DSS-induced colitis, which could be ameliorated by transplantation of wild-type, but not *Gal-9*$^{-/-}$ colonic organoids. Using epithelial organoids, we found addition of recombinant Gal-9 into *Gal-9*$^{-/-}$ organoid culture could restore most of the defects associated with stem cells and Paneth cells. Both *in vivo* and *in vitro*, Gal-9 directly regulates development and function of Lgr5^{+} stem cells and Paneth cells *via* pathways involving EGF, Wnt and Notch signaling. These findings therefore provide evidence that Gal-9 is essential for maintaining Lgr5^{+} stem cell-Paneth cell niche and any polymorphism of *Gal-9* gene might contribute to dysfunction of epithelial barrier, leading to intestinal inflammation.





OR.46, T20. Mucus Secreted from Inter-Crypt Goblet Cells is Required for Proper Mucus Layer Formation in Colon and Protectin Against Colitits

Elisabeth EL Nyström, George MH Birchenough, Beatriz Martinez Abad, Liisa Arike and Malin EV Johansson. University of Gothenburg, Gothenburg, Vastra Gotaland, Sweden

The colonic epithelium is protected against bacterial infection by a mucus layer covering the epithelial surface. Homeostasis of the mucus layer is maintained by a combination of mucus production, secretion and removal, and disruption of homeostasis is coupled to diseases such as colitis. Goblet cells (GCs) in the epithelium are responsible for the production and secretion of mucus and it has previously been shown that GCs have different properties dependent on their crypt axis position e.g. different mucus turnover rate and different abilities to endocytose and react to bacterial compounds. These studies indicate that GCs differentiate into discrete, functionally different subpopulations. The differentiation of GCs is partly determined by the transcription factor SPDEF. By morphological examination of colonic GCs in Spdef-deficient mice we found that lack of Spdef mainly affect the terminally differentiated GCs found in the inter-crypt regions at the mucosal surface. Investigation of the mucus phenotype in wild-type (WT) and Spdef-deficient mice using a novel *ex vivo* imaging method to study the mucus structure revealed a coherent mucus layer held together by mucus secreted from these inter-crypt GCs in WT that was largely absent in Spdef-deficient mice. The importance of a functional mucus layer was further demonstrated as the Spdef-deficient mice developed a more severe DSS-induced colitis and also developed spontaneous colitis over time. Taken together, this study provides new insight to the role of different GC subpopulations and the unique role for the inter-crypt surface GCs in maintaining functional protection of the epithelium.

OR.47, T37. Role of HDAC in Intestinal Barrier Integrity and Epithelial Regeneration in Inflammatory Bowel Disease

Marie Friedrich, Lorenz Gerbeth, Julia Ganther, Tilman Breiderhoff, Rita Rosenthal, Britta Siegmund and Rainer Glaube. Charité - Universitätsmedizin Berlin, Berlin, Germany

The integrity of intestinal mucosal barrier function is crucial in maintaining gut homeostasis as well as contributing to the pathophysiology of inflammatory bowel diseases (IBD). In particular, the ability of regeneration in inflamed and/or damaged epithelial tissue represents a key feature barrier integrity, while excessive proliferation and migration increases the risk of tumorigenesis. As pan-inhibition of histone deacetylases (HDAC) ameliorates experimental colitis and colitis associated tumorigenesis, HDAC moved into our focus as promising targets for diagnosis and therapy. While expression analysis of primary colonic epithelial cells revealed an overall reduction of HDAC in IBD, epithelial cell lines treated with the pan-HDAC inhibitor Givinostat demonstrated enhanced cell migration, increased secretion of regenerating IL-8. An enhanced barrier function through HDAC inhibitor treatment was demonstrated *in vitro* as well as in murine colitis models via measuring trans-epithelial electrical resistance (TER) and FITC-Dextran flux. On the other hand, functional analysis of single HDAC KO cells displayed divergent effects as shown by a reduced TER as well as delayed cell migration during wound healing assay in CRISPR/Cas9 HDAC7 KO cells. These effects were accompanied by a reduced expression of cell adhesion and migration molecules as shown by RNA sequencing. Our results indicate an integrated role of HDAC in the maintenance of the intestinal barrier and further point to a regulatory function of HDAC7 in the development of the intestinal epithelial barrier and the inflammatory response of colonic epithelial cells.

OR.48, T13. IL-33/ST2 Macrophage Crosstalk Promotes Epithelial Repair During Lung Injury

Rania Dagher[1], Alan M. Copenhaver[1], Valerie Besnard[2], Marielle Maret[3], Fatima Hamidi[4], Aron Berlin[1], Michel Aubier[5], Roland Kolbeck[1], Alison A. Humbles[1] and Marina Pretolani[4]. [1]MedImmmune - Respiratory, Inflammation & Autoimmunity, Gaithersburg, MD; [2]Université Paris, France [3] IRCAN Inserm UMR1081-CNRS 7284 UNSA, Universite de Nice, Nice, France; [4] Inserm UMR1152, Paris, Ile-de-France, France; [5] Hôpital Bichat-Claude Bernard, Paris, Ile-de-France, France

Increasing evidence points to a central role for macrophages in tissue regeneration, however, the molecular mechanisms underlying this function remain unknown. Here, using depletion and adoptive transfer approaches, we demonstrate a major role of alveolar macrophages in facilitating bronchial re-epithelialization. In addition, we identify a novel, and paradoxical, protective role for the IL-33/ST2 axis in epithelial repair following naphthalene (NA)-induced lung injury. We defined two subsets of ST2-expressing myeloid cells, namely recruited monocyte-derived cells and resident alternatively activated macrophages (AAMs), which infiltrate the airways during the regeneration of the bronchial epithelium. Further, ST2-deficient mice exhibited an incomplete epithelial repair which was associated with a dysfunctional AAM phenotype and a reduction in the proliferation of a self-renewing subset of Clara progenitor stem cells. Notably, reconstitution of $ST2^{+}$ AAMs, post NA, completely restored the epithelium in ST2-deficient animals. In contrast, the monocyte/macrophage-dependent CCL2/CCR2 axis was redundant for repair, however, anti ST2-antibody treatment to $CCR2^{-/-}$ mice resulted in ineffective epithelial regeneration and confirmed the observations seen with ST2-deficiency after NA-induced injury. Thus, the IL-33/ST2 pathway regulates effective Clara cell regeneration and bronchial re-epithelialization via AAM polarization and may lead to new therapeutic insights in acute respiratory diseases.

OR.49, T09. Secretory IgA Deficiency in Individual Small Airways in COPD Correlates with Local Activation of Innate Immunity

Vasiliy V. Polosukhin, Bradley W. Richmond and Timothy S. Blackwell. Vanderbilt University Medical Center, Nashville, TN

Persistent activation of innate immunity plays a critical role in the pathogenesis of chronic obstructive pulmonary disease (COPD). To investigate the role of secretory IgA (SIgA) deficiency in COPD, we analyzed small airways from 50 former smokers with COPD and 39 non-diseased controls. Based on measurement of IgA-immunospecific fluorescence on airway mucosal surface, we found an increased proportion of SIgA-deficient airways in COPD patients (< 5% airways in non-diseased controls vs. 47% airways in patients with mild/moderate COPD and 71% airways in patients with severe COPD). SIgA-deficient airways were characterized by thickened walls, accumulation of neutrophils, increased susceptibility to bacterial colonization, and NF-κB pathway activation in airway epithelial cells. Polyimmunoglobulin receptor (pIgR)-deficient mice, which are unable to produce SIgA, showed spontaneous development of COPD-like pathology in association with bacterial invasion into airway epithelium and persistent NF-κB activation. Treatment of pIgR-deficient mice with an antibiotic cocktail (Vancomycin, Neomycin, Ampicillin, and Metronidazole; VNAM) between 9 and 12 months of age prevented neutrophilic inflammation and COPD-like lung remodeling. Similarly, neutrophil depletion with anti-Ly6G antibodies abrogated COPD-like remodeling in pIgR-deficient mice. Together, our findings support the concept that loss of the mucosal immune barrier in small airways and persistent activation of innate immune response contribute to COPD progression. We propose that localized impairment of the mucosal immune barrier in small airways due to surface SIgA deficiency leads to impaired host defense and results in disproportional activation of innate immune responses to lung microbiota, thus promoting COPD pathology.

OR.78, T24. TGFBR1M318R$^{+/-}$ Knock-In Mice: A Monogenic Presentation of Spontaneous Eosinophilic Esophagitis

Karen Laky[1], Jessica Kinard[1,2], Anthony Guerrerio[3] and Pamela Frischmeyer-Guerrerio[1,2]. [1]National Institutes of Health, Bethesda, MD; [2] National Institute of Allergy and Infectious Diseases, DHHS, Bethesda, MD; [3] Johns Hopkins University School of Medicine, Baltimore, MD

Loeys-Dietz syndrome (LDS) is an autosomal dominant disorder caused by mutation of genes encoding proteins in the transforming growth factor beta (TGFb) signaling pathway. Patients heterozygous for a point mutation in the kinase domain of TGFβR1 (TGFBR1M318R) exhibit classic manifestations of LDS. Mice with this mutation were made in the hope that an animal model of LDS will lead to better understanding of the mechanisms underlying LDS symptoms. TGFBR1M318R$^{+/-}$ mice exhibit the hallmark skeletal, dental, pulmonary, and cardiovascular phenotypes of LDS. We sought to determine if TGFBR1M318R$^{+/-}$ mice also recapitulate the immune phenotypes characteristic of LDS. We find that like their human counterparts, TGFBR1M318R$^{+/-}$ mice spontaneously develop elevated serum IgE and eosinophilic esophagitis (EoE), characterized by esophageal dilation, food impaction, and increased infiltration of eosinophils, T cells, mast cells, and antigen presenting cells. Bone marrow (BM) chimeras were made to determine which cells with altered TGFβ signaling contribute to EoE. Lethally irradiated TGFBR1M318R$^{+/-}$ mice reconstituted with WT BM had EoE 8wk post-reconstitution, indicating that aberrant TGFβ signaling in radio-resistant cells was sufficient to cause EoE. In contrast, eosinophils and IgE were not elevated in WT mice reconstituted with TGFBR1M318R$^{+/-}$ BM. To confirm that EoE can develop in the absence of lymphocytes, we generated RAG2$^{-/-}$ TGFBR1M318R$^{+/-}$ mice and found that they too developed EoE. We looked for an alternate source of Type 2 cytokines and discovered that innate lymphoid cells (ILC) were increased in the esophagus of TGFBR1M318R$^{+/-}$ mice. Thus, we propose that altered TGFβ signaling in epithelial cells leads to EoE by stimulating ILC to proliferate and secrete cytokines that result in accumulation of eosinophils.

OR.94, F26. Retinoic Acid Receptor Alpha in Intestinal Epithelial Cells Controls Global Immunological Fitness

Humberto Jijon[1], Lucia Suarez-Lopez[2], Jaime De Calisto[3], Oscar Diaz[4], Michael Yaffe[2], J. Rodrigo Mora[5], Mikael Pittet[5], Yasmine Belkaid[6], Ramnik Xavier[7] and Eduardo J. Villablanca[4]. [1]University of Calgary, Calgary, Canada; [2] Massachusetts Institute of Technology, Cambridge, MA; [3] Universidad Mayor, Santiago, Region Metropolitana, Chile; [4] Karolinska Institutet, Stockholm, Stockholms Lan, Sweden; [5] Massachusetts General Hospital, Boston, MA; [6] National Institutes of Health, Bethesda, MD; [7] Broad Institute, Cambridge, MA

The Vitamin A metabolite, retinoic acid (RA), is crucial in maintaining intestinal homeostasis. RA acts on differenet intestinal immune cells such as, dendritic cells and lymphocytes, to modulate their lineage commitment and function. Although the role of RA has been characterized in immune cells, whether intestinal epithelial cells (IECs) sense RA to exert their immune-regulatory function has not been examined. Here we demonstrate that lack of retinoic acid receptor alpha (RARα) signaling in IECs results in deregulated epithelial lineage specification, leading to increased numbers of Paneth cells and Goblet cells. These changes are associated with reduced bacterial detection in the lumen, decreased AMP expression and a less developed intestinal immune system, as evidenced by an almost complete absence of gut resident mononuclear phagocytes and lymphoid follicles. This underdeveloped intestinal immune system shows a decreased ability to clear infection with *Citrobacter rodentium*. Collectively, our findings indicate that epithelial cell-intrinsic RARα signaling is critical to the global development of the intestinal immune system.

OR.95, F25. Epithelial Histone Deacetylase 3 Instructs Intestinal Immunity by Coordinating Local Lymphocyte Activation

Theresa Alenghat. Cincinnati Children's Hospital, Cincinnati, OH

Mucosal tissues require specialized fortification against pathogenic infection, however the cellular and molecular mechanisms that sense and instruct tissue-intrinsic innate immunity remain poorly understood. Here, we demonstrate that mammalian intestinal epithelial cell (IEC) histone deacetylase (HDAC) activity was induced by the enteric pathogen *Citrobacter rodentium*. Mice lacking the class I HDAC, HDAC3, in IECs (HDAC3$^{\Delta IEC}$ mice) were more susceptible to *C. rodentium* infection and this impaired host defense reflected significantly decreased IFNγ production by local intraepithelial CD8^{+} T cells. Further, HDAC3 was necessary for infection-induced epithelial expression of IL-18 and administration of IL-18 restored effector function to resident CD8^{+} T cells and reduced infection in HDAC3$^{\Delta IEC}$ mice. HDAC3 expression was not required in the absence of the microbiota to control *C. rodentium*, indicating that HDAC3-mediated antibacterial immunity is microbiota-dependent. Collectively, these results uncover that an epithelial HDAC senses infection and instructs cytokine-mediated interactions between IECs and resident lymphocytes. This discovery reveals a previously unrecognized level of regulation by which a host epigenetic modifier functionally primes mammalian cells to coordinate local innate protection against mucosal infection.

OR.96, F27. The Cytosolic Sensor STING is Required for Intestinal Homeostasis and Control of Inflammation

Maria Cecilia Campos Canesso[1], Luisa Lemos[1], Thalison Costa Neves[1], Fernanda Martins Marim[1], Émerson Soares Veloso[1], Camila Pereira de Queiroz[1], Helton da Costa Santiago[1], Flaviano dos Santos Martins[1], Juliana Alvez Silva[1], Enio Ferreira[1], Denise Carmona Cara[1], Glen Barber[2], Sergio Costa Oliveira[1] and Ana Maria C. Faria[1]. [1] Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; [2]University of Miami Miller School of Medicine, Miami, FL

STING (stimulator of interferon genes) is a cytosolic sensor for cyclic dinucleotides and also an adaptor molecule for intracellular DNA receptors. Although STING has important functions in the host defense against pathogens and in autoimmune diseases, its physiological relevance in intestinal homeostasis is largely unknown. In this study, we show that STING$^{-/-}$ mice presented defects in the protective mechanisms of intestinal mucosa including decreased number of goblet cells, diminished mucus production and lower levels of SIgA when compared with WT mice. We also observed that absence of STING lead to increase in ILC1 as well as ILC3 frequencies and decrease in ILC2 frequencies in the colon. Fecal content was able to activate STING, indicating a physiological trigger for this molecule in the gut. Development and function of Foxp3^{+} and LAP^{+} regulatory T cells were also compromised in STING$^{-/-}$ mice. Moreover, these mice were highly susceptible to DSS-induced colitis, showing increased mortality and morbidity when compared to WT animals. Interestingly, we observed a similar phenotype in DSS-treated IFNAR KO mice, suggesting that type I IFNs participate in STING-dependent control of gut homeostasis. Therefore, our results identify an important role of STING in maintaining gut homeostasis and a protective effect in experimental colitis.

Figure 1

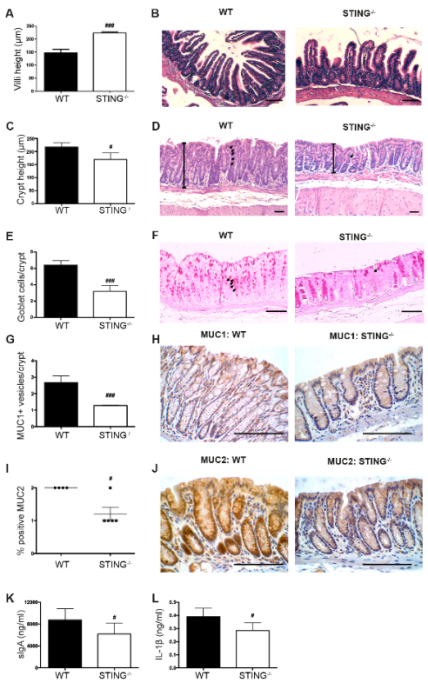


Figure 1: Alterations in mucosa structure in STING-/- mice. Small intestine and colon of WT and STING-/- mice were collected for analyses of (a) Villi height, (c) crypt height and (e) number of goblet cells per crypt. Representative photomicrographies of H&E-stained (b) ileum sections and (d) colon sections from WT and STING-/- mice evidencing crypt height. Representative photomicrographies of PAS-stained (f) colon sections from WT and STING-/- mice evidencing goblet cells (arrows). Quantification of MUC1 (g) and MUC2 (i) in colons of WT and STING-/- mice. Representative photomicrographies of MUC1 (h) and MUC2 (j) immunohistochemistry colon sections. (k) secretory IgA levels in feces and (l) IL-1 β levels in colons of WT and STING-/- mice. Bars represent 100um. Data represent two independent experiments with five mice/group. Data represent the mean +- SEM. #p < 0.05; ###p < 0.001.

Figure 2

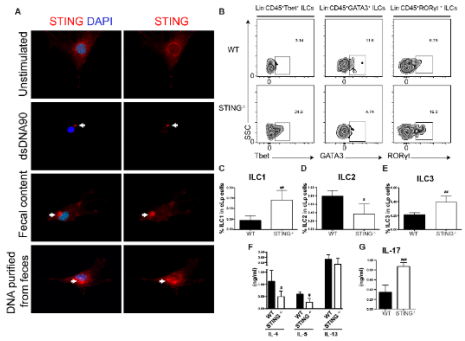


Figure 2: Activation of STING by commensals and altered frequency of immune cells in colon lamina propria of WT and STING-/- mice. (a) Confocal microscopy of WT MEFs stained with anti-STING and DAPI, unstimulated or stimulated for 4h with STING-activating dsDNA (dsDNA90 base pairs), fecal content or DNA purified from feces from WT mice. MEFs from STING-/- mice did not stain with anti-STING (data not shown). Arrows highlight STING punctual aggregation. (b) Representative plots displaying frequencies of ILC1 (Lin-CD45+CD127+Tbet+), ILC2 (Lin-CD45+CD127+GATA3+) and ILC3 (Lin-CD45+CD127+RORγt+) in colon lamina propria of WT and STING-/- mice. Frequencies of (c) ILC1, (d) ILC2 and (e) ILC3 cells in colon lamina propria of WT and STING-/- mice. Data are representative of six mice/group. Levels of (f) IL-4, IL-5 and IL-13 and (g) IL-17 cytokines in colon extract of WT and STING-/- mice. Data represent two independent experiments with three to five mice/group. Data represent the mean +- SEM. #p < 0.05; ##p < 0.01; ###p < 0.001.

Figure 3

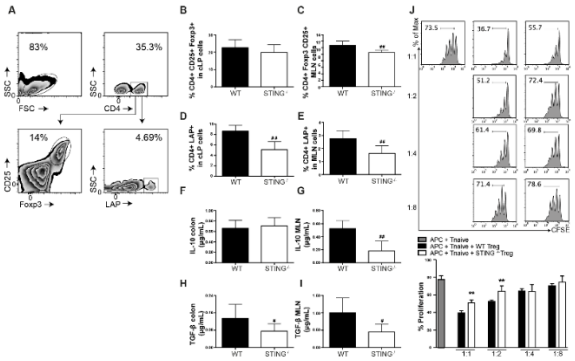


Figure 3: Frequency of Treg cells in colon lamina propria and MLN of WT and STING-/- mice. (a) Representative plots of gating strategy for Treg CD4+CD25+Foxp3+ and Treg CD4+LAP+ cells. (b) Frequency of Treg CD4+CD25+Foxp3+ in colon lamina propria and (c) MLN of WT and STING-/- mice. (d) Frequency of Treg CD4+LAP+ in colon lamina propria and (e) MLN of WT and STING-/- mice. (f) Levels of IL-10 and (h) TGF-β cytokines in colon extract of WT and STING-/- mice. (g) Levels of IL-10 and (i) TGF-β in MLN culture of cells stimulated with anti-CD3 and anti-CD28 for 48h and 72h, respectively. (j) CD4+CD62L+CD44low or CD4+CD25+ cells were purified by FACS sorting from spleen of 8 weeks WT and STING-/- mice, and co-cultured with APCs and anti-CD3 stimuli for 3d at different proportions. Data represent two independent experiments with three to five mice/group. Data represent the mean +- SEM. #p < 0.05; ##p < 0.01.

Figure 4

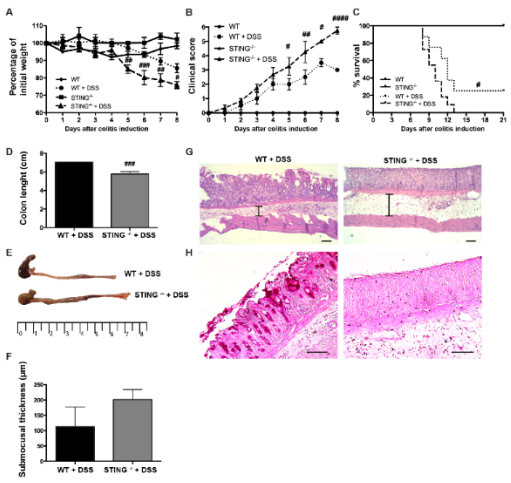


Figure 4: STING-/- mice are highly susceptible to DSS-induced colitis. WT and STING-/- mice were given 3% DSS in drinking water for 8d. (a) Weight change following DSS administration, monitored everyday. (b) Clinical score of WT and STING-/- mice during DSS treatment, where a higher score corresponds to increased pathology. (c) Survival of WT and STING-/- mice. Mice were given 3% DSS for 8d, followed by normal drinking water for another 13d, monitored everyday. (d) Quantification of the colon length described in E. (e) Colons from WT and STING-/- mice on day 8. Representative photomicrographies of H&E-stained (g) and PAS-stained (h) colon sections from WT and STING-/- mice evidencing submucosal thickness and goblet cells. Bars represent 100um. Data represent four independent experiments with five to eight mice/group. Data represent the mean +- SEM. #p < 0.05; ##p < 0.01; ###p < 0.001; ####p < 0.0001.

Figure 5

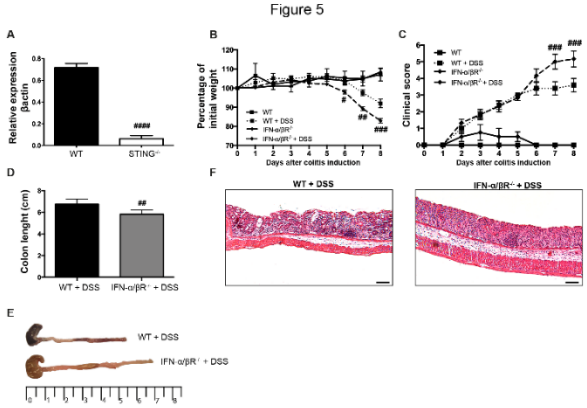


Figure 5: IFN-α/βR-/- mice are also susceptible to DSS-induced colitis. (a) qRT-PCR analysis of IFN-β mRNA in colons from WT or STING-/- mice. Data represent two independent experiments with four mice/group. WT and IFN-α/βR-/- mice were given 3% DSS in drinking water for 8d. (b) Weight change following DSS administration, monitored everyday. (c) Clinical score of WT and IFN-α/βR-/- mice during DSS treatment, where a higher score corresponds to increased pathology. (d) Quantification of the colon length described in E. (e) Colons from WT and IFN-α/βR-/- mice on day 8. Data are representative of six mice/group. Data represent the mean +- SEM. #p < 0.05; ##p < 0.01; ###p < 0.001; ####p < 0.0001.

OR.97, F23. Crohn's Disease Risk Gene, *Galectin-9* is Critical for Relieving Epithelial ER Stress Which Prevents Intestinal inflammation

Hsueh-Han Lu, Sudhakar Janaki, Ming-Che Liu, Fu-Tong Liu and Jr-Wen Shui. Institute of Biomedical Sciences, Academia Sinica, Taipei, Taipei, Taiwan

Glycan and gkycan-binding protein (i.e. galectins) is known to be essential components of ER-mediated autophagy and apoptosis machinery. In the intestine, Paneth cells and ER stress play a key role in inflammation associated with Crohn's disease. Characteristic features of Paneth-cells are their active secretion of anti-microbial products and niche support for neighboring Lgr5^{+}stem cells at the crypt base. Intriguingly, both Paneth-cells and Lgr5^{+}stem cells belong to those cell types which are likely more susceptible to ER stress. We therefore explore the possibility whether Crohn's disease risk gene galectin-9 is involved in homeostasis of these two cell types as well as has a role in inflammation and ER stress. We first determined Gal-9 is predominantly expressed in intestinal crypts, where stem cells and anti-microbial Paneth-cells are located. Further analysis indicated Gal-9 deficiency led to reduced crypt numbers (i.e. stem cell defect) at steady state. Also, Gal-9$^{-/-}$crypts showed impaired anti-bacterial ability (i.e. Paneth cell defect). Next, we found after dextran-sulfate-sodium(DSS) treatment, Gal-9$^{-/-}$mice showed 100% mortality, accompanied with more severe inflammation and UPR response. Furthermore, the inflammatory condition and ER stress of DSS treated Gal-9$^{-/-}$mice can be ameliorated by transplantation with WT organoid which is consistent with our *in vitro* findings that defective function of Gal-9$^{-/-}$organoid

could be restored by supplying recombinant galectin-9. Together, we have revealed a role for Gal-9 in ER stress which affects mutual homeostasis between Lgr5^{+}stem cells and Paneth-cells. Our findings therefore provide insights as to whether Gal-9 could be a potential target for treating intestinal inflammation associated with ER stress.

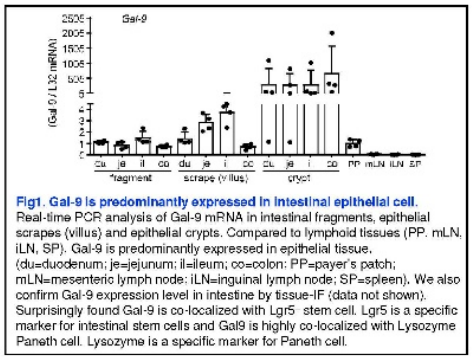


Fig1. Gal-9 is predominantly expressed in intestinal epithelial cell.
Real-time PCR analysis of Gal-9 mRNA in intestinal fragments, epithelial scrapes (villus) and epithelial crypts. Compared to lymphoid tissues (PP, mLN, iLN, SP). Gal-9 is predominantly expressed in epithelial tissue. (du=duodenum; je=jejunum; il=ileum; co=colon; PP=payer's patch; mLN=mesenteric lymph node; iLN=inguinal lymph node; SP=spleen). We also confirm Gal-9 expression level in intestine by tissue-IF (data not shown). Surprisingly found Gal-9 is co-localized with Lgr5^{+} stem cell. Lgr5 is a specific marker for intestinal stem cells and Gal9 is highly co-localized with Lysozyme Paneth cell. Lysozyme is a specific marker for Paneth cell.

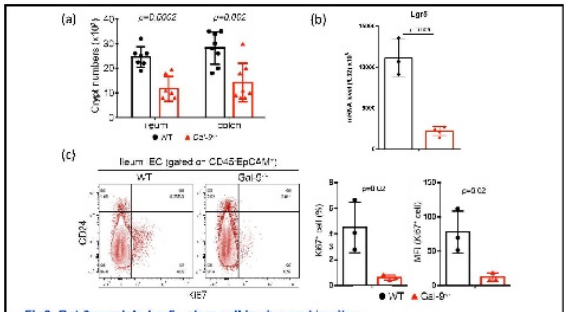


Fig2. Gal-9 regulate Lgr5+ stem cell in vivo and in vitro.
(a) Gal-9 deficiency in vivo leads to reduced number of crypts, where epithelial stem cell and anti-microbial Paneth cells are located. Crypts freshly isolated from WT or Gal-9$^{-/-}$ mice were manually counted under microscope and compared.
(b) Gal-9 deficiency leads to decreased Lgr5 expression level in organoid. Lgr5 is a specific marker for intestinal stem cells. Phenotype also confirmed by IF staining that KO (Lgr5-eGFP; Gal-9$^{-/-}$) mice showed reduced numbers of Lgr5^{+} stem cell in both ileum and colon, compared to WT (Lgr5-eGFP) mice (data not shown).
(c) Gal-9 deficiency leads to decreased Ki67^{+} proliferating intestinal epithelial cell (IEC, gated on CD45^{-} EpCAM^{+}), which were mostly shown in CD24^{low} (known to contain Lgr5^{+} stem cell), but not in CD24^{+high} (know to contain Paneth cell) populations. Edu were intraperitoneally administrated into mice and crypts were isolated 3hr later.

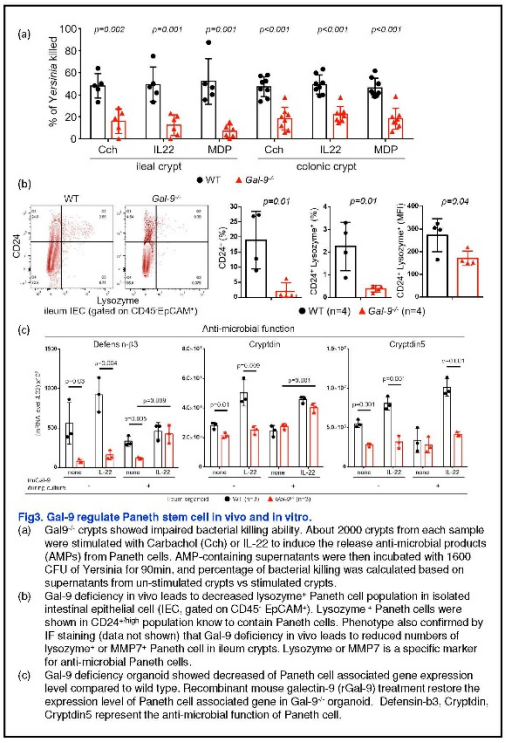


Fig3. Gal-9 regulate Paneth stem cell in vivo and in vitro.
(a) Gal9$^{-/-}$ crypts showed impaired bacterial killing ability. About 2000 crypts from each sample were stimulated with Carbachol (Cch) or IL-22 to induce the release anti-microbial products (AMPs) from Paneth cells. AMP-containing supernatants were then incubated with 1600 CFU of Yersinia for 90min, and percentage of bacterial killing was calculated based on supernatants from un-stimulated crypts vs stimulated crypts.
(b) Gal-9 deficiency in vivo leads to decreased lysozyme^{+} Paneth cell population in isolated intestinal epithelial cell (IEC, gated on CD45^{-} EpCAM^{+}). Lysozyme^{+} Paneth cells were shown in CD24^{+high} population know to contain Paneth cells. Phenotype also confirmed by IF staining (data not shown) that Gal-9 deficiency in vivo leads to reduced numbers of lysozyme^{+} or MMP7^{+} Paneth cell in ileum crypts. Lysozyme or MMP7 is a specific marker for anti-microbial Paneth cells.
(c) Gal-9 deficiency organoid showed decreased of Paneth cell associated gene expression level compared to wild type. Recombinant mouse galectin-9 (rGal-9) treatment restore the expression level of Paneth cell associated gene in Gal-9$^{-/-}$ organoid. Defensin-b3, Cryptdin, Cryptdin5 represent the anti-microbial function of Paneth cell.

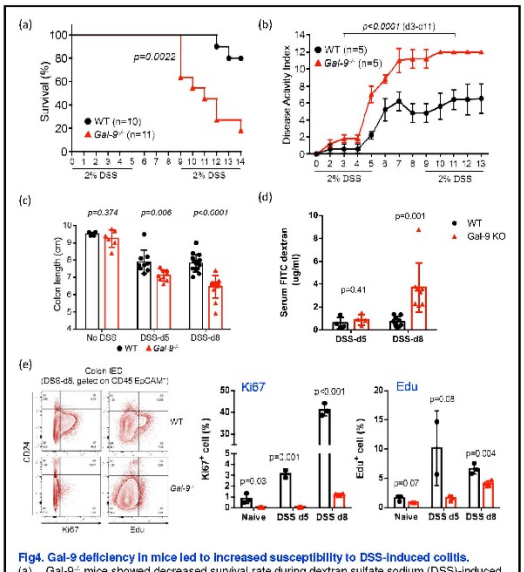


Fig4. Gal-9 deficiency in mice led to increased susceptibility to DSS-induced colitis.
(a) Gal-9$^{-/-}$ mice showed decreased survival rate during dextran sulfate sodium (DSS)-induced colitis.
(b) Gal-9$^{-/-}$ mice showed increased disease activity index (weight loss, rectal bleeding and stool consistency) during DSS colitis.
(c) Gal-9$^{-/-}$ mice showed shortened colon length, indicating more severe inflammation.
(d) Gal-9$^{-/-}$ mice showed increased barrier permeability during colitis. FITC-dextran was orally administrated into mice and serum was collected 6hr later to determine FITC-dextran level. Higher serum level of FITC-dextran indicates more tissue damage in the gut resulting in increased barrier permeability.
(e) During DSS induced colitis, Gal-9 deficiency leads to decreased Ki67^{+} or Edu^{+} proliferating intestinal epithelial cell (IEC, gated on CD45^{-} EpCAM^{+}), which were mostly shown in CD24^{low} (known to contain Lgr5^{+} stem cell), but not in CD24^{+high} (know to contain Paneth cell) populations. Edu were intraperitoneally administrated into mice and crypts were isolated 3hr later.

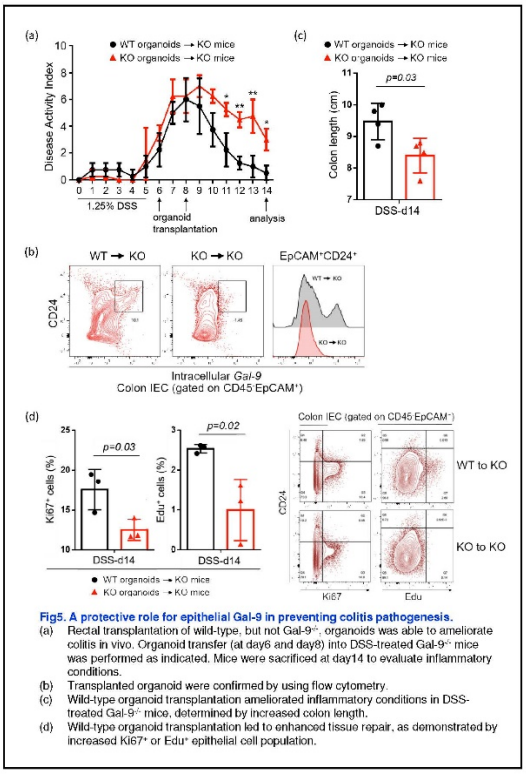


Fig5. A protective role for epithelial Gal-9 in preventing colitis pathogenesis.
(a) Rectal transplantation of wild-type, but not Gal-9$^{-/-}$, organoids was able to ameliorate colitis in vivo. Organoid transfer (at day6 and day8) into DSS-treated Gal-9$^{-/-}$ mice was performed as indicated. Mice were sacrificed at day14 to evaluate inflammatory conditions.
(b) Transplanted organoid were confirmed by using flow cytometry.
(c) Wild-type organoid transplantation ameliorated inflammatory conditions in DSS-treated Gal-9$^{-/-}$ mice, determined by increased colon length.
(d) Wild-type organoid transplantation led to enhanced tissue repair, as demonstrated by increased Ki67^{+} or Edu^{+} epithelial cell population.

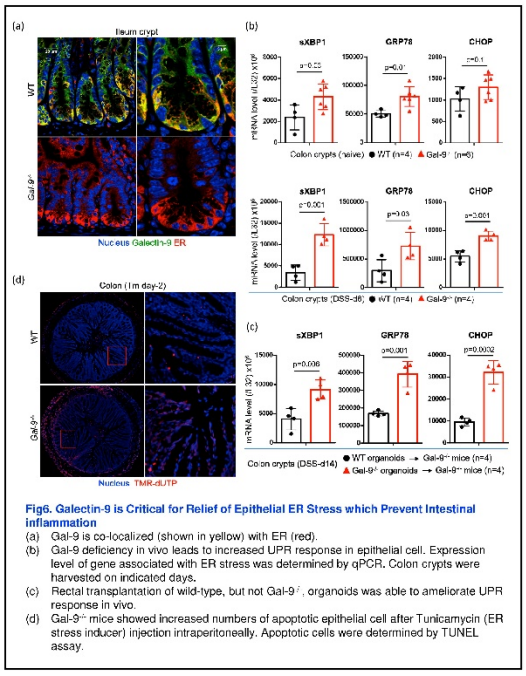


Fig6. Galectin-9 is Critical for Relief of Epithelial ER Stress which Prevent Intestinal inflammation
(a) Gal-9 is co-localized (shown in yellow) with ER (red).
(b) Gal-9 deficiency in vivo leads to increased UPR response in epithelial cell. Expression level of gene associated with ER stress was determined by qPCR. Colon crypts were harvested on indicated days.
(c) Rectal transplantation of wild-type, but not Gal-9$^{-/-}$, organoids was able to ameliorate UPR response in vivo.
(d) Gal-9$^{-/-}$ mice showed increased numbers of apoptotic epithelial cell after Tunicamycin (ER stress inducer) injection intraperitoneally. Apoptotic cells were determined by TUNEL assay.

OR.98, F24. Daxx Maintains Intestinal Homeostasis by Protecting the Epithelium against Necrosis

Ming-Che Liu, Sudhakar Janaki, Hsiu-Ming Shih, Hsueh-Han Lu, Yen-Chun Liu and Jr-Wen Shui. Institute of Biomedical Sciences, Academia Sinica, Taipei, Taipei, Taiwan

The components of Fas pathway, such as Fas-associated death domain (FADD), receptor interacting protein kinase 1 (RIPK1), and RIPK3, are important in IECs survival and epithelium-mediated inflammation. Death domain-associated protein 6 (Daxx) is a multifunctional nuclear protein which belongs to Fas pathway and regulates a wide range of biological processes, including epithelial cell death and gene transcription. The function of Daxx in intestinal inflammation remains unclear. Here, we show that Daxx is highly expressed in the intestine and IECs. Mice with IEC-specific ablation of Daxx (Daxx^{IEC-KO}) have colonic lymphocyte infiltration, indicating that Daxx^{IEC-KO} mice spontaneously develop chronic colitis. Further, Daxx^{IEC-KO} mice have lethal inflammatory response, increased apoptotic IECs, shorter colon length, severe colon damage, and compromised stem cell regeneration or tissue repair during dextran sulfate sodium (DSS)-induced colitis. The DSS-induced colitis phenotype of Daxx^{IEC-KO} mice can be rescued by transplantation of wild-type (WT) organoids, but not Daxx$^{-/-}$ organoids, indicating it is epithelial Daxx that contributes to inflammatory pathology. Necrostatin-1(Nec-1) is a necrosis inhibitor which blocks RIPK1 activity to protect cell necrosis. We found that Nec-1, but not apoptosis inhibitor, can restore DSS-induced colitis in Daxx^{IEC-KO} mice and Daxx$^{-/-}$ organoids were more susceptible to TNFα-induced cell death, suggesting that Daxx may interact with RIPK1 to control IEC necrosis or proliferation through TNFα signaling pathway during inflammation. Therefore, Daxx prevents intestinal inflammation by inhibiting RIPK1 kinase activity-mediated IEC necrosis, suggesting that RIPK1 inhibitors could be effective in the treatment of colitis in patients with Daxx mutations and possibly in IBD.

T08. IL-17 Promotes Intestinal Epithelial Homeostasis: Functional Use of Intestinal 3D Organoids and Implications for Potential IBD Therapies

Kristie L. Hilliard, Yanlin Jia, Michael Salmon, Milenko Cicmil, Ilona Kariv, George Addona and Hongmin Chen. Merck, Boston, MA

Inflammatory bowel disease (IBD) is characterized by chronic intestinal inflammation and epithelial injury, caused by genetic and environmental factors. GWAS studies have identified polymorphisms in genes associated with IBD along the Th17 pathway. Expression of IL-17, an inflammatory cytokine produced largely by Th17 cells, strongly correlates with disease severity in ulcerative colitis patients. IBD patients display elevated levels of IL-17 in serum and inflamed mucosa, indicating a potential pathogenic role of IL-17 in IBD. Therapeutic neutralization of IL-17A, however, exacerbated inflammation in Crohn's disease patients, prompting questions about its role in the disease mechanism. To understand IL-17's involvement in intestinal epithelial homeostasis, we utilized our 3D intestinal organoid model. Culturing of intestinal crypts in 3D matrix induces organoid growth, mimicking human intestine crypts with tight junctions, spheroid structure, and differentiated epithelial cells. We confirmed increased mucus production and expression of epithelial cell type markers following our differentiation protocol. After stimulation of colon organoids with rhIL-17A, we observed a significant increase in human beta-defensin 2 (HBD2) expression and significant epithelial tight junction-associated gene changes. Treatment with IL-17A/R neutralizing antibodies and TAK1 inhibitors inhibited expression of HBD2 suggesting that HBD2 expression was dependent on IL-17 signaling and TAK1 function. Lamina propria lymphocytes secreted increased IL-17A concentrations after PMA/Ionomycin stimulation, suggesting a local source for IL-17 in the gut. Taken together, these data suggest that the intestinal epithelium relies on gut IL-17 signaling to induce antimicrobial peptides and preserve the epithelial barrier during inflammation.

T10. Different Tissue Phagocytes Sample Apoptotic Cells to Direct Distinct Homeostasis Programs

Ryan Barbet[1], Gaetan Barbet[2], Sergio Lira[1] and Julie Blander[2,3]. [1]ICAHN School of Medicine at Mount Sinai, New York, NY; [2]Weill Cornell Medical College, New York, NY; [3]The Jill Roberts Institute for Research in Inflammatory Bowel Disease, New York, NY

Despite the fact that death of intestinal epithelial cells (IEC) constitutes an important part of the physiology of the intestine, little is known about how IEC apoptosis influences the regulatory and inflammatory processes within the intestine. Importantly, excessive IEC death is a hallmark of inflammatory bowel diseases (IBD) and has been proposed to constitute a pathogenic mechanism driving Crohn's disease. In this project, we developed and published a new mouse model to characterize the mononuclear phagocytes (MP) involved in the clearance of IECs. In this mouse model the IECs express a fusion protein, the diphtheria toxin receptor (DTR) coupled to enhanced green fluorescent protein (eGFP), controlled by the villin promoter. Therefore, induction of IEC death by injecting diphtheria toxin allowed us to track and characterize the MP populations responsible for the engulfment of apoptotic $eGFP^+$ IEC. We precisely determined the identity of two subsets of macrophages and one of dendritic cells that phagocytose apoptotic IEC during turnover of the intestinal epithelium in the absence of inflammation. We characterized the unique transcriptional profiles of apoptotic IEC-sampling MPs, showing that they all shared a common "suppression of inflammation" signature. Furthermore, several of the genes differentially expressed by phagocytes bearing apoptotic IEC overlapped with susceptibility genes for IBD. We also discovered that only dendritic cells carrying apoptotic IECs were able to induce T cell differentiation into regulatory T cells. Those findings provide new insights into the consequences of apoptotic cell sampling and set the stage for the development of novel therapeutics.

T11. Epithelial iNOS Induction by IL22 Depends on IL22RA1 Activation And STAT Phosphorylation In A Murine Model of Colitis-Associated Cancer

Guanyu Gong[1], Hilda Holcombe[1], Evan Conaway[2], Erin Bryant[1], Sureshkumar Muthupalani[1], Dylan Puglisi[1], Vasudevan Bakthavatchalu[1], Steven Tannenbaum[1], James Fox[1] and Bruce Horwitz[2,3]. [1]Massachusetts Institute of Technology, Cambridge, MA; [2]Brigham and Women's Hospital, Boston, MA; [3]Harvard University, Boston, MA

The risk of colon cancer is higher in patients with inflammatory bowel diseases than the general population. The etiologic link between chronic inflammation and cancer is incompletely understood. However, since cancer is a disease of the genome, it has been hypothesized that colitis-associated cancer is associated with the excessive oxidative stress, which damages DNA, generates mutations and contributes to carcinogenesis. Previously we modeled these events using 129SvEvRAG2$^{-/-}$ mice infected with *Helicobacter hepaticus* (Hh). These mice develop persistent colitis and progressively develop cancer. *Hh*-infected RAG2$^{-/-}$ mice demonstrated increased straining with gH2AX, a marker of DNA damage, within crypt epithelial cells. Remarkably, DNA damage is promoted by IL-22-dependent induction of iNOS. In the present study, we evaluated mice that lack the interleukin 22 receptor subunit α1 (IL22RA1$^{-/-}$). IL22RA1$^{-/-}$ mice were infected with *Hh* for 2 weeks. These mice showed significantly less epithelial DNA damage than wild-type littermates, and this was associated with significantly less epithelial iNOS induction, demonstrating that IL-22-dependent iNOS induction in epithelial cells requires signaling through IL22RA1. Next, because members of the STAT family of transcription factors are targets of IL22RA1, we asked whether phosphorylation and activation of these proteins following Hh infection was dependent on IL-22 signaling. Hh infection induced higher percentage of nucleus pSTAT1 and pSTAT3 in the epithelial cells and these percentages were significantly lower in IL22RA1$^{-/-}$ mice. Ongoing investigations, including studies of epithelial-specific IL22RA1 knock-out mice, will increase our understanding of the relationship between IL-22 pathway, iNOS induction and colitis-associated carcinogenesis.

T12. Exposure to Cigarette Smoke Condensate Induces Paneth Cells Alterations in Mice

Loni Berkowitz, Francisco Salazar-Echegarai, Jorge Flández, Catalina Pardo-Roa, Geraldyne A. Salazar, Gigliola Ramirez, Alexis M. Kalergis, Susan M. Bueno and Manuel M. Álvarez-Lobos. Pontificia Universidad Catolica de Chile, Santiago, Region Metropolitana, Chile

Paneth cells (PCs) are important components of the intestinal barrier. Alterations in its function are associated with an imbalance of the normal microbiota and inflammatory processes, such as Crohn's disease (CD). Various studies have shown that cigarette exposure, the main environmental factor for CD, affects the intestinal barrier of the small bowel, increasing intestinal bacterial translocation. Considering the role of PC in intestinal homeostasis, we propose to evaluate whether exposure to cigarette smoke condensate (CHC) alters the integrity of PC in mice. For that, C57BL/6 mice received 200µg CHC, 400µg CHC or vehicle, intraperitoneally or intragastric (n=4 per group), 3 times a week for 2 weeks. After 3 weeks, ileum samples were obtained for histopathological analysis, evaluation of PCs integrity and quantification of their bactericidal peptides expression by qPCR. Mice treated with intragastric CHC (400ug) exhibited signs of inflammation and distortion of the ileal mucosal architecture, lower number of PCs per crypt (p=0,0017), and reduced expression of cryptdin-1 (p=0,001), cryptdin-4 (p=0,001) and RegIIIγ (p=0,001) compared to the vehicle group. Mice treated with CHC intraperitoneally only showed a reduced cryptdin-1 expression at the higher dose (p=0,031), compared to the vehicle group. The distribution of the PCs granules, stained with Alcianblue/PAS, was not significantly different under any of the treatments. Therefore, our results show that cigarette components alter the ileal mucosa, affecting PCs. Intraperitoneal CHC generates lower changes than intragastric CHC, suggesting that the alterations depend on a local effect. Further investigation is required to understand the molecular pathways involved.

T14. Lypd8 Protects Colonic Mucosa from Pathogenic Enteric Bacteria

Ryu Okumura and Kiyoshi Takeda. Osaka University, Suita, Osaka, Japan

Intestinal mucosa is protected against microorgnisms including pathogenic bacteria by various kinds of barriers. Ly6/Plaur containing 8 (Lypd8) is a highly glycosylated GPI-anchored protein. This molecule is highly and selectively expressed on the uppermost epithelial layer of the colon and shed into the intestinal lumen. We found that Lypd8 promotes the segregation of intestinal bacteria and colonic epithelia by inhibiting bacterial invasion of the inner mucus layer and colonic epithelia. In particular, Lypd8 suppressed the invasion by flagellated microbiota such as *Proteus* and *Escherichia* through inhibition of their motility. *Citobacter rodentium* is a Gram-negative pathogenic bacterium belonging to *Enterobacteriaceae* including *Escherichia* and *Proteus*. Orally-infected *C. rodentium* induces attaching/effacing (A/E) lesion in the mouse intestine and causes intestinal inflammation, like enterohemorrhagic *Escherichia coli*. In order to test whether Lypd8 is involved in the protection against *C. rodentium* infection, we orally infected wild-type mice and Lypd8 knockout (KO) mice with *C. rodentium*. A larger number of *C. rodentium* attached to the colonic epithelia of Lypd8 KO mice after the infection, accompanied by the higher expression of several genes related to production of reactive oxygen species (ROS) in intestinal epithelial cells. In accordance with the fact that ROS promotes induction of Th17 cells, Lypd8 KO mice showed more severe colitis with the dramatically increased number of Th17 cells and neutrophils in the colonic lamina propria. These findings demonstrate that Lypd8 plays an important role in the protection against pathogenic enteric bacteria such as *C. rodentium* by inhibiting their adhesion on intestinal epithelial cells.

T15. Diet-Derived Short Chain Fatty Acids Stimulate Intestinal Epithelial Cells to Induce Mucosal Tolerogenic Dendritic Cells

Gera Goverse[1], Rosalie Molenaar[2], Laurence Macia[3], Jian Tan[3], Martje Erkelens[2], Tanja Konijn[2], Marlene Knippenberg[2], Emma C. L. Cook[2], Diana Hanekamp[2], Marc Veldhoen[4], Anita Hartog[5], Guus Roeselers[6], Gianluca Matteoli[7], Charles R. Mackay[3] and Reina Mebius[2]. [1]University of Leuven, Leuven, Vlaams-Brabant, Belgium; [2] VU University Medical Center, Amsterdam, Noord-Holland, Netherlands; [3] Monash University, Clayton, Victoria, Australia; [4] Braham Institute, Cambridge, England, United Kingdom; [5] Utrecht Institute for Pharmaceutical Sciences, Utrecht, Netherlands; [6] Microbiology and Systems Biology, TNO, Zeist, Utrecht, Netherlands; [7] KU Leuven, Translational Research Center for Gastrointestinal Disorders, Leuven, Vlaams-Brabant, Belgium

The gastrointestinal tract is continuously exposed to many environmental factors thereby influencing intestinal epithelial cells and the underlying mucosal immune system. Here, we demonstrate that both dietary fibers and short chain fatty acids (SCFAs) were able to induce the expression of the vitamin A converting enzyme RALDH1 in intestinal epithelial cells *in vivo* as well as *in vitro*, respectively. Furthermore, our data showed that RALDH1 expression levels in small intestinal epithelial cells correlated with the activity of vitamin A converting enzymes in MLN dendritic cells along with increased numbers of intestinal regulatory T cells and a higher production of luminal IgA. Moreover, we show that the consumption of dietary fibers can alter the composition of SCFA producing microbiota and SCFA production in the small intestines. In conclusion, our data illustrate that dietary adjustments affects small intestinal epithelial cells, which can be used to modulate the mucosal immune system.

T17. The Effects on Pro-Inflammatory Cytokines and Chemokines by *Pseudomonas aeruginosa* in Primary Human Nasal Epithelial Cells

Jae Chan Ryu, Su-Jin Kim, Youn-Wook Chung, Ji-Hwan Ryu and Joo-Heon Yoon. Yonsei University College of Medicine, Seoul, Seoul-t'ukpyolsi, Republic of Korea

The airway epithelium is the first line of host defense against inhaled particles including pathogens. Although the epithelium always contacts infectious agents and commensal organisms, efficient immune responses remain host healthy. However, airway diseases can be occurred on the occasion of the immune-compromised state or pathogenic bacterial overgrowth. *Pseudomonas aeruginosa* is one of critical infectious pathogens found in chronic airway diseases because it is congenitally resistant to many antibiotics and can survive on mucus secreted from epithelial cells. Nasal epithelial cells (NECs) contribute to maintain the immune homeostasis due to efficient elimination of the fatal pathogen by the innate immune responses. At ALI 7-day, re-differentiated human NECs were infected by *P. aeruginosa* wild-type PA01, excluding the influence of cell death. This study found that when polarized NECs recognized the pathogen, NECs secreted more pro-inflammatory cytokines toward apical surface which faces the nasal cavity, and more chemokines toward basal surface which is close to blood vessels. Interaction between pathogens and receptors in plasma membrane or cytosol activates NECs to secrete immune response mediators. In this study, I examined toll-like receptor (TLR) subfamily TLR1 to TLR9; then, the gene expression of TLR 2, 4, 7 significantly increased following PA01 infection. According to inhibitor tests, mitophagy had an influence on the secretion of pro-inflammatory cytokines and chemokines despite there were different responses depending on other inhibitors. As a result, this study has shown that the secretion of pro-inflammatory cytokines and chemokines from airway epithelial cells intricately connected with many mechanisms.

T18. Interleukin-33 Production by Intestinal Epithelial Cells in Response to Bacterial Flagellin Promotes Type 2 Immunity

Joo-Hong Park, François Déjardin and Gérard Eberl. Institut Pasteur, Paris, Ile-de-France, France

The toll-like receptor 5 (TLR5) regulates innate and adaptive immunity in response to bacterial flagellin. Flagellin can promote Th1, Th2, and Th17 responses depending on the immune context. The mechanism by which flagellin regulates immune responses at intestinal mucosal surface remains to be elucidated. We have generated a transgenic mouse model expressing flagellin in intestinal epithelial cells under control of doxycycline-inducible villin-rtTA system. Epithelial expression of flagellin increases intestinal regulatory T cells (Tregs) that express Gata3 and the IL-33 receptor. The expansion of $Gata3^{+}$ Tregs is mediated by TLR5 and IL-33. Flagellin promotes the elevation of type 2 cytokines, including IL-33, IL-25, and TSLP, as well as pro-inflammatory cytokine IL-6 and IL-1β in intestinal epithelial cells. These results demonstrate that flagellin-TLR5 signalling can induce mucosal repair responses through IL-33 production in intestinal epithelial cells.

T19. Cross-Talk of Let-7f and C/EBPβ is Critical for the Maintenance of Immune Homeostasis in the Cervico-Vaginal Mucosal Interface

Kanchana K. Ayyar and KVR Reddy. National Institute for Research in Reproductive Health, Mumbai, Maharashtra, India

Epithelial cells lining the mucosa of lower female reproductive tract mount innate immune response via several Pattern Recognition Receptors eg. Toll-like Receptors (TLRs). Studies have shown that vaginal infections increase the risk of acquiring HIV; there is a paucity of data on the mechanism(s) underlying pathogen-induced cellular immune responses. Here, we demonstrate the role of microRNA, let-7f in the modulation of immune responses at the human cervico-vaginal mucosal interface. let-7f was knocked down in human endocervical epithelial cell line (End1/E6E7) using siRNA. Microarray was performed and data was validated by qPCR and western blot. Expression of C/EBPα, C/EBPβ, etc. was checked. Cells were treated for 3hrs with C/EBP inhibitor, Betulinic acid (20μM) followed by stimulation with TLR3 ligand, poly(I:C) (10μg/mL) for a total period of 24hrs and let-

7f expression was determined by qPCR. Cells were transfected with pCMV-C/EBPβ-LIP plasmid to knockdown C/EBPβ. Chromatin Immunoprecipitation (ChIP) was performed to study binding of C/EBPβ to let-7f promoter. Knockdown of let-7f, led to significant upregulation of C/EBPα and C/EBPβ. let-7f promoter contains six C/EBPβ binding sites. Knockdown of C/EBPβ resulted in repression of let-7f. ChIP confirmed binding of C/EBPβ to let-7f promoter. Poly(I:C) stimulation resulted in a change in the preferential binding position of C/EBPβ on let-7f promoter. The results indicate that let-7f and C/EBPβ form a feedback-loop which modulates immune responses at the cervico-vaginal mucosal interface. This information would be critical for designing safe and effective vaginal microbicides and strategies to regulate mucosal damage caused by vaginal infections.

T21. *Shigella* Infection Impairs SUMOylation to Promote Bacterial Entry

Pierre Lapaquette[1], Sabrina Fritah[2], Nouara Lhocine[2], Alexandra Andrieux[2], Joelle Mounier[2], Philippe Sansonetti[2] and Anne Dejean[2]. [1]Universite de Bourgogne, Dijon, Bourgogne, France; [2] Institut Pasteur, Paris, Ile-de-France, France

Shigella, the causative agent of bacillary dysentery, has developed a highly specialized strategy to invade epithelial cells and to subsequently disrupt intestinal barrier. SUMOylation is a post-translational protein modification that governs several cellular processes in eukaryotes, including intestinal homeostasis, immune response, cell proliferation and DNA repair. Recent studies demonstrate that some pathogenic microorganisms manipulate host SUMOylation system for their own benefit. Here, we show that *Shigella* switches off host SUMOylation during epithelial cells infection by inducing a calpain-mediated degradation of the SUMO E1 enzyme SAE2. Furthermore, we describe an original mechanism by which *Shigella* favors RhoGTPases activation and thereby promotes its own invasion by altering the SUMOylation state of RhoGDIa, a master negative regulator of RhoGTPases activity. Together, our results clearly reinforce the role of SUMOylation as a gatekeeper mechanism that acts on frontline to restrain invasive bacteria entry.

T22. Modulation of Autophagy by Probiotics Bacteria: Selecting and Engineering Strains able to Stimulate Autophagy in Intestinal Epithelial Cells

Jana Al Azzaz[1], Aurélie Rieu[1], Luis G. Bermúdez-Humarán[2], Philippe Langella[2], Frédéric Dalle[1], Jean Guzzo[1] and Pierre Lapaquette[1]. [1]Université de Bourgogne, Dijon, Bourgogne, France; [2] INRA, Jouy-en-Josas, Ile-de-France, France

Autophagy is a lysosomal degradation process representing a critical component of the innate immunity by selectively eliminating invading bacteria and modulating inflammation. Defects in autophagy are associated with several human diseases including inflammatory bowel diseases (IBD). In this context, autophagy insufficiencies lead to an increase persistence of intracellular pathogenic bacteria and an exacerbated inflammatory response, negatively affecting clinical outcome. Thus, novel therapies designed to enhance autophagy in the gut might represent an attractive strategy to overcome autophagy insufficiencies associated with IBD. In this study, we aim to: **(i)** identify probiotic lactobacillus strains that stimulate autophagy in intestinal epithelial cells and **(ii)** develop food-grade lactic acid bacteria designed to secrete a cell-permeable autophagy-inducer peptide in the gut. Among a panel of *Lactobacillus* probiotic strains, some strains of *L. casei*, *L. Rhamnosus* and *L. fermentum*, and their supernatants, were able to strongly stimulate autophagy in contact to differentiated human intestinal epithelial (Caco-2) as measured by immunoblotting and confocal analysis of the autophagic marker LC3. In addition, these strains stimulate autophagy at transcription level by enhancing expression of autophagy-related genes (*Atg5*, *Atg16L1*, *Vps34*, *Ulk1*) in host cells. Altogether, these results indicate that some *Lactobacillus* strains, beyond their well-recognized immunomodulatory properties, displayed the ability to stimulate autophagy. We also genetically engineered *Lactococcus lactis* strains secreting an autophagy inducer peptide and characterized their abilities to induce autophagy in vitro. In conclusion, we characterized probiotic strains (natural and genetically-modified) that stimulate autophagy in human epithelial cells and might have beneficial role in IBD by restoring autophagy.

T23. Dynamic Chromatin Binding Regulates IL-33 Extracellular Release During Necrosis

Cora E. Miracle, Jared Travers, Mark Rochman and Marc E. Rothenberg. Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Interleukin 33 (IL-33) is a member of the IL-1 cytokine family with key roles in allergic diseases, including asthma, atopic dermatitis, and eosinophilic esophagitis. Under normal conditions, IL-33 is retained within the nucleus of the cell, bound to the nucleosome acidic patch. Upon necrosis, IL-33 is released and activates immune cells through its receptor, ST2. While most studies focus on the extracellular function of IL-33, the physical properties and functional significance of IL-33–chromatin binding remain largely understudied. Herein, we examined the molecular characteristics of IL-33–chromatin binding and tested the hypothesis that it regulates IL-33 extracellular release in esophageal epithelial cells. Wild-type IL-33 (IL-33^{WT}) was localized to the nucleus and was enriched in heterochromatic regions. In contrast, truncated IL-33 (IL-$33^{112\text{-}270}$), which lacks the chromatin binding domain, exhibited nuclear and cytoplasmic localization. Fluorescence recovery after photobleaching revealed that IL-33^{WT} has a 10-fold slower mobility ($p < 0.0001$) within the nucleus than that of the classic nuclear cytokine IL-1a. IL-$33^{112\text{-}270}$ was freely mobile similarly to the GFP control. These results indicate that IL-33 demonstrates slow, dynamic chromatin binding. After induction of cellular necrosis, IL-33^{WT} exhibited decreased extracellular release compared to IL-$33^{112\text{-}270}$. Time-lapse microscopy revealed intracellular retention of H2B and IL-33^{WT}, but not IL-1a or IL-$33^{112\text{-}270}$, after induction of necrosis. Under these conditions, IL-33^{WT} had a slow, linear release over time that was not observed for H2B. From our findings, we propose that IL-33–chromatin binding counter-regulates IL-33 extracellular release during necrosis to curtail downstream effects.

T25. Attenuation of Pulmonary ACE2 Activity Impairs Inactivation of Des-Arg9 Bradykinin/BKB1R Axis and Facilitates LPS-Induced Neutrophil Infiltration

Hongpeng Jia[1], Chhinder Sodhi[1], Christine Wohlford-Lenane[2], Thomas Prindle[1], William B. Fulton[1], Paul B. McCray[2], Mark Chappell[3] and David Hackam[1]. [1]Johns Hopkins University School of Medicine, Baltimore, Baltimore, MD; [2] University of Iowa, Iowa City, IA; [3] Wake Forest University, Winston-Salem, NC

Angiotensin converting enzyme 2 (ACE2) is a terminal carboxypeptidase with important functions in the renin angiotensin system and plays a critical role in inflammatory lung diseases. ACE2 cleaves single terminal residues from several bioactive peptides such as angiotensin II. However, few of its substrates in the respiratory tract have been identified and the mechanism underlying the role of ACE2 in inflammatory lung disease has not been fully characterized. In an effort to identify biological targets of ACE2 in the lung, we tested its effects on des-arg^9 bradykinin (DABK) in airway epithelial cells based upon a hypothesis that DABK is a biological substrate of ACE2 in the lung and ACE2 plays an important role in the pathogenesis of acute lung inflammation partly through modulating DABK/BKB1R axis signaling. We found that loss of ACE2 function in mouse lung in the setting of endotoxin inhalation led to activation of the DABK/BKB1R (bradykinin receptor B1) axis, release of the proinflammatory chemokine CXCL5 from airway epithelia, and an increased neutrophil infiltration. These results indicate that a reduction in pulmonary ACE2 activity contributes to the pathogenesis of lung inflammation, in part due to an impaired ability to inhibit DABK/BKB1R axis mediated signaling, resulting in more promptly occurring neutrophil infiltration in the lung. Our study identifies a biological substrate of ACE2 within the airways, as well as a potential new therapeutic target for inflammatory diseases.

T26. Estradiol Modulates the Inflammatory Response Preventing the Intestinal Epithelial Barrier Dysfunction in Endotoxemic Female Rats

Aline Barbosa Ribeiro, Celso Rodrigues Franci, Humberto Giusti and Rafael Simone Saia. University of São Paulo, Ribeirão Preto, Sao Paulo, Brazil

Intestinal injury is one of the serious complications of sepsis, associated with the loss of intestinal barrier integrity and the disruption of the tight junctions. Estradiol has been demonstrated as an anti-inflammatory hormone and responsible for maintaining the architecture of the intestinal epithelium. Our aim was to investigate the role of estradiol on lipopolysaccharide (LPS)-induced intestinal epithelial barrier dysfunction. The female rats were ovariectomized and allowed to recover for 10-12 days before the experiment. For three consecutive days, rats were pretreated with estradiol cypionate (50 or 100 µg/kg, subcutaneous) or corn oil (vehicle). At 6h after endotoxemia induction (LPS, 1.5 mg/kg, intravenous), the intestinal permeability was evaluated by injecting FITC-dextran 4 kDa in the ileum and the colon. Mesenteric lymph nodes were collected for microbiological analysis and also cytokines were quantified in the plasma and intestinal mucosa. Additionally, the integrity of the tight junctions was determined by the expression of tight junction proteins (occludin, claudin-1, claudin-2, junctional adhesion molecule-A). Our results demonstrated that estradiol reduces intestinal permeability and prevents the bacterial translocation to the mesenteric lymph nodes. The concentration of pro-inflammatory cytokines in ileal and colonic mucosas was reduced in estradiol-treated rats. Estradiol treatment reverted the LPS-induced epithelial barrier dysfunction, increasing the expression of the tight junction proteins investigated and attenuating the histological damages. These results suggest clearly a protective role for estradiol preventing the intestinal barrier dysfunction induced by systemic inflammation, possibly modulating the inflammatory response and the expression of proteins of the intestinal tight junctions.

T27. Il-33 protects during hypervirulent *Clostridium difficile* infection.

Alyse Frisbee[1], Carrie Cowardin[2], Mahmoud Saleh[1], Erica Buonomo[1] and William A. Petri[1]. [1]University of Virginia, Charlottesville, VA; [2] University of Washington, St. Louis, MO

Clostridium difficile infection is the leading cause of hospital acquired antibiotic-associated diarrhea in the US (Bartlett, 2006). The increased prevalence of circulating *C.difficile* strains poses a significant health threat to US health care facilities. Strains expressing the toxin *C. difficile* Transferase (CDT), in addition to Toxins A and B (TcdA and TcdB), are more virulent and associated with higher mortality rates (Bacci et al., 2011). The standard treatment for *C. difficile* is antibiotic therapy which further disrupts the beneficial microbiota and increases the risk for relapse or reinfection. We have recently identified a protective role for eosinophils against *C.difficile* pathogenesis (Buonomo et al., 2016). We have also defined CDT's ability to increase host inflammation and suppress protective eosinophils through a TLR2 dependent mechanism. Mice lacking TLR2 signaling are protected from CDT induced mortality (Cowardin et al., 2016). How CDT promotes virulence and inflammation via TLR2 is still under investigation. We employed a genome-wide microarray approach to reveal divergent transcriptional profiles between protected ($TLR2^{-/-}$) and unprotected (WT) mice infected with either CDT expressing or CDT mutant strains of *C. difficile*. This work revealed activation of IL-33 and alternatively activated macrophage markers by CDT infection in wildtype mice. Treatment with IL-33 was sufficient to protect mice from CDT associated mortality and weight loss. We are currently investigating whether dampening detrimental TH17 inflammation is involved in IL-33 mediated protection. This work advances our fundamental understanding of the immune response which protects the gut mucosa during *Clostridium difficile* infection.

T28. IL-17 Factors Have Ancient Roles as Early Mediators in the Gut Epithelium During Inflammatory Response

Katherine M. Buckley[1] and Jonathan P. Rast[2]. [1]George Washington University, Washington, DC; [2] University of Toronto, Toronto, ON, Canada

The IL-17 cytokines are central mediators of mammalian innate and adaptive immunity. These widely-expressed factors derive from diverse cellular sources, including a variety of lymphocytes and myeloid cells, as well as epithelial cells situated at immune barriers (*e.g.,* gut and lung). Because this cytokine family is phylogenetically widespread, invertebrates can provide efficient models to investigate IL-17 function in the intact organism. Sea urchins share an important molecular heritage with chordates that includes the IL-17 system. The feeding sea urchin larva mounts complex transcriptional and cellular immune responses to gut-associated bacterial perturbation. Here, we characterize the role of epithelial expression of IL-17 in the course of this response. The purple sea urchin genome encodes 34 *IL17* genes that are organized into ten subfamilies (32 genes) and two IL-17 receptors. Genes within two of the ligand subfamilies are upregulated strongly in the gut epithelium in response to bacterial disturbance in the gut lumen. Perturbation of IL-17R signaling results in reduced expression of downstream effector genes. An additional IL-17 subfamily is strongly expressed in adult circulating immune cells in response to bacterial challenge. Analysis of the genome sequences from other echinoderm species indicates many of these subfamilies have been conserved throughout this phylum. These dual roles of the *IL17* genes within the epithelial tissues of the larva and immune cells of the adult sea urchin immune responses reflects an ancient evolutionary origin for the functional dichotomy of these fundamental cytokines within vertebrates.

T29. NOD-Like Receptors: Guardians of Intestinal Mucosal Barriers

Shu Zhu[1], Siyuan Ding[2], Penghua Wang[3], Erol Fikrig[1], Harry Greenberg[2] and Richard Flavell[1]. [1]Yale University, New Haven, CT; [2] Stanford University, Stanford, CA; [3] New York Medical College, Valhalla, NY

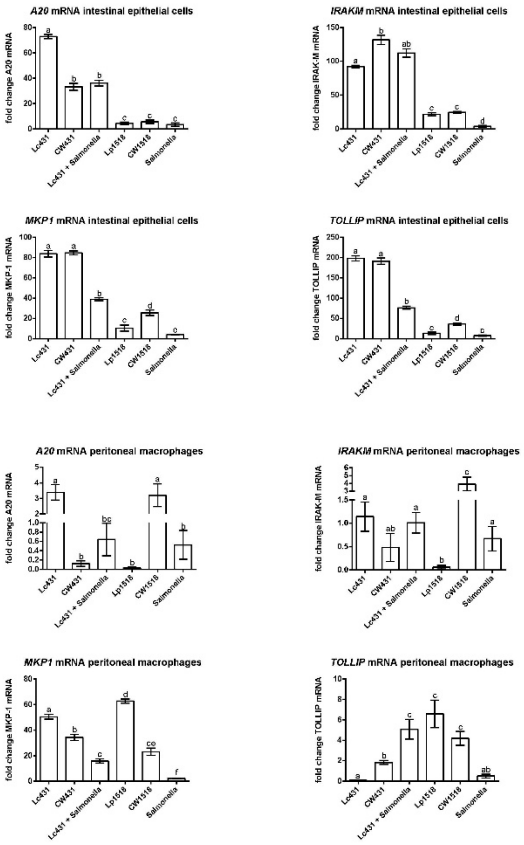
Cytosolic NOD-like receptors (NLR) are a newly identified pattern recognition receptors (PRRs), which form inflammasomes to elicit robust host defense against a variety of exogenous pathogens and endogenous stress signals by activating caspase-1 for cytokine maturation and cell death. Despite the indispensable function of NLRs in the intestine to recognize virulent bacteria pathogen such as *Salmonella*, it's not known how NLRs recognize and defense enteric viruses. We have reported Nlrp6, for the first time as an intestinal specific RNA sensor, controls enteric virus infection in the intestine by interacting with a RNA helicase, Dhx15, to trigger a MAVS-dependent antiviral responses, particularly type I/III IFNs and ISGs (*Wang, Zhu et al. Science. 2015*). Most recently, we found another intestinal epithelial cell- specific NLR-Nlrp9, uses the adaptor protein Asc and Caspase-1, to promote the maturation of interleukin (IL)-18 and Gasdermin D (Gsdmd)-induced pyroptosis in respond to enteric viruses. Our current studies highlight cooperative innate immune signalings that mediated by different NLRs in IECs and may present as useful targets in the modulation of host defenses against viral pathogens.

T30. Modulation of Signaling Pathways by Probiotics in the Gut Mucosa

José María Lemme-Dumit, Lucila Saavedra, Elvira María Hebert, Gabriela Perdigón and Carolina Maldonado Galdeano. CERELA-CONICET, San Miguel de Tucumán, Tucuman, Argentina

Probiotic may modulate the gut immune functions and ameliorate diseases. The aim of the current study was to determine the signaling pathways send by two probiotics and their cell walls (CW), compared with an enteropathogen analyzing the regulatory genes expression of TLRs after activation triggered by bacteria interaction with intestinal epithelial cells (IECs) and peritoneal macrophages (PMQ). BALB/c mice were given with *Lactobacillus casei* CRL431 (Lc431) or its cell walls (CW431) and *Lactobacillus paracasei* CNCM I-1518 (Lp1518) or its CW1518 during

7 or 5 days, respectively. After that, expressing genes (*a20*, *irak-m*, *mkp-1* and *tollip*) were determined on IECs and PMQ by RT-qPCR. Probiotics bacteria and their CW were able to increase the expression of regulator genes of TLRs pathways (*a20*, *irak-m*, *mkp-1* and *tollip*) on IECs compared to *Salmonella* (negative control), while on PMQ showed lower expression of regulatory genes. Considering previous study, we demonstrated that probiotics interact with immune and non-immune cells through TLRs (TLR2 and TLR4) and MyD88; we propose that probiotics modulate the NF-κB pathway by expression of regulators of signaling pathways on IECs. In the presence of pathogenic microorganisms, IECs secrete cytokines that are crucial for the recruitment and the activation of inflammatory cells. PMQ does not increase the expression of regulatory genes because of these cells are key players in the host response to microorganisms. We demonstrate the way by which probiotics are able to modulate the gut mucosal immune system, without interfere in the activation of the immune cells distant to the gut.



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T31. Biallelic Inherited DUOX2-Inactivating Mutations as a Cause of Very Early Onset Inflammatory Bowel Disease

Marianna Parlato[1], Fabienne Charbit-Henrion[1,2], Patti Hayes[3], Antonio Tiberti[4], Marina Aloi[4], Salvatore Cucchiara[4], Bernadette Begue[1], Marc Bras[1,5], Aurore Pouliet[1], Sabine Rakotobe[1], Frank Ruemmele[2], Ulla Knaus[3] and Nadine Cerf-Bensussan[1]. [1]Institut IMAGINE- Inserm UMR 1163, Paris, France; Hôpital Necker-Enfants Malades, Paris, France; [3]University College Dublin, Dublin, Ireland; [4]University Hospital Umberto I, Rome, Italy; [5]Université Paris-Descartes, Paris, France

By catalyzing generation of hydrogen peroxide (H_2O_2) in enterocytes, DUOX2 participates in epithelial defense against intestinal microbes. We report here biallelic, inherited mutations of *DUOX2* as a Mendelian cause of VEO-IBD in a boy born from unrelated parents of Italian origin. The patient developed pancolitis with bloody diarrhea at the age of 3. Whole exome sequencing identified compound heterozygous variants in *DUOX2.* The first variant resulted in non-conservative change of proline into serine residue at position 609 (P609S) within the first N-terminal (NT) intracellular loop. The second variant changed an arginine to histidine at the position 286 (R286H) in the NT peroxidase-like domain. Western blot showed comparable amounts of WT DUOX2, R286H and P609S mutants in total cell lysates. In contrast, flow cytometry revealed reduced surface expression of P609S compared to WT, while R286H was undetectable at the plasma membrane, indicating that the mutated proteins were not completely translocated to the cell surface, but rather retained in the endoplasmic reticulum. Indeed, staining of DUOX2 carried out on patient's biopsies showed markedly decreased protein expression compared to control. Catalytic activity, assessed in DUOXA2-H661 cells by measuring H_2O_2 generation upon thapsigargin stimulation, was absent in cells expressing the R286H mutant, while H_2O_2 production was partially but significantly decreased in cells expressing the P609S mutant. Overall our data provided evidence that rare, inherited, biallelic mutations in *DUOX2* were the molecular cause of this case of VEO-IBD confirming the important role of DUOX2 in gut homeostasis.

T33. Ulcerative Colitis-Associated Endoplasmic Reticulum Stress Induces Trypsin Activity Affecting Innate Immune Response

Nuria Sola-Tapias, Frederick Barreau and Nathalie Vergnolle. Inserm U1220, Toulouse, Midi-Pyrenees, France

Inflammatory bowel disease (IBD) is associated with excessive induction of endoplasmic reticulum stress (ERS) in intestinal epithelial cells (IEC), leading to intestinal barrier disruption and inflammation. Increased serine protease activity, is associated with IBD. We investigated the interplay between ERS and serine protease activity. I*n situ* zymography and enzymatic assays were used to assess Trypsin activity, western-blot, qRT-PCR and immunohistochemistry to assess Interleukin-8 (IL8), Trefoil factor-3 (TF3), and human beta-defensins (HbD) expression. Monolayers of human intestinal epithelial cells (Caco-2 and HT-29) were cultured and stimulated with Thapsigargin or Tunycamycin to induce ERS. Barrier function was assessed by FITC-dextran passage. In tissues from Ulcerative colitis patients, Trypsin-like activity was detected in epithelial cells and correlated with Trypsin-3 overexpression. ERS induction in epithelial cell monolayers increased Trypsin-like activity and Trypsin-3 release on the apical compartment. Activity-based probes used in supernatants demonstrated that Trypsin-3 was responsible for Trypsin-like activity. ERS-induced increase in permeability was blocked by Trypsin inhibitors and protease-activated receptor (PAR) antagonists. Trypsin-3 exposure also increased permeability in a PAR-dependent manner. ERS-induced overexpression of TF3 and HbD by intestinal epithelial cells was inhibited by Trypsin and PAR inhibitors, while IL8 production was potentiated by the same treatments. We concluded that ERS-associated Trypsin activity in IEC has a dual role, being responsible for the loss of barrier function but also for the production of protective factors limiting an overactivation of mucosal immunity. Trypsin-3 seems to be the protease responsible for IEC-associated Trypsin activity.

T34. Tight Junction Development is Altered in Ganglionated Versus Aganglionic Bowel in Mice with Hirschsprung Disease

Korah P. Kuruvilla[1,2], Pradeep Shukla[2], Frederic Cailleux[1,2], Radhakrishna Rao[2] and Ankush Gosain[1,2]. [1]Le Bonheur Children's Hospital, Memphis, TN; [2] University of Tennessee Health Science Center, Memphis, TN

Tight junction (TJ) dysfunction contributes to bowel inflammation. Hirschsprung disease (HD) results from failed development of the Enteric Nervous System (ENS) in the distal hindgut. Hirschsprung-Associated Enterocolitis (HAEC), the most severe complication of HD, has a complex pathophysiology. Our goal was to determine if TJ's are altered in HD. Using the neural-crest conditional deletion of Endothelin Receptor-B (EdnrB-null) model of HD, we examined the temporal [post-natal (P) 14,18,22] and spatial [ileum, proximal colon (PC), distal colon (DC)] gene and protein expression of Occludin (Ocln), Zona occludens-1 (ZO-1), Claudin-2 (Cldn2) and -3 (Cldn3). We found no changes in the ileum. In the PC, we observed significant upregulation of multiple TJ genes/proteins in the EdnrB-nulls at all time points. However, in the aganglionic DC of EdnrB-nulls, we found significant downregulation of TJ's. These findings of temporal and regional variability in TJ gene expression and protein localization in HD are consistent with recent studies that suggest that mucosal immune defects extend beyond the aganglionic bowel in HD and contribute to HAEC pathogenesis. Potential roles for the microbiota and/or ENS as drivers of these alterations are under investigation.

T35. Optimizing the Development and Characterization of Canine Small Intestine Crypt Enteroids as a Research Model

Dawn Kingsbury, Liping Sun, Yijun Qi, Jorrell Fredericks, Qun Wang, Michael Wannemuehler, Jonathan Mochel, Albert Jergens and Karin Allenspach. Iowa State University, Ames, IA

Orthotopic transplantation of intestinal organoids has the potential to repair or replace damaged epithelial tissues associated with chronic gastrointestinal disorders. However, progress in transplantation of intestinal organoids has been hampered due to the limited size and lifespan of mice. There remains,

therefore, a critical need to develop well-characterized cell lines and protocols for cultivation and investigation of organoid delivery, efficacy and long-term safety following transplantation in large animal models. Dogs are more similar to people, when compared to mice, in many aspects including genomic makeup, anatomy/physiology, and susceptibility to infectious disease. This is the first report of successful propagation of canine enteroids *ex vivo*. Ten centimeter pieces of proximal jejunum were acquired from 4 young healthy dogs, washed in PBS and minced. Intestinal crypts were enriched, using EDTA chelation, released via trituration, embedded in matrigel, and grown in intestinal stem cell media. Optimal factors for canine organoid isolation and culture including rho-associated kinase ROCK inhibitor Y27632, glycogen synthase kinase 3β inhibitor CHIR99021 and wnt-3a were evaluated to maximize growth performance. Preliminary morphological characterization included live cell brightfield imaging, Trypan Blue viability staining, H&E, Alcian Blue and PAS staining. Canine organoid culture has been successfully carried greater than 8 passages. Preliminary results suggest that the addition of 50 ng/mL of rho-associated kinase inhibitor produced greater colony-forming efficiency over 3 generations. Morphological characteristics apparent from H&E and IHC stainings include proliferation via relatively abundant mitotic figures, classic ordered simple columnar epithelial polarity with basal nuclei and clear cytoplasm of goblet cells.

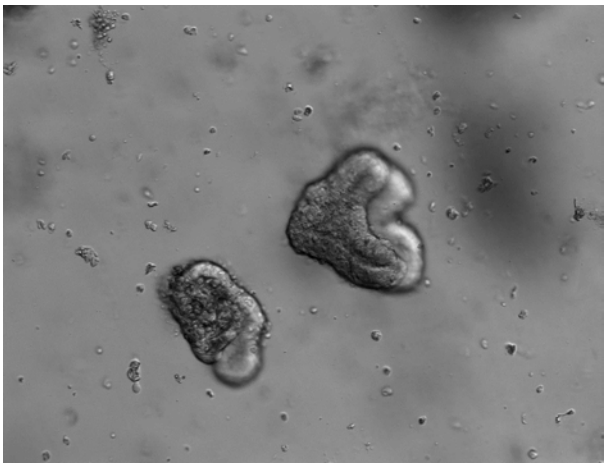


Image 1. Canine small intestinal spheroid on day 2 of passage 6. Live cell brightfield image at 400x magnification.

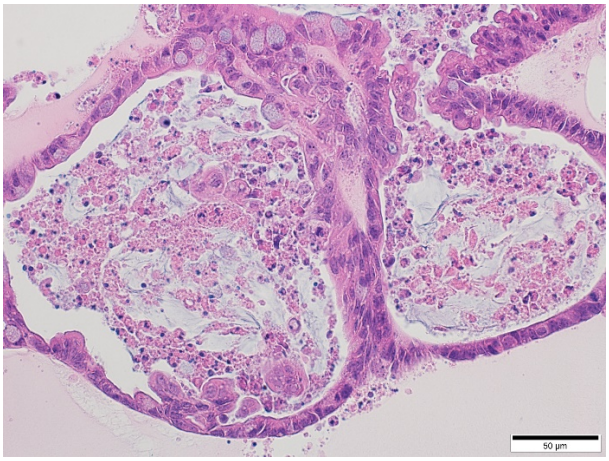


Image 2. Canine small intestinal organoid on day 14 of passage 5. 5um thick, paraffin-embedded, formaldehyde-acetic acid-ethanol-fixed section stained with hematoxylin & eosin (H&E) at 400x magnification.

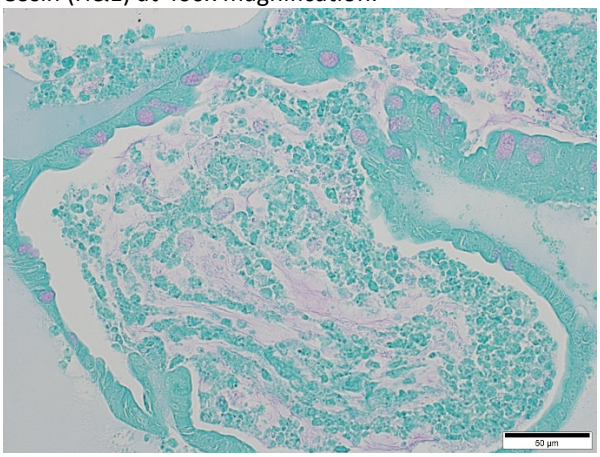


Image 3. Canine small intestinal organoid on day 14 of passage 5. 5um thick, paraffin-embedded, formaldehyde-acetic acid-ethanol-fixed section stained with Periodic-Acid Schiff diastase (PAS) at 400x magnification. Red staining indicates goblet cells.

T36. Live Imaging Analysis of Human Gastric Organoids Reveals Spontaneous Rupture, Inversion, Rotation, and Fusion Events.

Andrew Sebrell, Barkan Sidar, Rachel Bruns, James Wilking and Diane Bimczok. Montana State University, Bozeman, MT

Organoids are 3-dimensional spheroid cultures of primary epithelial cells that have become increasingly popular for studies of gastrointestinal development, mucosal immunology and epithelial infection. However, little is known about the behavior of these complex living spheres in their three-dimensional culture matrix. Therefore, we performed extended time-lapse imaging analysis (up to 4 days) of human gastric organoids generated from adult tissue

samples in order to visualize the dynamics of these spheroids in detail. In our hands, human gastric organoids grew to an average diameter of 252 ± 142 µm after 10 days, with 6.3% of the organoids reaching a diameter of > 500 µm. Live imaging analysis revealed that organoid growth was frequently associated with cyclic rupture of the epithelial shell, which led to the release of luminal contents. Organoid rupture usually resulted in an initial collapse, followed by spontaneous re-formation of the spheres. In some cases, organoid rupture led to inside-out inversion of the organoid shell, resulting in transient amoeboid-like motility followed by disintegration. Moreover, organoids frequently rotated around their axes within the Matrigel matrix, possibly propelled by basolateral pseudopodia-like formations of the epithelial cells. Interestingly, adjacent organoids occasionally underwent luminal fusion, as visualized by injection of individual organoids with FITC-dextran (40 kDa). In summary, our analysis has revealed multiple unexpected dynamics in human gastric organoids that challenge our current view of cultured epithelia as static entities and that need to be considered when performing organoid infection experiments.

T39. Protein Malnutrition Modifies Innate Immunity, Gene Expression by Intestinal Epithelial Cells and Human Rotavirus Infection in Neonatal Gnotobiotic Pigs

Anastasia N. Vlasova[1], Francine C. Paim[1], Sukumar Kandasamy[1], Moyasar A. Alhamo[1], David D. Fischer[1], Stephanie N. Langel[1], Loic Deblais[1], Anand Kumar[2], Juliet Chepngeno[1], Lulu Shao[3], Huang-Chi Huang[1], Rosario A. Candelero-Rueda[1], Gireesh Rajashekara[1] and Linda J. Saif[1]. [1]Ohio State University, Wooster, OH; [2] Los Alamos National Laboratory, Los Alamos, NM; [3] University of Pittsburgh, Pittsburgh, PA

Malnutrition affects millions of children in developing countries, compromising immunity, and contributing to increased mortality from infectious diseases. Rotavirus is a major etiological agent of childhood diarrhea in developing countries, where malnutrition is prevalent. However, the interactions between the two and their combined effects on immune and intestinal functions are poorly understood. In this study, we used neonatal gnotobiotic (Gn) pigs transplanted with fecal microbiota from a healthy 2-month-old infant (HIFM) and fed protein deficient or sufficient bovine milk diets. Protein deficiency induced hypoproteinemia, hypoalbuminemia, hypoglycemia, stunting and generalized edema in Gn pigs as observed in protein malnourished children. Irrespective of the diet, human rotavirus (HRV) infection early, at post HIFM transplantation day 3 (PTD3) resulted in adverse health effects and high mortality (45-75%) as compared to later HRV infection (PTD10). Protein malnutrition exacerbated HRV infection and affected the morphology and function of the small intestinal epithelial barrier. In the pigs infected with HRV at PTD10, there was a uniform decrease in the function and/or frequencies of natural killer cells, plasmacytoid dendritic cells, CD103+ and apoptotic mononuclear cells, and altered gene expression profiles of intestinal epithelial cells [Chromogranin A (CgA), mucin 2 (MUC2), proliferating cell nuclear antigen (PCNA), SRY-Box 9 (SOX9) and villin]. Thus, we established the first HIFM transplanted neonatal pig model that recapitulates major aspects of protein malnutrition in children and can be used to evaluate physiologically relevant interventions. Our findings provide an explanation as to why nutrient-rich diets alone may lack efficacy in malnourished children.

T40. Allograft Inflammatory Factor 1 (Aif1) is a Regulator of Transcytosis In M Cells

Shintaro Sato[1], Sari Kishikawa[2] and Hiroshi Kiyono[2]. [1]Osaka University, Suita, Osaka, Japan; [2]University of Tokyo, Minato-ku, Tokyo, Japan

Microfold (M) cells in follicle-associated epithelium (FAE) are specialised antigen-sampling cells that take up various intestinal luminal antigens. Transcription factor Spi-B is known to regulate M-cell maturation, but the molecules that promote transcytosis within M cells are unidentified, with the exception of glycoprotein 2 (GP2), a receptor for FimH-positive bacteria such as *Salmonella* Typhimurium. Here, we report that mouse allograft inflammatory factor 1 (Aif1) is specifically expressed in M cells and contributes to M-cell transcytosis. Whole-mount staining revealed that Aif1 was expressed in GP2-positive mature M cells, and its expression was dependent on the presence of Spi-B. To investigate the function of Aif1 *in vivo*, we generated Aif1-deficient mice. FAE in *Aif1*$^{-/-}$ mice showed suppressed uptake of particles and commensal bacteria compared with that in wild-type mice. Translocation of *Yersinia enterocolitica*, but not of *S.* Typhimurium, leading to

the generation of antigen-specific IgA antibodies, was also diminished in Aif1-deficient mice. Although β1 integrin, which acts as a receptor for *Y. enterocolitica* via invasin protein, was expressed on the apical surface membranes of M cells, its active form was rarely found in *Aif1*$^{-/-}$ mice. Taken together, these findings show that Aif1 is important for bacterial and particle transcytosis in M cells.

T41. NLRP3 Inflammasome- A Key Player at The Tipping Point of Colitis into Colorectal Cancer

Rajaraman D. Eri and Agampodi Promoda Perera. University of Tasmania, Launceston, Australia

Colitis is associated with colorectal cancer but the exact mechanism has not been established. Many studies have indicated a role for NLRP3 inflammasome in colitis and tumorigenesis but the results have been controversial and inconclusive due to different chemical models of colitis induction. To address the controversial role of NLRP3, we designed the world first murine model deficient in NLRP3 in a spontaneous chronic colitis mouse model Winnie (*Muc2* mutant), arguably the best available murine model to study colitis. Our *in vitro* studies have shown that Winnie colonic IL-1β levels are inhibited by MCC950, specific inhibitor of NLRP3 inflammasome. Therefore, we hypothesised that the lack of NLRP3 in Winnie x NLRP3$^{-/-}$ would ameliorate colitis. Our results show multiple dysplastic polyps in colon of Winnie x NLRP3$^{-/-}$ at 12 weeks. NLRP3$^{-/-}$x Winnie mice had significantly shorter colons, and a higher ratio of colon weight to body weight than the control groups indicating the severity of colitis and tumorigenesis. Histopathology of NLRP3$^{-/-}$x Winnie colon revealed severe crypt distortion and goblet cell depletion with high-grade dysplasia and adenoma. Extracted RNA from colonic segments was used for the analysis of proinflammatory and cancer biomarker gene expression using PCR micro array. Colon organ cultures were performed and the supernatants were assayed for cytokines and biomarkers. Our data from the generation of spontaneous colon cancer in NLRP3 deficiency indicates that blockade of chronic inflammation with NLRP3 inhibition could lead to carcinogenesis in the colon. Thus, providing direct relevance to therapeutic implications following long-term NLRP3 inhibition.

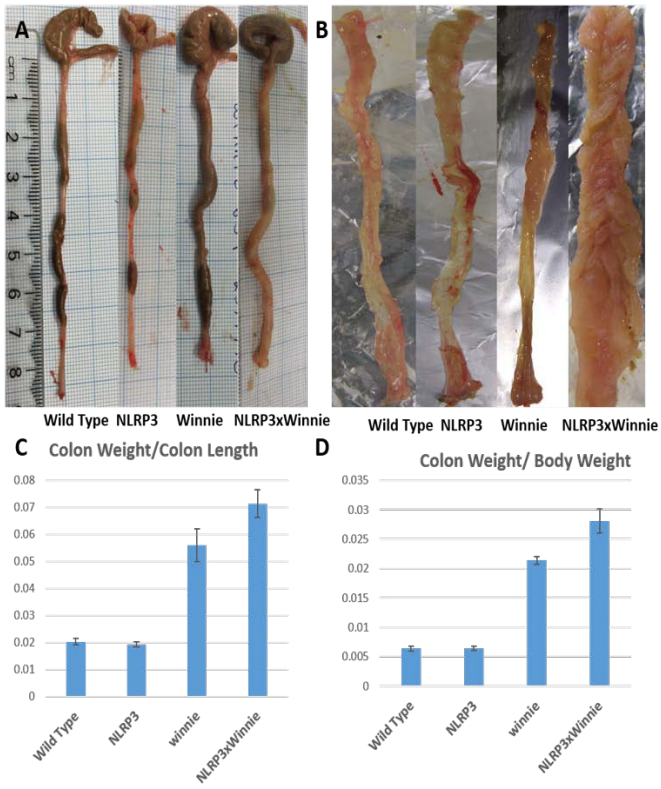


Fig 1: Clinical features of 12 week Wild type, NLRP3$^{-/-}$, NLRP3$^{-/-}$x Winnie. A: Gross appearance of the colon at termination. **B**: The longitudinally opened mouse colon illustrating the gross appearance of the colon surface **C**. Colon Weight/Colon length ration of n=10 Error bars depict standard error (SE) of mean. **D**. Colon Weight/Colon length ration of n=10. Error bars depict standard error (SE) of mean.

T42. Gamma Delta IELs in Maintenance of Intestinal Epithelial Integrity

Angela M. Patterson, Stephanie Roy, Aimée Parker, Devina Divekar, Isabelle Hautefort, Simon Carding and Alastair Watson. Institute of Food Research, Norwich, England, United Kingdom

Shedding of intestinal cells in a controlled manner is critical to maintenance of barrier function. During this shedding process barrier function is maintained by a redistribution of tight junctional proteins to facilitate closure of the gap left by the shedding cell. Excessive epithelial cell shedding and loss of epithelial barrier integrity is triggered by exposure to lipopolysaccharide (LPS) or tumour necrosis factor (TNF). Little is known about the mechanisms through which the gut microbiota and mucosal immune system regulate epithelial cell shedding. Intraepithelial lymphocytes are located within the intestinal epithelial layer and it is hypothesised that these cells are intricately associated with apoptotic epithelial cell shedding and prevention of loss of barrier function. Using a short-term murine model of pathological small

intestinal epithelial apoptosis and shedding the role of γδ IELs was investigated. Mice lacking the δ chain (C57BL/6-TCRδ$^{-/-}$) had a profound reduction in LPS-induced cell shedding with a significant reduction of apoptotic caspase-3 positive epithelial cells (4.3% ± 0.9%) in comparison to wild-type mice (16.0% ± 0.9%) ($p < 0.05$) (figure). The mice with the γδ T cell receptor abnormalities also had a dramatic contraction of the lamina propria within the villus after administration of LPS (59.9µm ± 8.9µm vs 27.5µm ± 3.3µm) ($p < 0.05$) (figure). This data indicates that γδ IELs are involved in the regulation of apoptotic shedding of epithelial cells. Further microbiological and immunological analysis indicates that the γδ IELs are an important component in the complex signalling systems that preserve the viability of the epithelium.

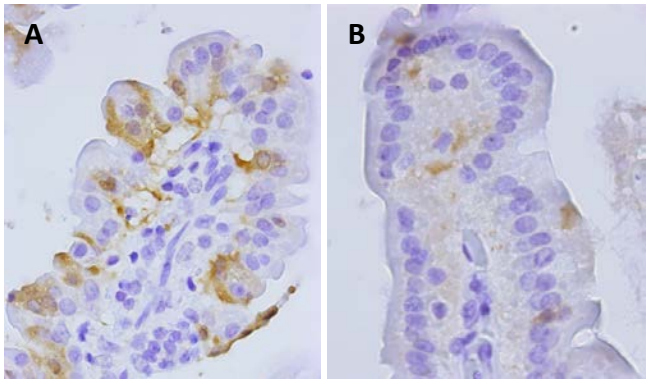


Figure: Differences in LPS induced caspase activation in villus intestinal epithelial cells of γδ IEL-deficient mice. Sections of duodenal tissue obtained from C57Bl6 (WT) mice (A), and TCRδ$^{-/-}$ mice (B) labelled with cleaved caspase-3 (brown) antibody, counterstained with haematoxylin (blue) and visualised by light microscopy. Arrow indicates lamina propria retraction of TCRδ$^{-/-}$ mice (B).

Food Allergy

L.03, F28. Systemic IL-2/Anti-IL-2Ab Complex Combined with Sublingual Immunotherapy Suppresses Experimental Food Allergy through Induction of Mucosal Tolerance

Guillermo H. Docena[1], Paola Smaldini[1], Fernando Trejo[1], José Cohen[2] and Eliane Piaggio[3]. [1]CONICET y Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina; [2]Hôpital Pitié-Salpêtrière, Paris, France; [3]Institut Curie, PSL Research University, Paris, France

Cow's milk allergy (CMA) is mediated by an aberrant immunological reaction to cow's milk proteins (CMP). Although oral and sublingual immunotherapies show promise as potential disease-modifying therapies no therapy has yet been approved. Based on recent data demonstrating that systemic human IL-2/ anti-IL-2 complex selectively expands regulatory T cells (Tregs), we combined sublingual immunotherapy (SLIT) with IL-2/anti-IL2 to improve SLIT. Balb/c mice were sensitized with CMP and cholera toxin and then treated with PBS (Sens) or CMP (CMP_{des}) (sublingual) plus intraperitoneal injections of IL-2/anti-IL-2 (C or CMP_{des}/C). Mice were orally challenged with CMP and the immune response was *in vitro* (serum IgE, IL-5 and IFN-γ secretion by splenocytes, lamina propria Tregs, IL-10 and TGF-β) and *in vivo* (clinical score and cutaneous tests) evaluated. We found a lower medium clinical score in treated mice compared with sensitized mice, with a reduced skin mast cell reactivity, mainly in CMP_{des}/C group. Immunological changes included decreased specific IgE (2,1±0,3OD Sens vs 1,3±0.1CMP_{des}; 1,4±0,9C; 1,1±0,8CMP_{des}/C), decreased levels of Th2 cytokines and induction of IL-10 and Tregs in duodenal lamina propria (14,6±1,2% Sens vs 28,0±0,6% CMP_{des}; 35,2±3,1% C; 42,5±1,8% CMP_{des}/C) and in sublingual mucosa of treated mice (pdes/C than CMP_{des}(pdes/Cshowed increased lamina propria tolerogenic dendritic cells $CD11c^{+}CD11b^{-}$ $CD8\alpha^{+}$ (p). We demonstrated in a murine model of CMA that SLIT down-modulated the mucosal and systemic allergic immune response in sensitized mice and that the IL-2/anti IL-2 complex improved the sublingual immunotherapy.

OR.75, T49. Intestinal Secretory Epithelial Cell Antigen Passages (SAPs) Sample Food Allergens and Control the Onset of Food-Induced Anaphylactic Reactions

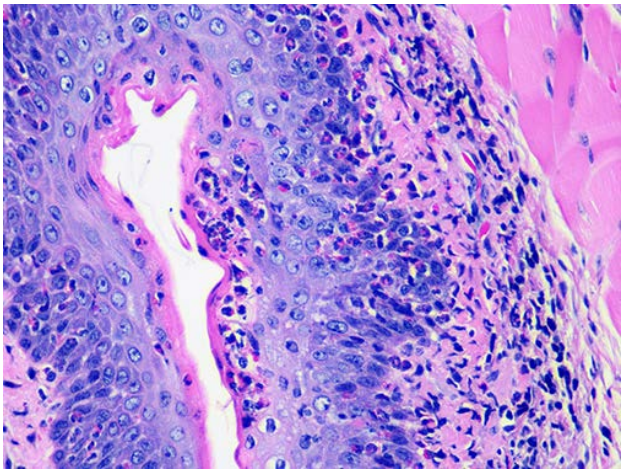
Taeko Noah[1], Kathryn Knoop[2], Keely G. McDonald[2], Jenny Gustafsson[2], Lisa Waggoner[1], Simone Vanoni[1], Matthew Batie[1], Maxime Mahe[1], Michael Helmrath[1], Rodney Newberry[2] and Simon Hogan[1]. [1]Cincinnati Childrens Hospital Medical Center, Cincinnati, OH; [2] Washington University School of Medicine, Saint Louis, MO

Food allergy is currently on the rise in the Western world. Clinical and experimental analyses have identified that food allergen: IgE complex cross linking of the FceR on mast cells and basophils drives the secretion of autacoid mediators which act on target organs including gastrointestinal, cutaneous, respiratory and cardiovascular systems resulting in the clinical manifestations of disease. While there has been extensive delineation of the immune pathways involved in the downstream effector response, very little is known about the process by which food allergens cross the intestinal epithelium and stimulate the allergic IgE-dependent inflammatory reaction. Here, we identify the presence of intestinal secretory epithelial antigen passages (SAPs) in the small intestine (SI) of food allergic mice that act as a conduit for food allergens to passage through the SI epithelium. We show that the SI SAPs are of the secretory epithelial lineage and consist of villus and cryptic goblet cells (GCs), enteroendocrine cells and Paneth cells; are induced by the key allergic cytokine, IL-13 in a CD38/cADPR dependent manner and are conserved in humans. *In vivo* analyses revealed that SAPs are required for passage of luminal food allergens across of the intestinal epithelium and induction of a food-induced anaphylactic phenotype in mice. SAPs are a mechanism by which food allergens cross the SI epithelium and permit IgE-mast cell activation and onset of the food allergic phenotype.

OR.77, T46. Ablation of NF-κB Inducing Kinase (NIK) Results in Eosinophilic Esophagitis (EoE) and Gastric Hyperplasia

Irving Coy Allen, Kristin Eden, Dylan McDaniel and Bettina Heid. Virginia Tech, Blacksburg, VA

NF-κB inducing kinase (NIK) is a central molecule in the noncanonical NF-κB signaling cascade and is essential for the production of unique effector molecules that significantly impact innate and adaptive immunity. Mice lacking NIK have been previously shown to develop a hypereosinophilic-like syndrome (HES). However, characterization of the gastrointestinal tract of these mice has been lacking. Here, we show that *Nik*$^{-/-}$ mice display significant eosinophilic esophagitis (EoE) that precedes many of the features associated with the previously characterized HES and has many features in common with human EoE. These mice demonstrate significant intraepithelial eosinophil accumulation and degranulation, microabscess formation, fibrosis, and basal cell hyperplasia. Additionally, the *Nik*$^{-/-}$mice display significant gastric hyperplasia most prominently at the gastroesophageal junction suggestive of chronic irritation due to reflux. Interestingly, eosinophil infiltration is localized to the esophagus and around the gastroesophageal junction; the caudal stomach, small intestines, and colons of these mice are unaffected, again similar to the human disease. Esophageal tissue of *Nik*$^{-/-}$ mice contains elevated mRNA levels of thymic stromal lymphopoietin (TSLP), a gene associated with EoE, as well as, major Th2 mediators IL-4 and IL-13. In a bioinformatics analysis of gene expression metadata from human EoE biopsy specimens, we found significant differences in gene expression associated with dysregulated noncanonical NF-κB signaling that is consistent with our findings in the *Nik*$^{-/-}$ mice. Together, these findings suggest that *Nik*$^{-/-}$ mice may useful as a model of human EoE and highlights a novel role for noncanonical NF-κB signaling in eosinophilic gastrointestinal disease.



T43. Intestinal Regulatory T Cells Induced by Kakkonto, a Traditional Japanese Herbal Medicine, Improve Therapeutic Efficacy of Oral Immunotherapy in Food Allergy Model Mice

Takeshi Yamamoto, Yuka Nagata, Michie Hayashi and Makoto Kadowaki. University of Toyama, Toyama, Toyama, Japan

Despite the increasing prevalence of food allergy (FA), no therapeutic drugs have been developed. Oral immunotherapy (OIT) has been considered as a hopeful therapy for FAs. However, the current OIT has a limit concerning the long-term efficacy and safety. We have previously demonstrated that kakkonto suppresses the occurrence of allergic symptoms in a murine FA model by inducing regulatory T cells in the colon. In this study, we developed an OIT model using the FA mice with already established allergic symptoms and evaluated the combined therapy of OIT with kakkonto in the OIT model. Methods: BALB/c mice were systemically sensitized and then orally challenged with ovalbumin. An OIT for the FA mice was performed by oral administration with increasing doses of OVA. Allergic symptoms were reduced in the OIT-treated FA mice. Furthermore, the combined therapy significantly enhanced the effectiveness of OIT on the allergic symptoms. OIT significantly downregulated the expression of IL-4, IL-5 and IL-13 mRNA in the FA mouse colon. The combined therapy further suppressed these Th2 cytokine mRNA expressions. In addition, OIT significantly elevated the population of $Foxp3^{+}$ $CD4^{+}$ regulatory T cells in the FA mouse colon, which was further increased by the combined therapy. Furthermore, the combined therapy reduced the expression of retinoic acid

metabolizing enzyme mRNA in the FA mouse colon. Kakkonto enhanced the therapeutic efficacy of OIT by inducting the intestinal regulatory T cells. These findings indicated that the combined therapy represents a promising approach for FA treatment.

T44. TGFβ Receptor Mutations Alter Peritoneal Mast Cell Development and Function

Mathilde Turfkruyer, Philip Dugan, Jessica Kinard and Pamela Frischmeyer-Guerrerio. National Institute of Allergy and Infectious Diseases, NIH, DHHS, Bethesda, MD

Mutations in *TGFBRI*, encoding the receptor for TGFβ, cause Loeys-Dietz syndrome (LDS), a Mendelian disorder associated with an increased risk of developing nearly all forms of allergic disease. To better understand the mechanisms responsible for this association, knock-in mice harboring LDS mutations have been developed, which recapitulate nearly all aspects of the human phenotype. Mast cells are the main effector cells of allergic disease and several studies have identified TGFβ as a critical mediator of mast cell development and function. We hypothesized that LDS mast cells are altered in a manner that would promote greater susceptibility to allergic outcomes. To address this possibility, *in vitro* assays were performed with peritoneal mast cells (pMCs) cultured in IL-3 and SCF and stimulated with IL-33, TGFβ, or IgE crosslinking agents. LDS pMCs expressed higher levels of ST2, the receptor for IL-33, and greater secretion of IL-9 following IL-33 stimulation. Furthermore, LDS pMCs were more resistant to apoptosis, exhibited greater degranulation following IgE receptor crosslinking, and showed less downregulation of FcεRI and c-kit following exposure to rTGFβ1. This was associated with reduced pSmad2/3 levels, a key signaling molecule in the TGFβ pathway. Furthermore, we demonstrated that following food allergy induction, LDS mice exhibited greater MC hyperplasia in the small intestinal lamina propria as well as in the peritoneum where LDS peritoneal mast cells showed enhanced secretion of IL-9. *In vivo* studies are ongoing to further examine the role of altered mast cell frequency and/or function resulting from altered TGFβ signaling in promoting food-induced anaphylaxis.

T45. Mast Cells Play a Critical Role in the Inflammatory Response in Food Allergy

Goele Bosmans[1], Morgane Florens[1], Iris Appeltans[1], Pedro J. Gomez-Pinilla[2], Thorsten B. Feyerabend[3], Hans-Reimer Rodewald[3], Gianluca Matteoli[1] and Guy E. Boeckxstaens[1]. [1]KU Leuven, Leuven, Vlaams-Brabant, Belgium; [2]University of Leuven, Leuven, Vlaams-Brabant, Belgium; [3]German Cancer Research Center, Heidelberg, Baden-Wurttemberg, Germany

Mast cells (MCs) have been identified as key effector cells in allergic inflammation. This insight is mainly based on experiments using Kit mutant mice or MC depleting methods, not only affecting MCs but also other cell types. To avoid this confounding effect, we used *Cpa3*$^{Cre/+}$ MC-deficient mice, shown to have a normal immune system, to investigate the exact contribution of MCs in a model of food allergy. We confirmed that naïve *Cpa3*$^{Cre/+}$ mice were devoid of MCs in the mucosa of the small bowel. After induction of food allergy, MCs significantly accumulate in the gut mucosa of *Cpa3*$^{+/+}$ mice upon OVA challenges. MC accumulation was completely abolished in *Cpa3*$^{Cre/+}$ mice. The increase in MC number and activity coincided with the development of severe diarrhea in *Cpa3*$^{+/+}$ littermates (80-90%), while none of the *Cpa3*$^{Cre/+}$ mice developed diarrhea. Induction of food allergy resulted in a significant increase of Th2-associated genes IL-4 and IL-5 and inflammatory genes IL6, CXCL1 and CXCL2 in *Cpa3*$^{+/+}$ while lower inflammatory response was present in *Cpa3*$^{Cre/+}$ mice. Of note, lack of MCs did not affect OVA-specific IgE, IgG1 or IgG2a production, as serum levels of these antibodies were similarly increased in both *Cpa3*$^{+/+}$ and *Cpa3*$^{Cre/+}$ mice. Mast cells seem to play an important role in augmenting the Th2 inflammatory response in food allergy. Furthermore, our data confirm that MCs are the crucial effector cells for the development of diarrhea, making them an interesting target for treatment in food allergy.

T48. Desensitization of Mucosal Mast Cells Promotes Intestinal-Tolerance in Oral Immunotherapy

Yosuke Kurashima[1], Yoshihiro Takasato[2], Daiki Yamamoto[2] and Hiroshi Kiyono[2]. [1]Chiba University, Minato-ku, Tokyo, Japan; [2]University of Tokyo, Minato-Ku, Tokyo, Japan

Food allergy is estimated to affect about 5 percent in children and adults, and occasionally causes life-threatening reactions. Especially food allergy in infant and child shows gastrointestinal symptoms, including diarrhea, vomiting, and abdominal pain. To cure the food allergy, allergen inoculation from oral rout to the patient called oral immunotherapy, OIT, is potentially effective and new developed therapeutic; however, most patient experience allergic reactions during the therapy due to the lack of basic understandings of immunological events at intestinal mucosa. In this study, we analyzed OIT murine model and newly found OIT effectively suppressed degranulation of mucosal mast cells and simultaneously promoted mast cell-mediated regulatory T cell (Treg) inductions in the local intestinal mucosa. The gene profiling of activated and desensitized mast cells revealed that the desensitization process prone mast cells to allergic type to immune regulatory type. Thereby, mice depleting mast cell during OIT process led diminish of Treg. The finding newly uncovered that effective modification of mast cells by transient desensitization of OIT is important for the safe and successful determination and Treg-dependent tolerance induction of OIT.

HIV

M.05, F29. Myeloid Cells from Human Cervical Tissue Express Siglec-1 and Capture HIV-1

Jon Cantero-Pérez[1], Daniel Perez-Zsolt[2], Itziar Erkizia[2], Alba Hernández-Gallego[3], Laia Pérez-Roca[3], Irian Lorencés[3], Julio Garrido[4], Antoni Tarrats[3], Javier Martinez-Picado[2,5], Nuria Izquierdo-Useros[2] and Meritxell Genescà[1]. [1]Health Sciences Research Institute Germans Trias i Pujol Foundation, Badalona, Catalonia, Spain; [2]AIDS Research Institute IrsiCaixa, Badalona, Catalonia, Spain; [3]Hospital Universitari Germans Trias i Pujol, Badalona, Catalonia, Spain; [4]Hospital Municipal de Badalona, Badalona, Catalonia, Spain; [5]Institució Catalana de Recerca i Estudis Avançats, Badalona, Catalonia, Spain

The HIV/AIDS pandemic is primarily sustained by heterosexual transmission of HIV-1. Antigen presenting cells located in the cervical mucosa may disseminate and amplify viral infection through different mechanisms, including *trans*-infection. *Trans*-infection allows for viral internalization within myeloid cells, which enhances bystander $CD4^+$T cell infection. Viral capture by myeloid cells relies on the sialic acid binding immunoglobulin-like lectin-1 (Siglec-1/CD169), which is a type-I interferon (IFN) inducible receptor highly expressed on activated myeloid cells. Here we addressed Siglec-1 expression by flow cytometry in human cervicovaginal tissue obtained from women undergoing hysterectomy for benign non-inflammatory causes. Siglec-1-mediated viral capture was assessed by incubating cell suspensions with fluorescent HIV-like particles lacking the envelope glycoprotein (VLP), which are captured as efficiently as infectious HIV-1 via Siglec-1. Sub-cellular localization analyses were performed using Amnis® equipment. Siglec-1 was expressed in $HLA\text{-}DR^+CD3^-$ myeloid cells from cervical mucosa, being more frequent in $CD11c^{dim}CD14^+$ cells, followed by $CD11c^+CD14^+$ cells and $CD11c^+CD14^-$ cells. Moreover, in the $CD14^+$ subsets, Siglec-1^+ cells were more frequent in ectocervix than endocervix ($P < 0.05$). Viral capture assays revealed that fluorescent VLP co-localized with Siglec-1 and, accordingly, this capture was more frequent in ectocervix-derived cells. Thus, myeloid subpopulations present at the cervical mucosa express Siglec-1 and are able to capture HIV-1. Since IFNa is upregulated during HIV-1 primary infection and Siglec-1 expression is potently enhanced by this cytokine, we propose that Siglec-1^+ myeloid cervical cells may facilitate HIV-1 capturing during the early stages of infection, allowing transfer to bystander $CD4^+$T cells and thus facilitating initial infection and dissemination.

OR.11, W19. IFN ε Has Potent Immune Functions and Protects Primary Macrophages Against HIV

Carley Tasker[1], Darine El-Naccache[1], Selvakumar Subbian[1], Pan Gao[2], Jennifer Couret[1], Carly Levine[1], Saleena Ghanny[1], Patricia Soteropoulos[1], Xilin Zhao[1], Nathaniel Landau[3], Wuyuan Lu[2] and Theresa Li-Yun Chang[1]. [1]Rutgers, New Jersey Medical School, Newark, NJ; [2]University of Baltimore School of Medicine, Baltimore, MD; [3]New York University, New York, NY

Interferon ε (IFNε) is a unique type I IFN that is not induced by pattern-recognition response elements. IFNε is constitutively expressed in mucosal tissues including the female genital mucosa. IFNε exhibits a potent protective activity against *Chlamydia muridarum* and herpes simplex virus 2 in mice, possibly through immune regulation, despite the fact that the direct anti-viral activity of IFNε is weak compared to IFNα. Our results indicated that IFNε stimulated a broader range and higher levels of cytokines/chemokines than IFNa2 in macrophages and PBMCs. It induced an anti-HIV state in primary macrophages through a mechanism independent from known type I IFN-induced HIV host restriction factors. Transcriptome analysis indicated that IFNε elicited a recognizable type I IFN signature, but that the IFNε signature exhibited unique features distinguishing it from the IFNa2 signature. IFNε induced phagocytosis and reactive oxygen species that contributed to anti-HIV activity. Our findings indicated that IFNε induced an antiviral state that was mediated by different factors than those induced by IFNa in primary macrophages. Understanding the mechanism of IFNε-mediated HIV inhibition through immune modulation has implications for prevention.

OR.50, T51. Association of Gut Microbiome with Mucosal Immune Activation and SHIV Viral Transmission

Yongjun Sui[1,2], Amiran Dzutsev[2], David Venzon[2], Blake Frey[2], Giorgio Trinchieri[2] and Jay Berzofsky[2]. [1]National Institues of Health, Bethesda, MD; [2] National Cancer Institutes, Bethesda, MD

It is unknown whether the gut microbiome affects HIV transmission. In our recent SHIV vaccine study, in which 44% vaccine efficacy (p = 0.028) was achieved, we found that the naïve rhesus macaques from two different sources had significantly different rates of infection against repeated low-dose intrarectal challenge with SHIVSF162P4. Because the two naïve groups came from different sources, we hypothesized that their rectal mucosal immune activation and microbiomes may differ. We measured the $CD4^+CCR5^+Ki67^+$ T cells by flow cytometry using rectal mucosal single suspension cells, and examined the gut microbiome by 16S rDNA MiSeq using the fecal samples collected one-week before the serial SHIV viral challenges. We found that the more susceptible group of 7 macaques (N7) in the original shipment (as well as the 21 vaccinated animals from the same source in pre-immunization rectal biopsies) had significantly more activated $CD4^+CCR5^+Ki67^+$ T cells in the rectal mucosa than the more resistant group of 11 naive macaques from a different source. The prevalence of pre-challenge activated rectal CD4 T cells in the naïve macaques correlated inversely with the number of challenges required to infect. The groups also differed significantly by principal component analysis of activation markers, with only N7 matching the vaccinated group. Furthermore, after sequencing 16s rRNA, the preliminary principle component analysis of the gut taxa showed that these two cohorts had different microbial compositions even after more than 5 months of co-housing. We found differences between the two naive groups that correlated with immune activation status. For example, significantly lower ratios of *Bacteroides* to *Prevotella*, and significantly lower levels of *Firmicutes* were found in the susceptible cohort. These parameters also inversely correlated with high levels of immune activation in the rectal mucosa. We are currently validating some of the results using qPCR. Host-microbiome interactions might influence the HIV/SIV mucosal transmission through effects on mucosal *T cell* activation.

OR.51, T50. Simultaneous Expression of Interferon-Gamma and Interleukin-22 from Innate Lymphoid and Natural Killer Cells in the Colon of SIV-Infected Rhesus Macaques

Andrew Cogswell[1], Stephanie Dillon[2], Moriah Castleman[2] and Cara Wilson[2]. [1]Rush University Medical Center, Chicago, IL; [2] University of Colorado Anschutz Medical Campus, Aurora, CO

Breakdown of intestinal epithelial integrity occurs during HIV-infection leading to microbial translocation, which correlates with increased mucosal and systemic immune activation. Maintenance of intestinal barrier integrity relies in part on the presence of interleukin (IL)-22. Recent findings indicate that innate lymphoid cell type 3 (ILC3) display a degree of functional plasticity and can produce IL-22 or inflammatory cytokines depending on the local cytokine milieu produced by mucosal myeloid dendritic cells (MDCs). In our studies, colonic MDCs of SIV-infected rhesus macaques spontaneously produced IL-23, IL-1b and IL-12 while the same cells from uninfected animals only produced IL-23 and IL-1b. We therefore hypothesized that colonic ILC1s, ILC3s and natural killer cells (NK) cells in context of HIV-infection lead to production of IL-22 and IFN-g. Multi-color flow cytometry was utilized to identify the various ILC and NK cell populations and measure expression of transcription factors (Tbet, RORgt) and frequencies of cytokine-expressing cells in the absence of stimulation (*i.e.,* spontaneous production) to best reflect their *in vivo* cytokine profiles. Regardless if colonic innate lymphocytes were ILC1s, ILC3s or NK cells, the majority co-expressed both T-bet and RORgT and co-produced IFN-g and IL-22 when acquired from colons of SIV-infected rhesus macaques. These same cells from colons of SIV-uninfected animals expressed predominately IL-22 with little to no IFN-g. These findings indicate that the presence of inflammatory cytokines not loss of IL-22 by ILCs and NK cells during SIV infection may increase intestinal epithelial barrier permeability and enhanced bacterial transcytosis.

OR.52, T56. Expression of Immune Activation Markers does not Correlate Across Cervix, Rectum and Blood

Catia T. Perciani[1], Walter Jaoko[2,3], Bashir Farah[3], Omu Anzala[2,3], Mario Ostrowski[1], Kavi Team[3] and Kelly MacDonald[2,4]. [1]University of Toronto, Toronto, ON, Canada; [2]University of Nairobi, Nairobi, Kenya; [3]Kenya AIDS Vaccine Initiative, Nairobi, Kenya; [4]University of Manitoba, Toronto, ON, Canada

The lack of normative immune activation values in mucosal sites can mislead study designs. Differentiating cellular phenotype and disposition in the genital and gastrointestinal tracts will direct studies in the HIV prevention and treatment fields. In this study, we analyzed a diverse set of immune activation parameters in samples obtained from healthy Kenyan women (n=45) enrolled at KAVI-VZV001 trial (ClinicalTrials.Gov: NCT02514018). Plasma, cervico-vaginal and rectal secretions were tested for a combination of 14 cytokines, including IL-8, MIP-1α, IL-1α, IL-1β, IL-17 and TNFα, using MSD U-PLEX platform. Fresh blood, cervical and rectal $CD4^+$*T cell*s were analyzed by flow cytometry for expression of CCR5, α4, β7, CD69, HLA-DR, CD38, and Ki67. Friedman, Wilcoxon matched-pairs tests and Spearman's correlations were performed using SPSS, and p-values adjusted for multi-comparisons (step-down procedure). With the exception of MIP-3α, all cytokines measured were significantly higher in cervico-vaginal secretions than in plasma and rectal secretions ($p < 0.0001$). Cervix also harbored higher frequency of $CD4^+$Tcells expressing CCR5 and co-expressing HLA-DR and CD38 ($p < 0.01$). Overall the frequency of $CD4^+$Tcells expressing CCR5, α4β7, α4β1, CD69, Ki67, HLAD-DR and CD38 as well as secreted cytokines were significantly different across the three tissues studied. CD69 expression in $CD4^+$Tcells was the only marker showed to correlate between rectum and cervix ($r_s=0.525$, $p=0.005$) and between rectum and blood ($r_s=0.485$, $p=0.026$) after adjusting for multiple comparisons. Hence, due to their unique immune activation signatures, responses in one compartment may not be predictive across other sites, which should be taken into account when developing potential HIV prevention strategies.

OR.54, T53. Rectal and Vaginal Biopsies from Men and Women Infused Intravenously with VRC01 Show Protection in *ex vivo* HIV-1 Challenge

Maria P. Lemos[1], Rena Astronomo[1], Sandeep Narpala[2], Julie Czartoski[1], Katharine Westerberg[1], Lamar Fleming[1], Mary Gross[1], John Hural[1], Hong Van Tieu[3], Lindsey Baden[5], Benigno Rodriguez[6], Scott Hammer[7], Ian Frank[8], Christina Ochsenbauer[9], Nicole Grunenberg[1], Julie Ledgerwood[2], Adrian McDermott[2], Kenneth Mayer[10] and Juliana McElrath[1]. [1]Fred Hutchinson Cancer Research Center, Seattle, WA; [2] National Institute of Health, Washington, DC; [3] New York Blood Center, New York, NY; [5] Brigham and Women's Hospital, Boston, MA; [6] Case Western Reserve University School of Medicine, Cleveland, OH; [7] Columbia University Medical Center, New York, NY; [8] University of Pennsylvania, Philadelphia, PA; [9] University of Alabama at Birmingham, Birmingham, AL; [10] Fenway Health, Boston, MA

VRC01 is a potent, broadly neutralizing monoclonal antibody whose clinical safety, pharmacokinetics and functionality were tested in HVTN104; efficacy trials are ongoing. Our sub-study evaluated intravenous infused VRC01 distribution and protection at mucosal sites of HIV-1 entry. Of 20 eligible HVTN104 subjects, 7 men and 5 women were enrolled and had received at least two 10 or 30 mg/kg VRC01 infusions every 2 months. Serum, rectal (men) and vaginal (women) biopsies and secretions were collected once 4-13 days post-infusion. Similar samples were collected from 10 male and 8 female untreated controls. VRC01 levels were quantifiable by Singulex in serum (median 0.0046 VRC01/protein, IQR 0.0031-0.0083), rectal tissue (median 0.00011 VRC01/protein; IQR $5.41x10^5$-0.00015), rectal secretions (median 0.00014 VRC01/protein; IQR $2.65x10^5$-0.00018), vaginal tissue (median $5.83 x10^5$ VRC01/protein; IQR 4.49x105-0.00022) and cervical secretions (median 0.00083 VRC01/protein; IQR 0.00042-0.00266) from all VRC01 recipients, but undetectable in the controls, indicating that infused VRC01 reaches cervicovaginal and rectal mucosa. Serum VRC01/IgG ratios from VRC01 recipients correlated with paired rectal tissue ($r=0.86$, $p=0.02$) and rectal secretions ($r=0.93$, $p=0.007$), but serum, vaginal tissue, and cervical secretion ratios were not significantly correlated. *Ex vivo* $HIV\text{-}1_{Bal26}$ challenge infected 4/21 rectal explants from 7 male VRC01 recipients whereas 16/19 explants from 5

controls were infected (Mann-Whitney p=0.0012). 0/13 vaginal explants from 5 VRC01 recipients, compared to 23/28 explants from 8 controls were infected (Mann-Whitney p=0.019). Thus, infused VRC01 distributes into female genital and male rectal tissue, and can partially protect against *ex vivo* mucosal challenge with a VRC01-sensitive HIV strain.

T52. Sex-dependent Memory Impairment in HIV-1-transgenic (Tg)26 mice: Potential regulation by gut microbiota?

Shamsudheen Moidunny[1], Michael Benneyworth[2], Joyce Meintz[2], Mark Thomas[2] and Sabita Roy[3]. [1]Miller School of Medicine, University of Miami, Miami, FL; [2] University of Minnesota, Minneapolis, MN; [3] Department of Surgery, Miller School of Medicine, University of Miami, Miami, FL

Progressive HIV-1 infection, despite aggressive anti-retroviral regimens, elicits demonstrable neurocognitive impairments (NCI) such as anxiety, depression and dementia. In order to develop efficient treatment strategies against NCI, a detailed understanding of the underlying mechanisms is important. Here, we investigated whether the HIV-1-transgenic (Tg)26 mice that express 7 out of 9 HIV-1 viral genes, is a suitable animal model to study molecular mechanisms as well as sex differences in HIV-associated dementia (HAD). We observed that adult Tg26 males show an impaired spatial reference memory and contextual-fear memory, compared to their wild-type littermates. However, learning and memory in Tg26 females was similar to that of their wild-type controls. The memory impairment in Tg26 male animals was independent of neurotoxicity, as the number of neurons in their hippocampus was similar to that of wild-type littermate controls. On the other hand, Tg26 males showed reduced neurogenesis in the hippocampal dendate gyrus, which has been associated with Alzheimer's dementia and HAD. Emerging evidence indicate that altered gut microbiota could influence development of glial cells in the central nervous system and modulates microglial activity in a mouse model of Alzheimer's disease. We propose that the observed sex-dependent memory impairment in Tg26 mice might be due to an alteration in the gut microbial diversity.

T54. Bad Romance: Defining the Link Between *Neisseria gonorrhoeae* and HIV

Stacey X. Xu, Danila Leontyev, Rupert Kaul and Scott D. Gray-Owen. University of Toronto, Toronto, ON, Canada

Human immunodeficiency virus (HIV) is a sexually transmitted infection affecting millions of people worldwide. With no existing cure and development of resistance against current antiviral drugs, there is a pressing need to reduce transmission of the virus and limit disease progression to acquired immune deficiency syndrome (AIDS). Clinical observations show that gonorrhea infection increases HIV shedding and transmission, and enhances an individual's susceptibility to HIV infection upon exposure. While *in vitro* studies of interactions between HIV and *Neisseria gonorrhoeae* (Ngo)have elucidated processes with the potential to drive transmission, these have not been considered *in vivo* because HIV and Ngo are both human-restricted pathogens. Here, we describe the development and initial findings from the first animal model of HIV and Ngo co-infection, using immunodeficient NSG mice reconstituted with a human immune system via hematopoietic stem cell transplantation. While it had not previously been appreciated, we observe that systemic HIV infection results in local shedding of virus in the female genital tract of humanized mice, reflecting the phenomenon seen in humans. While plasma levels of virus were not notably impacted by vaginal Ngo infection, the co-infected mice showed elevated shedding of HIV into the genital tract. Ongoing work aims to reveal the relative contribution of Ngo-derived factors and infection-induced inflammatory responses to this localized increase in HIV within the co-infected mucosa. Moreover, this model lays the foundation for future work aiming to intervene in the pathogenic synergy between these two devastating pathogens.

T55. Opioid-Induced Chronic Intestinal Inflammation Accelerates HIV Disease Progression

Jingjing Meng, Santanu Banerjee, Li Zhang, Bin Li, David Robbins and Sabita Roy. University of Miami, Miami, FL

Previous clinical and laboratory studies have provided substantial evidence establishing that opioid abuse is associated with higher risk of HIV infection and accelerated HIV disease progression. However, the mechanisms by which opioid use promotes disease progression in HIV-infected individuals still remain elusive. In the current study, the analysis of intestinal tissues from patients and BLT humanized mice indicate that opioids compromise intestinal epithelial barrier function in infected individuals and the compromised barrier function was associated with goblet cell hyperplasia in the gut epithelium with over-expression of pro-inflammatory cytokines such as IL-6. To investigate whether intestinal barrier dysfunction and goblet cell hyperplasia result from the direct effects of opioid exposure, 3D culture of mouse intestinal crypts was developed and treated with morphine, lipopolysaccharide (LPS), or IL-6. Treatment of intestinal crypt cells with LPS and IL-6 inhibited crypt cell proliferation and modulated crypt cell differentiation, resulting in disorganization of intestinal tight junction proteins. We further demonstrate that opioid treatment in the context of HIV infection exacerbated goblet cell hyperplasia and compromised epithelial barrier function thus contributing to accelerated HIV disease progression.

T57. Elevated Frequencies of Myeloid and CD4 T-Cells Presenting Tolerogenic/Regulatory Profiles in the Genital Mucosa of HIV-1 Highly Exposed Seronegative (HESN) Female Commercial Sex Workers

Lyvia Fourcade[1], Valérie Thibodeau[1], Annie-Claude Labbé[2], Michel Alary[3], Fernand Guédou[4], Johanne Poudrier[1] and Michel Roger[1]. [1]CRCHUM, Montreal, PQ, Canada; [2]Université de Montreal, Montreal, PQ, Canada; [3]Centre de Recherche du CHU de Québec, Quebec, PQ, Canada; [5]Dispensaire des IST, Cotonou, Benin

The majority of HIV infections are acquired by women through heterosexual intercourse, particularly in sub-Saharan Africa. We established an ongoing cohort of highly HIV-1-exposed female commercial sex workers (CSWs), in Cotonou (Benin), in which we have identified individuals who remain HIV-1-uninfected (resistant) after more than 4 years of active prostitution. This is a model of natural immunity against the virus, and they maintain low genital inflammatory conditions to prevent HIV infection. HIV-1 interacts with many receptors such as toll-like receptors (TLR)-7/8 to induce interferon (IFN)-a, an important antiviral and immunomodulatory cytokine, which act together with interleukin (IL)-10, human leukocyte antigen (HLA)-G and immunoglobulin-like transcript (ILT)-4 to initiate a “tolerogenic/regulatory” anti-inflammatory loop. We have characterised TLR-7, IFN-a, IL-10, HLA-G and ILT-4 expression profiles in the genital tract of female CSWs and HIV-1-uninfected non-CSWs from Benin. Endocervical myeloid HLA-DR$^+$ cells from HESN CSWs expressed higher levels of IFN-α, TLR-7, IL-10 and HLA-G than those from both HIV-1-infected CSWs and HIV-1-uninfected non-CSWs. Further characterization of the endocervical myeloid HLA-DR$^+$ cells in HESN CSWs revealed a population of ‘’tolerogenic’’ CD103$^+$CD14$^+$CD11c$^+$ myeloid cells expressing high levels of IFN-α and IL-10. Concomitantly, HESN CSWs had higher frequencies of endocervical regulatory CD4$^+$ *T cell*s when compared to those from the two other groups of women. These data suggest thattolerogenicmyeloid cells and regulatory *T cell*s could have a role in the modulation of mucosal responses associated with the protection against HIV.

Host-Microbiota Interactions

F30. Diet-Induced Obesity Increased the Host Susceptibility to *Mycobacterium tuberculosis* Infection and is Associated with Alterations of Gut Microbiota

Sandra Palma Albornoz, Thaís Barbosa Bertolini, Daniela Carlos, Rômulo Silva De Oliveira, Ana Flávia Gembre, Isis Do Carmo Kettelhut, Leandra Zambelli Ramalho and Vânia Luiza Deperon Bonato. University of São Paulo, Ribeirão Preto, Sao Paulo, Brazil

Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis (TB) that affects 10.4 million people worldwide and it causes at least 1.8 million died. TB is attributed to social and economic factors, host's genetic background and malnutrition. Furthermore, obesity increases the risk factor for pulmonary diseases that are associated with lung injury and inflammation response. Therefore, our aim was to evaluate if the obesity affects the outcome of Mtb infection in murine model. C57BL/6 female mice were fed either with low-fat diet (LFD) or high-fat diet (HFD) for 90 days. At 60 days, mice were infected with $1x10^5$ bacilli of Mtb by intra-tracheal route. At 30 days post infection, the glucose tolerance test, the body weight gain and adipose tissue accumulation were determined. Lungs were collected to quantify Colony Forming Unit (CFU), histopathological and flow cytometry analysis. In parallel, gut microbiota composition was evaluated from stools. Compared with LFD infected mice, HFD infected mice showed higher body weight, adipose tissue deposition, more glucose intolerance, a higher CFU number and inflammatory infiltrate in the lung, also a higher percentage of $IFN\gamma^+$ and $IL\text{-}17^+$ producing $CD4^+$ cells. Finally, HFD infected mice exhibited an abundance of *Firmicutes* over *Bacteroidetes* phylum compared to the LFD infected mice. These results show that HFD infected mice were more susceptible to Mtb infection and exhibited a higher inflammatory immune response in the lung. Futher experiments are undergoing to evaluate whether progression of infection and magnitude of lung inflammation could associate with alterations of the gut microbiota.

F31. *Helicobacter hepaticus* is Protective Against *Citrobacter rodentium* Infection

Theodore James Sanders, Johannes Pettmann, Sofia Nordlander and Kevin Maloy. University of Oxford, Oxford, England, United Kingdom

Although *Helicobacter hepaticus* infection induces colitis in mice lacking IL-10, it persistently colonises the caecum and colon of wild-type mice without inducing clinical pathology. This led us to examine whether chronic *H. hepaticus* infection confers any host benefits. The mouse enteric pathogen *Citrobacter rodentium*, which is closely related to enteropathogenic *E. coli*, causes an acute, self-limiting colitis associated with the formation of attaching and effacing lesions. We have previously observed that mice lacking the inflammasome adaptor protein ASC are highly susceptible to *C. rodentium* infection. However, we found that prior colonisation with *H. hepaticus* led to significantly reduced weight loss during *C. rodentium* infection of $Asc^{-/-}$ mice and was associated with reduced colonisation and pathology of the caecum. Similar protection was also observed when using a *H. hepaticus* mutant lacking a functional type 6 secretion system, as well as in mice lacking the inflammasome sensor protein NLRC4. Furthermore, $Asc^{-/-}$ mice pre-colonised with *H. hepaticus* were completely protected when inoculated with *C. rodentium* via the faecal-oral route, including a dramatic reduction in pathology of the caecum and distal colon. *H. hepaticus* therefore provides a host benefit in colonization resistance against *C. rodentium*, which is especially apparent following infection by the natural faecal-oral route. Insight into the mechanism behind such resistance could be used to develop novel therapies against human enteric infection.

F32. Sialylated Milk Oligosaccharides Promote Microbiota-Dependent Immune and Skeletal Development in a Model of Infant Undernutrition

Carrie Cowardin[1], Philip Ahern[1], Janaki Guruge[1], Michael Barratt[1], Daniela Barile[2], David Mills[2] and Jeffrey Gordon[1]. [1]Washington University in St. Louis, St. Louis, MO; [2] University of California at Davis, Davis, CA

Childhood undernutrition is a pressing global health challenge, contributing to over 3 million deaths annually. Impaired linear growth (stunting) is a major complication of undernutrition. The mechanisms that contribute to stunting in children with undernutrition remain unclear. We previously reported (Cell ***164***,859 (2016)) that breast milk from Malawian mothers of undernourished infants contains significantly lower levels of sialylated oligosaccharides compared to breast milk from mothers of healthy infants. Moreover, gnotobiotic mice colonized with bacterial strains generated from the fecal microbiota of a severely stunted, underweight Malawian infant, and fed a representative Malawian diet supplemented with purified sialylated bovine milk oligosaccharides (S-BMO) exhibited significantly greater increases in lean body mass gain compared to controls. Microcomputed tomography of femurs demonstrated that dietary supplementation with S-BMO also affects bone growth. S-BMO produces significant reductions in mature osteoclasts in tibial trabecular bone, significant reductions in bone marrow granulocyte-monocyte progenitors that give rise to osteoclasts, and significant reductions in serum C-Terminal Telopeptide of Type I Collagen (CTX-I), a biomarker of osteoclast activity. Studies of a cohort of Bangladeshi children prior to and after treatment for severe acute malnutrition (SAM) revealed that serum CTX-I levels were significantly increased prior to and subsequently fell after treatment with ready to use therapeutic foods. Serum proteomics likewise demonstrated significant alterations in immune mediators following treatment for SAM. Together these results emphasize the role of increased osteoclastic activity in the stunting that accompanies severe acute malnutrition, and suggest S-BMO as a tool for further understanding its underlying molecular mechanisms.

F33. Distinct Bacteria Dictating IgA and TGF-β Expression in the Intestine

Hyun-Dong Chang, Alexander Beller and Andreas Radbruch. DRFZ Berlin, Berlin, Germany

Immunoglobulin A (IgA) is the predominant class of antibodies in mucosal intestinal immune responses and is an integral part of the mucosal intestinal barrier. While originally IgA expression in the mucosa was considered to be an intrinsic property of the mucosal immune system, later evidence suggested that it is induced by stimulation of the mucosal immune system by the microbiota as such. However, it has been unclear which bacteria and how they enhance expression of mucosal IgA. By comparing the microbiota composition of conventionally held mice with high intestinal IgA levels to ones with low intestinal IgA levels, we have now identified distinct bacteria which selectively induces IgA expressing germinal center B cells in Peyer's patches and IgA secreting plasma cells in the small intestinal lamina propria, resulting in increased mucosal IgA. These bacteria control IgA expression presumably by inducing the IgA class switch inducing cytokine TGF-beta in T follicular helper cells in the Peyer's patches. Taken together our data underline that intestinal IgA dominance can be conferred by distinct members of the microbiota which qualify as essential players in the dialogue between the microbiota and the immune system.

F34. IL-6 Producing Intraepithelial Lymphocytes Protect Against *C. Rodentium* Infection through Modulation of Epithelial Barrier Integrity

Kristine A. Kuhn, Hanna M. Schulz, Alyssa K. Whitney, Eric L. Campbell and Sean P. Colgan. University of Colorado School of Medicine, Aurora, CO

Citrobacter rodentium infection of mice is frequently used as a model to study host responses during pathogenic gastrointestinal infection. IL-6 signaling in the colon is required for protection against *C. rodentium* infection; however, the cellular source of IL-6 for this protection is unclear. Our previous work has identified IELs as one source of IL-6 in the colon. Therefore, we hypothesized that IL-6 produced by colonic IELs was sufficient to provide protection during *C. rodentium* infection. To test our hypothesis, we orally infected mice with *C. rodentium* following transfer of donor IL-$6^{+/+}$ or IL-$6^{-/-}$ IELs into IL-$6^{+/+}$ or IL-6^{-}

$^{/-}$ recipients. IL-6$^{-/-}$ mice that received IL-6$^{-/-}$ IELs had significantly more weight loss, increased intestinal histopathology, and increased bacterial translocation after 12 days of infection compared to transfer of IL-6$^{+/+}$ IELs into recipient IL-6$^{-/-}$ mice and IL-6$^{+/+}$ IELs into IL-6$^{+/+}$ mice. IEL-derived IL-6 is functionally important in the maintenance of the epithelial barrier as IL-6$^{-/-}$ mice were noted to have increased paracellular permeability, decreased claudin-1 expression, and a thinner mucus-gel layer, all of which were reversed by transfer of IL-6$^{+/+}$ IELs. *In vitro* studies utilizing model epithelia confirmed IL-6 was able to signal and increase claudin-1 and mucin-2 expression. Therefore, we conclude that IL-6 expression by IELs is sufficient to restore protection against *C. rodentium* infection through modulation of the epithelial barrier integrity.

F35. Human Gut Microbes Are Susceptible to Antimicrobial Food Additives: *In vitro* Studies

Lucia Hrncirova[1], Tomas Hudcovic[2], Jan Krejsek[3] and Tomas Hrncir[2]. [1]Charles University, Faculty of Medicine in Hradec Kralove, Novy Hradek, Kralovehradecky kraj, Czech Republic; [2] Institute of Microbiology of the CAS, Novy Hradek, Kralovehradecky kraj, Czech Republic; [3] Charles University, The Faculty of Medicine in Hradec Kralove, Hradec Kralove, Kralovehradecky kraj, Czech Republic

The role of gut microbiota in human health and disease is becoming increasingly evident. Environmental factors, especially food and its components, are one of the major factors influencing the composition of gut microbiota and its function. The aim of this project was to test the hypothesis that antimicrobial food additives may alter the composition of human gut microbiota by selectively suppressing the growth of susceptible gut microbes. We have found that some human gut microbes are highly susceptible to food additives while others are resistant. Surprisingly, gut microbes with known anti-inflammatory properties were susceptible to additives while microbes with known pro-inflammatory or colitogenic properties were mostly resistant. The most potent antimicrobial food additive was sodium nitrite and its combinations, and a benzoate-nitrite-sorbate combination was the most synergistic. Our data show that some human gut microbes are highly susceptible to antimicrobial food additives. We speculate that permanent exposure of human gut microbiota to even low levels of additives may modify the composition and function of gut microbiota and thus influence the host's immune system. Whether the effect of additive-modified gut microbiota on the human immune system could explain, at least in part, the increasing incidence of allergies and autoimmune diseases remains to be shown.

F36. Deficiency of IgA Induces Microflora Alteration and Ileal Inflammation

Takashi Nagaishi, Taro Watabe, Akinori Hosoya, Nisha Jose, Yudai Kojima, Naoya Tsugawa, Takahiro Adachi and Mamoru Watanabe. Tokyo Medical and Dental University, Tokyo, Tokyo, Japan

Background & Aim: It has been reported that the lack of Ig class-switching in B cells results in aberrant composition of microflora in the gut. However, it is still unclear whether this was directly caused by the lack of secreted IgA. Therefore, we generated an animal model of IgA deficiency (IgA$^{-/-}$) by using the CRISPR/Cas9 system. Methods & Results: The guide RNAs specific for IgE and IgA cytoplasmic domains and Cas9 mRNA were injected into C57BL/6 zygotes. Sequencing of the region between IgE and IgA tails from the offsprings revealed that one of these had a deletion of the entire IgA region and thus identified as IgA$^{-/-}$. Histopathologcal analysis revealed inflamed ileal mucosa and no significant changes in other organs. Flow cytometry analysis showed increased CD4^{+} T cells in the lamina propria associated with increased production of IFN-γ and IL-17 in the IgA$^{-/-}$ mice. Electron microscopy and intra-vital imaging revealed increased segmented filamentous bacteria on the epithelia and activated B cells in Peyer's patches, respectively, at the IgA$^{-/-}$ ileum. Conclusion: Our results imply that the lack of IgA may induce altered microflora and disregulated mucosal homeostasis in the gut. Additional experiments are currently ongoing.

F37. Antimicrobial Food Additives Influence the Composition and Diversity of the Human Gut Microbiota: Studies in Germ-Free Mice

Tomas Hrncir[1], Lucia Hrncirova[2], Tomas Hudcovic[1] and Jan Krejsek[2]. [1]Institute of Microbiology of the CAS, Novy Hradek, Kralovehradecky Kraj, Czech Republic; [2]Charles University, Hradec Kralove, Kralovehradecky Kraj, Czech Republic

The incidence of allergies and autoimmune diseases is increasing worldwide. Recent data suggest that gut microbiota can modulate not only local but also systemic immune responses. In this study, we focus on environmental factors, specifically food additives, which may modify the composition of gut microbiota and thus influence host's immune responses. To address this issue, we have administered either sterile water or water supplemented with antimicrobial food additives to wild-type and Nod2-deficient C57BL/6 mice colonized with human microbiota. The daily intake of additives was calculated to match the maximum daily intake reached in human populations in Europe. We have analyzed the effect of additives on microbial composition and diversity by amplification and high-throughput sequencing of the hypervariable regions of the 16S rDNA genes. The resulting sequences were processed using CLcommunity software. Our experimental data indicate a significant effect of antimicrobial food additives on the composition and diversity of the human gut microbiota.

F38. Differential Effects of In-Feed Antibiotic Bacitracin on Composition of Cecal Microbiota and Host Cecal Transcriptome in Commercial Turkeys

Matthew J. Sylte, Looft Torey and Timothy A. Johnson. United States Department of Agriculture-Agriculture Research Service National Animal Disease Center, Ames, IA

In January 2017, the FDA restricted the use of many in-feed administered antimicrobial compounds in food-producing animals. These compounds have been traditionally used in young animals for disease prevention or treatment, and improved feed efficiency. Bacitracin methylene disalicylate (BMD) is exempt, and continues to be used in turkey production. We sought to determine the effects of BMD on microbial communities and host transcriptional response in cecal tissue of growing turkeys. Day of hatch poults were split into three treatment groups (non-medicated, sub-therapeutic (50g BMD/ton feed for 11 weeks), and therapeutic (200g BMD/ton of feed for 5 weeks, and transitioned to sub-therapeutic BMD for the remaining 6 weeks)). Throughout the study, cecal contents and cecal tissue were collected to characterize microbial population shifts using high-throughput 16S rRNA gene sequence analysis, and RNA-Seq to evaluate differentially expressed genes. Both concentrations of BMD had immediate and lasting impacts on the microbiota structure, reducing species richness in the BMD-treated turkeys through the end of the study. In contrast, therapeutic BMD dramatically impacted cecal gene expression compared to non-medicated, at the end of the study, and included upregulaiton of genes for nutrient acquisition, innate (*C5, DUOXA1, DUOXA2, LYZ)* and acquired immunity (*CTLA4*, *CD3*ε, *CD40*, *CCL19*), while sub-therapeutic concentrations minimally impacted gene expression compared to non-medicated. This change in host-gene expression may be due to the accumulative effect of cecal microbiota composition disturbed by a therapeutic dose of antibiotic, resulting in enhanced cecal immunity to pathogens that may improve animal health and production.

F39. Antibiotics Ameliorate Lupus-Like Symptoms in Mice

Qinghui Mu and Xin Luo. Virginia Tech, Blacksburg, VA

Gut microbiota and the immune system interact to maintain tissue homeostasis, but whether this interaction is involved in the pathogenesis of systemic lupus erythematosus (SLE) is unclear. Rheumatologists and patients have observed that antibiotics can control lupus flares, suggesting a role for pathogenic microflora in SLE. Here we report that oral antibiotics given during active disease removed harmful bacteria from the gut microbiota and attenuated lupus. Using MRL/lpr mice, we showed that antibiotics ameliorated systemic autoimmunity and kidney histopathology. They also decreased IL-17-producing cells and increased the level of circulating IL-10. In addition, antibiotics removed *Lachnospiraceae* and increased the relative abundance of *Lactobacillus* spp., two groups of bacteria previously shown to be associated

with deteriorated or improved symptoms in MRL/lpr mice, respectively. Furthermore, we showed that the attenuated disease phenotype could be recapitulated with a single antibiotic vancomycin, suggesting the use of vancomycin as a potential treatment in SLE.

F40.Intestinal Inflammatory Leukocytes Determine Efficacy of Colonization Resistance against *Salmonella*

Denise N. Bronner, Erin Olsan and Andreas Baumler. University of California Davis, Davis, CA

The gastrointestinal tract consists of a complex microbial ecosystem that provides nutrients and colonization resistance for the host. Previous microbiotic models (e.g., ASF, SIHUMI) were insufficient in providing colonization resistance against enteric pathogens (*Salmonella*). Recently, Stecher et al. developed a minimal bacterial community (Oligo-MM^{12}) that provides conventional like colonization resistance to *Salmonella enterica* serovar Typhimurium SL1344 in gnotobiotic mice. We adopted the Oligo-MM^{12} microbiota to elucidate the mechanisms by which the microbiota confers colonization resistance. Notably, when we challenged gnotobiotic mice with *Salmonella enterica* serovar Typhimurium IR715, the level of colonization resistance provided by Oligo-MM^{12} microbiota was lower when compared with SL1344. The higher bacterial burden due to IR715 was accompanied by significant *Clostridia* depletion, a phenomenon not observed with SL1344. Previous studies have illustrated that *Clostridia* depletion is neutrophil dependent and that *Clostridia* can aid in limiting *Salmonella* growth. We hypothesized that neutrophil recruitment would be higher in IR715-infected mice thus removing the main *Salmonella* competitor. We assessed the infiltrating leukocyte population in fecal pellets and observed that IR715-infected mice exhibited high KC and MPO levels, whereas SL1344-infected mice exhibited high CCL2 and neopterin levels. Neutrophils can generate NETs that aid in killing microbes via histones and other neutrophil enzymes. From our *in vitro* killing assay, we discovered that *Clostridia* spp in the Oligo-MM^{12} microbiota were more sensitive to killing when incubated with purified histones when compared to their *Bacteroides* counterparts. Further investigation is needed to discover the host and *Salmonella* effector(s) that regulate intestinal neutrophil recruitment.

F41. Helminth-Induced Alterations of the Gut Microbiota Exacerbate Bacterial Colitis

Chien Wen Su. Massachusetts General Hospital - Harvard Medical School, Charlestown, MA

Infection with the intestinal helminth parasite *Heligmosomoides polygyrus* exacerbates the colitis caused by the bacterial enteropathogen *Citrobacter rodentium*. To clarify the underlying mechanism, we analyzed fecal microbiota composition of control and helminth-infected mice and evaluated the functional role of compositional differences by microbiota transplantation experiments. Our results showed that infection of Balb/c mice with H. polygyrus resulted in significant changes in the composition of the gut microbiota, characterized by a marked increase in the abundance of *Bacteroidetes* and decreases in *Firmicutes* and *Lactobacillales*. Recipients of the gut microbiota from helminth-infected wide-type, but not STAT6-deficient, Balb/c donors had increased fecal pathogen shedding and significant worsening of Citrobacter-induced colitis compared to recipients of microbiota from control donors. Recipients of helminth-altered microbiota also displayed increased regulatory T cells and IL-10 expression. Depletion of $CD4^+CD25^+$ T cells and neutralization of IL-10 in recipients of helminth-altered microbiota led to reduced stool *C. rodentium* numbers and attenuated colitis. These results indicate that alteration of the gut microbiota is a significant contributor to the *H. polygyrus*-induced exacerbation of *C. rodentium* colitis. The helminth-induced alteration of the microbiota is Th2-dependent and acts by promoting regulatory T cells that suppress protective responses to bacterial enteropathogens.

F43. Antibiotic induced Changes in Host Resistance to Helminthes in Murine Small Intestine

Motoko Morimoto. Miyagi Universitu, Sendai, Miyagi, Japan

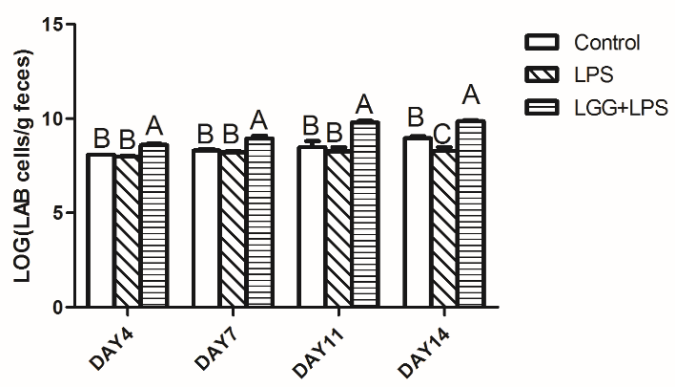
Infection with *Heligmosomoides polygyrus* (Hp), which is a gastrointestinal nematode in rodents, leads to a highly polarized Th2 response with an elevated IL-4 and IL-13 expression and induces M2 macrophages and Tregs. Hence, it is considered that intestinal nematode parasites possess potential immune-modulatory activity. In addition, helminth infections change the gut environment, such as permeability of the epithelium and mucus secretion. These alterations may affect the intestinal microbiome; at the same time, intestinal bacteria may interact with helminth-induced host immune responses. If the microbiome can modulate the development of host immunity, changes in the gut flora might affect the development of Th2 responses against the nematode. In this study, we investigated whether the administration of antibiotics to mice, including the newborn, during the weaning period affects Th2 immune responses against Hp infection. Balb/c mice were administered ampicillin (1 mg/ml) after birth through drinking water and infected with Hp at 8 weeks. On day 8 after infection, tissues were taken from the small intestine, and analyzed by real-time PCR for IL-4, IL-13, IL-10, IFNγ, TGFβ, and ARG1. Analysis of intestinal flora using next-generation sequencing was performed with freshly collected feces in the colon. The gene expression of IL-4, IL-13, and ARG1 was significantly low in the antibiotic group compared with that in the control group. Analysis of intestinal flora revealed that the *Lachnospiraceae* decreased after antibiotic administration but reappeared with Hp infection, while the Prevotella completely disappeared after antibiotic administration and did not reappear with infection. The details of the mechanism have not been identified yet, but these results suggested that intestinal flora interact with the induction of Th2 immune responses by nematode infection.

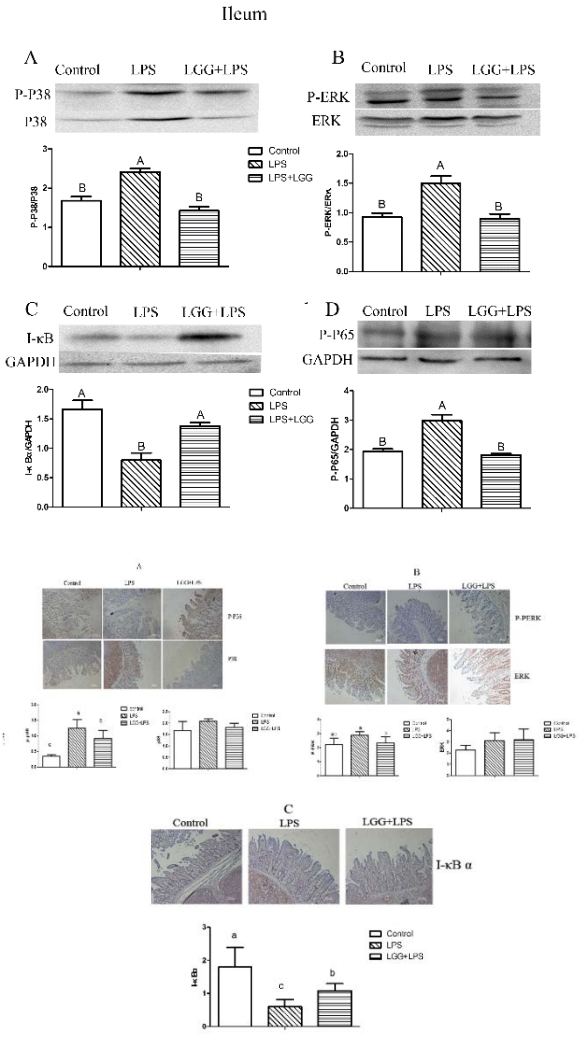
F44. *Lactobacillus rhamnosus* GG Modulates MAPK/NF-κB Signaling Pathway and Intestinal Bacterial Community and Metabolomics Response to Lipopolysaccharide Stimulation in the Intestine of Piglets

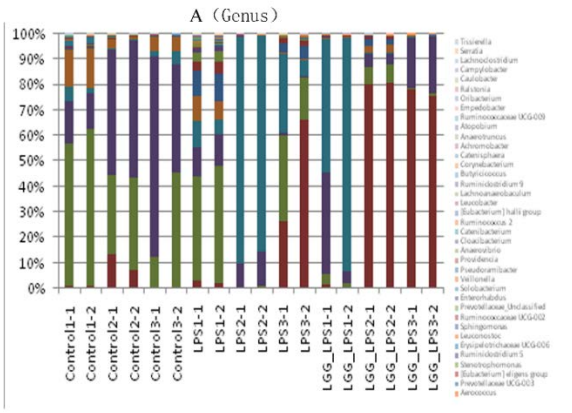
Haifeng Wang, Xiaoxiao Dou, Kan Gao, Yanjun Cui and Jianxin Liu. Zhejiang University, Hangzhou, Zhejiang, China

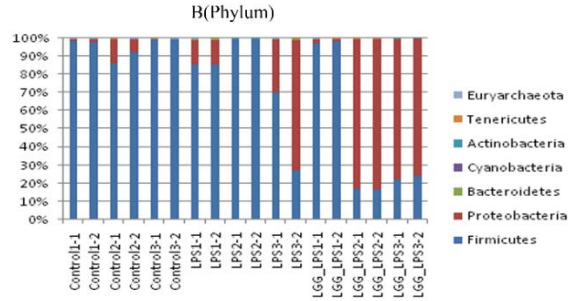
Probiotics plays a vital role in maintain the health of intestinal, dysfunction of which will lead to the pathophysiology of a varity of gastrointestinal disorder. This study will investigate possible protective activity of feeding *Lactobacillus rhamnosus* GG (LGG) against intestinal injury for weaned pigs stimulated by lipopolysaccharide and elucidate the underlying mechanisms. Nine weaned piglets were randomly assigned to three LGG or/and LPS treatment groups: none-LGG and LPS (Control), none-LGG but with LPS (LPS), and with LGG and LPS (LGG^+LPS). The piglets were fed with LGG (8x1010 CFU/d) for 2 weeks in LGG^+LPS groups. The piglets were intraperitoneal injected with LPS in LPS and LGG^+LPS groups as stimulation, and with saline in Control groups. After 4 hours of injection, all pigs were euthanized and intestinal contents and intestinal tissue were collected. LGG alleviated the LPS-induced increase of IL-6 and TNF-α, and upregulated the IL-10. Compared with the control, LPS stimulation induced significantly higer P38 and ERK1/2 phosphorylation ratio and NF-κBp65 level, whereas a significantly lower IκBα level in the ileumby western blot. The feeding of LGG alleviated the activation of signial pathway and restored the p-P38/P38, ERK1/2, NF-κBp65 and IκBα to the normal level in ileum. Immunohistochemistry for p38, p-p38, ERK, p-ERK and IκBα in ileal tissue verified the result of western blot. In bacterial community analysis, LGG reduced the proportion of *Clostridium sensu* stricto 1, *Streptococcus* and *Lactobacillus* in genus level compared with the LPS group. LGG decreased the proportion of *Firmicutes* and increased the proportion of *Proteobacteria* in phylum level. There were 22, 21 and 26 metabolites and 5, 4 and 4 major metabolic pathways with significant differences ($VIP > 1.5$, $P < 0.05$) between the Control and LGG+LPS groups, the Control and LPS groups, and the LPS and LGG+LPS groups, respectively. LGG alleviated LPS-induced inflammatory of weaned piglets by inhibiting MAPK and NF-κB signaling to decrease inflammatory cytokine, which indicated that LGG had beneficial modulatory effects on the pathway

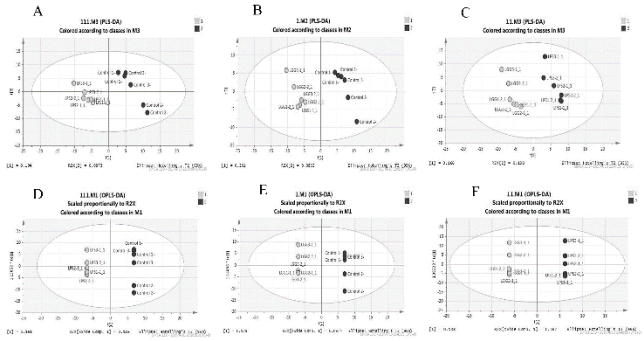
of inflammatory in weaned piglets induced by LPS. The changed metabolites induced by LGG may partly account for the anti-inflammation that LGG exerts on the intestine.











F45. Microbiota Modify Inflammatory Arthritis through Mucosal Inflammation and Autoantibody Generation

Widian Jubair, Jason Hendrickson, Sumitra Adhikari, Diana Ir, Charles Robertson, Daniel Frank, Nirmal Banda and Kristine A. Kuhn. University of Colorado School of Medicine, Aurora, CO

Given the observations of mucosal dysbiosis and inflammation in patients with rheumatoid arthritis (RA), microbial-mucosal interactions are a potential trigger of RA. However, a causal link has yet to be demonstrated. To study the role of the intestinal microbiota in the pathogenesis of RA, we utilized the murine collagen-induced arthritis (CIA) model. CIA is established by immunization of bovine type II collagen in complete Freund's adjuvant on days 0 and 21; inflammatory arthritis can be observed after day 21.

We found that significant intestinal dysbiosis and mucosal inflammation occurred as early as day 14 in CIA, stabilized through day 21, and again increased at day 35. Mucosal inflammation was manifest in colon histology, barrier impairment, and elevation of small and/or large intestinal IL-17A, IL-22, IL-23 and TNF-α tissue cytokines. Early depletion of the microbiota using broad-spectrum antibiotics prior to induction of CIA reduced disease severity, serum inflammatory cytokines, and serum autoantibodies to collagen. Intriguingly, late depletion of microbiota with antibiotics at the time of the second immunization resulted in a more profound suppression of arthritis compared to early microbial depletion. In this late-antibiotic treated group, serum and intestinal inflammatory cytokines decreased significantly ($p < 0.001$), but serum autoantibodies were minimally changed. Together, these data suggest a model in which intestinal dysbiosis triggers mucosal immune responses that stimulate T and B cell activities leading to inflammatory arthritis. Although the precise mechanisms are still under investigation, our data indicate that microbiota have differing effects on CIA development at different stages of the disease process.

F46. Dietary Resistant Starch Modulates Intestinal Microbial Populations and Immune Status in Pigs

Crystal L. Loving[1], Julian M. Trachsel[1,3], Cassidy Briggs[2], Nicholas K. Gabler[3] and Heather Allen[1]. [1]United States Department of Agriculture-Agriculture Researcb Service-National Animal Disease Center, Ames, IA; [2]Washington State University Veterinary Medical School, Pullman, WA; [3]Iowa State University, Ames, IA

With recent limitations to antibiotic use in livestock species, alternatives are sought to maintain swine health and production. Dietary resistant starch, such as raw potato starch (RPS), is known to modulate intestinal microbial populations, increase short chain fatty acid (SCFA) levels, and modify mucosal gene expression. To better describe the effects of dietary RPS on swine peripheral and intestinal immune status, pigs were fed a diet containing 5% RPS or non-amended diet for 3 weeks immediately post-weaning. Pigs fed RPS had increased levels of butyrate in the cecum, and mucosa-associated bacterial communities were significantly different between the two groups. *Proteobacteria*, commonly associated with intestinal inflammation, was reduced in the RPS-fed pigs. Flow cytometric analysis of the cecum revealed no differences in the percentage of T cells, but shifts in the phenotype of T cells present. Specifically, piglets fed RPS had a reduction in $CD8\alpha^{+}CD25^{+}$ population and a greater proportion of regulatory T cells ($CD4^{+}CD25^{+}FoxP3^{+}$). No significant changes in blood or intestinal lymph node regulatory T cell populations were detected. Immunohistochemical staining of the cecum substantiated no overall difference in $CD3^{+}$ cells, and no difference in the number of IgA-secreting cells. Cecal expression of *MUC2* and *IL-6* mRNA was increased in pigs fed RPS, but no difference in levels of *IL-17A*, *IL-22*, *IL-10* or *TGF-b* mRNA were detected. Overall, these data indicate the prebiotic RPS modulates intestinal immune status towards a less inflamed state and may serve as a potential alternative to antibiotics to improve swine enteric health.

F47. Diacylglycerol Kinase and Histamine Mediated Crosstalk Between *Lactobacillus reuteri* and Mammalian Intestinal Epithelium

Bhanu Priya Ganesh[1,2,3], Anne Hall[1], Sriram Ayyaswamy[1], Robert Fultz[1], Mark Whary[2], James Fox[2] and James Versalovic[1,3]. [1]Baylor College of Medicine, Houston, TX; [2]Massachusetts Institute of Technology, Cambridge, MA; [3]Texas Children Hospital, Houston, TX

Lactobacillus reuteri ATCC PTA 6475, probiotic bacterium, with an intact *hdc* gene cluster is known to synthesize and secrete histamine, and can suppress inflammation in mammalian systems via specific activation of the type 2 histamine receptor (H2R). However, it is unclear if *L. reuteri*-derived histamine also modulates activity of pro-inflammatory H1 receptors in the intestinal epithelium. In this work, we identified a soluble secreted isoform of diacylglycerol kinase (Dgk) from *L. reuteri* 6475. DGKs belong to a distinct, conserved family of intracellular lipid kinases that phosphorylate diacylglycerol (DAG), catalyzing conversion of DAG to phosphatidic acid (PA). This reaction may diminish DAG quantities in the cell membrane possibly modifying host intracellular signaling downstream of DAG. Histamine binding to H1R can cause phosphorylation of PKC via DAG activation. We found that *L. reuteri* 6475 suppressed basal levels of the pro-inflammatory cytokines IL-6, IL-1, Eotaxin (eosinophilic chemoattractant proteins) and G-CSF in the luminal mucosa and in blood plasma. *L. reuteri* lacking Dgk could not suppress the

aforementioned pro-inflammatory biomarkers. In addition, we demonstrated that histamine synthesized by *L. reuteri* 6475 activates both H1 and H2 receptors, but Dgk synthesized by the bacterium suppresses H1R downstream signaling. Inhibition of signaling downstream of H1R was supported by diminished PKC phosphorylation in the intestines of wild-type (WT) *L. reuteri* treated but not in *ΔdgkA* mutant treated germ-free (GF) mice. In addition, we also report suppression of $CD11b^{+}Gr1^{+}$ $Ly6G^{hi}$ immature myeloid cells (IMCs) after WT *L. reuteri* treatment. The proportion was consistent with *in vivo* experiments in our mouse model, PKC phosphorylation was reduced in human epithelial cells after treating the cells with *L. reuteri* derived conditioned media. *L. reuteri* suppressed immune responses by direct effects of the metabolite, histamine, and a secreted bacterial enzyme, diacylglycerol kinase, which converts a membrane lipid signal to an inactive form. Additionally, 1% DSS treated conventional BALB/c mice treated with 10^{9} cells of WT *L. reuteri* showed reduced proinflammatory $CD11b^{+}F4/80^{+}MHCII^{hi}$ Mφ compared to the mice that received *ΔdgkA L. reuteri* in the intestinal mucosa. Also, $CD4^{+}CD25^{+}Foxp3^{+}$ T-reg cells were increased with WT *L. reuteri* treatment in intestinal mucosa. These findings provide a deeper mechanistic understanding of intestinal immunomodulatory probiotics and microbiome: host interaction.

F48. Host Immune Response Supports Fecal Microbiota Transplant-Mediated Clearance of *Clostridium difficile* Infection

Michael C. Abt, Rebecca Carter, Eric R. Littmann, Boj Susac and Eric G. Pamer. Memorial Sloan Kettering Cancer Center, New York, NY

Clostridium difficile is an opportunistic pathogen that infects the large intestine following perturbation of the intestinal microbiota causing epithelial damage and debilitating, potentially fatal, colitis. Current antibiotic treatment options result in high recurrence rates, highlighting the need to identify alternative approaches to control this disease. Fecal microbiota transplantation (FMT) is clinically proven to clear recurrent *C. difficile* infection. Despite remarkable efficacy, implementation of FMT therapy is limited due to inadequate understanding of FMT mechanism of action. Here, we use a murine model of chronic *C. difficile* infection to demonstrate a critical role for the host's immune system in determining FMT success. C57BL/6 and T & B cell deficient $Rag1^{-/-}$ mice were infected with *C. difficile* and exhibited comparable establishment of chronic infection with similar bacterial burden and toxin titers. Following FMT, however, C57BL/6 mice resolved *C. difficile* infection within two weeks while cohoused $Rag1^{-/-}$ mice failed to clear the infection. FMT in *C. difficile* infected mice lacking B cells, $CD8^{+}$ T cells or $CD4^{+}$ T cells reveal a necessary role for $CD4^{+}$ T cells, but not B cells or $CD8^{+}$ T cells, in resolution of *C. difficile* following FMT. Analysis of intestinal bacterial communities following FMT demonstrated the microbiota of C57BL/6 mice assimilated toward the composition of the FMT donor, while the microbiota of $Rag1^{-/-}$ and $CD4^{+}$ T cell deficient mice did not acquire the bacterial composition of the FMT donor. These data suggest that FMT engraftment and efficacy is dependent on an intact $CD4^{+}$ T cell compartment.

F49. NLRP3 Deficiency Gives Rise to an IgA-Suppressive Microbiota Resulting in Enhanced Susceptibility to Intestinal Inflammation and *Streptococcus pneumoniae* Challenge

Craig P. McEntee[1], Natalia Munoz-Wolf[1], Brian Flood[1], Sinead Kenealy[1], Luke AJ O'Neill[1], John MacSharry[2], Emma M. Creagh[1] and Ed C. Lavelle[1]. [1]Trinity College Dublin, Dublin, Ireland; [2]University College Cork, Cork, Ireland

The NLRP3 inflammasome has been implicated as a mediator of disease pathogenesis in several auto-inflammatory conditions. However, paradoxically, NLRP3 is known to be protective in colitis, although a definitive mechanism remains elusive. Herein, we investigated the impact of NLRP3 deficiency on constitutive IgA responses. Strikingly, we observed significantly compromised IgA levels in $Nlrp3^{-/-}$ mice, a phenotype which could be reversed upon co-habitation with wild-types (WTs). Microbiota analysis revealed marked reductions in *Clostridia* in $Nlrp3^{-/-}$ mice prior to co-habitation, microbes which promote gut homeostasis and attenuate intestinal inflammation through their ability to ferment dietary fibre into short chain fatty acids (SCFAs) including butyrate, propionate, and acetate. Signalling of these metabolites results in the up-regulation of genes associated with IgA CSR including *Il10* and *Aldh1a1*, the latter being involved in the synthesis of retinoic acid (RA) from dietary Vitamin A. Dietary supplementation with SCFAs or exogenous

administration of RA completely restored IgA CSR in *Nlrp3*$^{-/-}$ mice *in vivo*. Moreover, this SCFA-mediated restoration could be blocked via administration of the RA receptor antagonist BMS-493 and was thus elicited in a RA-dependent manner. In two distinct *in vivo* models of mucosal disease, DSS-induced colitis and invasive pneumococcal pneumonia, both of which induce significantly greater pathology in *Nlrp3*$^{-/-}$ mice compared to WTs, SCFA-treated mice were significantly more resistant to disease pathogenesis. Thus, microbial metabolite-driven natural IgA is capable of conferring significant protection at the mucosal surfaces of the gastrointestinal and respiratory tracts in murine models of inflammatory bowel disease (IBD) and invasive pneumococcal pneumonia respectively.

F50. The Intracellular Life of *Pseudomonas aeruginosa*

Emmanuel Faure[1], Julie Bérubé[1], Manon Ruffin[2], Emmanuelle Brocchiero[1], Dao Nguyen[1] and Simon Rousseau[1]. [1]McGill University, Montreal, PQ, Canada; [2]Centre Hospitalier Universitaire de Montreal, Montreal, PQ, Canada

Pseudomonas aeruginosa is mainly considered as an extracellular opportunistic pathogen, responsible for chronic lung infections by persisting extracellularly in a biofilm lifestyle. However, although *P. aeruginosa* owns suitable weapons for intracellular lifestyle, no study assessed the possibility of an intracellular persistence. We hypothesized that *P. aeruginosa* may persist in epithelial cells and that intracellular persistence may contribute to pathogenicity. To this end, using bronchial epithelial cells, a long-term persistence model was performed. BEAS-2B (B2B) and CFBE cells were infected for 2 hours with PAO1 and related mutant strains. Thereafter, Tobamycin, known to have poor intracellular diffusion, was added in the culture medium. Cells were incubated for 120 hours. Intracellular and extracellular bacterial burden and cytotoxicity were assessed after 2 hours, 24 hours and 120 hours, by serial dilutions and LDH release respectively. At each time point, Western Blot analysis and quantitative PCR expression were performed to assess host immune response. Concomitantly, gene expression of intracellular bacteria and their motility were assessed. Finally, B2B-CRISPR for targeted genes were generated to identify critical intracellular host pathways involved in response to pathogen. Here we show that *P. aeruginosa* can persist intracellularly in B2B and CFBE during 5 days, inducing low cytotoxicity. Moreover, we demonstrate that *P. aeruginosa* can replicate intracellularly, independently of the presence of flagella, Type-3 secretion system and Type-6 secretion system. Finally, intracellular *P. aeruginosa* represses flagellar expression. Taken together, our data suggest that *P. aeruginosa* can persist intracellularly into airway epithelial cells *in vitro*.

F51. The Potential Role of ELMO-1 in Regulating Autophagic Induction and Bacterial Clearance During Enteric Infection

Arup Sarkar[1], Courtney Tindle[2], Rama F Pranadinata[2], Peter B Ernst[2] and Soumita Das[2]. [1]Trident Academy of Creative Technology, Bhubaneswar, Orissa, India; [2] University of California, San Diego, San Diego, CA

Macrophages are specialized innate immune arsenals, phagocytose invading pathogens and thereby eliminate foreign intruders either by phagolysosomal or autophagic degradation. Previously, we reported that the Engulfment and Cell Motility protein 1 (ELMO1) regulates both bacterial internalization and inflammation. Here, we hypothesize that the ELMO1 plays a crucial role in bacterial clearance by modulating host cell immune responses. By performing subcellular fractionation we have showed the presence of ELMO1 protein in the phagosomes. *Salmonella* infection in J774 increases accumulation of an autophagic marker LC3B in an ELMO1-dependent manner. Silencing ATG5 in ELMO1-knockdown cells confirms that ATG5 is essential for ELMO1-mediated LC3B regulation. It shows the involvement of ELMO1 in conventional autophagy and partially to the LC3 mediated phagocytosis. As evidence of changes in the proteolytic capacity, ELMO1-depleted macrophages showed a time-dependent increase in pH and a decrease in Cathepsin B activity associated with the increase in bacterial survival. However, in this study we have observed an inefficient killing of pathogens in ELMO1-deficient macrophages. This clearly hinted us the involvement of ELMO1 in pathogen clearance. Together, these data show that ELMO1 is crucial for regulating clearance of *Salmonella* infection that can be targeted in the enteric infection.

F53. Immunomodulatory Activity of The Conditioned Media of *Lactobacilli* in Blot Extract-Stimulated PBMCS

Jian-Ming Lamony Chew[1], Chiung-Hui Huang[1], I-Chun Kuo[1] and Lynette Pei-Chi Shek[2]. [1]National University of Singapore, Singapore; [2] Khoo Teck Puat-National University Children's Medical Institute, Singapore

Lactobacilli have been shown to prevent or treat allergic diseases. However, the exact mechanisms through which *Lactobacilli* modulate hosts' immune responses in allergic diseases are still not fully understood. Here, we examined the role of *Lactobacilli* conditioned media on modulating Th2 cytokine profiles and attempted to unravel its underlying molecular mechanisms. PBMCs of 10 atopic subjects were stimulated with *Blomia tropicalis* (*Blo t)* extract in the presence or absence of the conditioned media, heat-treated or proteinase K-treated conditioned media of 7 different *Lactobacilli*. Cell culture supernatants were harvested after 5 days and measured for the concentration of IL-5, IL-9, IL-10, IL-13, and IFN-γ using Milliplex assay. Conditioned medium of *Lactobacillus reuteri* ATCC 55730 was shown to elicit the strongest suppression of IL-5, IL-9, IL-13, and IFN-γ, and enhancement of IL-10 in *Blo t* extract-stimulated PBMCs, as compared to the conditioned media of 6 other *Lactobacilli*. Furthermore, heat-treated or proteinase K-treated *L. reuteri* conditioned medium was also shown to retain its immunomodulatory effects of suppressing IL-5, IL-9, IL-13, and IFN-γ, and enhancing IL-10 in *Blo t* extract stimulated PBMCs. The results suggest that *L. reuteri* can modulate hosts' immune responses through secretion of heat-stable and proteinase K-resistant molecules. Identification of these molecules can potentially enhance the management of allergic diseases.

F54. Postnatal Development of The Murine Gut Microbiota

Niels van Best[1], John Penders[2], Paul Savelkoul[2] and Mathias Hornef[1]. [1]Uniklinik RWTH Aachen, Germany; [2]Maastricht University, Maastricht, Netherlands;

The enteric microbiota represents a dense and highly dynamic microbial community which exerts a major influence on many aspects of the host. In adults, the microbiota displays a dense community with relatively stable composition. In contrast, neonates are born essentially sterile with the establishment of the microbiota starting immediately after birth. The most dramatic changes in the density and composition of the microbiota are observed during early life. Therefore, this period might critically influence the ultimate composition of the enteric microbiota, the life-long maintenance of host-microbial homeostasis and the susceptibility to disease. Therefore, we conducted a systematic analysis of the time kinetic of bacterial colonization during the immediate postnatal period in mice. 16S rDNA V4 sequencing and the use of bacterial group specific PCR primers was complemented by profiling of metabolic and environmental factors at various time points and anatomical sites after birth. We observed a rapid colonization of the neonate intestine, increase in richness (choA1) early after birth combined with a major shift in composition during weaning. The post-weaning microbiota was closely related to the maternal adult microbiota. The microbiota composition was found to be highly individual directly after birth, but shifted towards a more homogenous pattern within one week. Different sites of the intestine harbored a comparable microbiota composition during the pre-weaning period. Our results illustrate the critical influence of environmental factors and are consistent with the existence of selective host mechanisms that shape the initial colonization pattern and ensure the development of a beneficial mature microbiota composition.

F56. The Role of Gut Microbiota in the Pathogenesis of Experimental Autoimmune Encephalomyelitis

Eiji Miyauchi[1], Seok-Won Kim[1], Wataru Suda[1], Masahira Hattori[2], Todd D. Taylor[1] and Hiroshi Ohno[1]. [1]RIKEN Center for Integrative Medical Sciences, Yokohama, Kanagawa, Japan; [2]Waseda University, Shinjuku, Tokyo, Japan

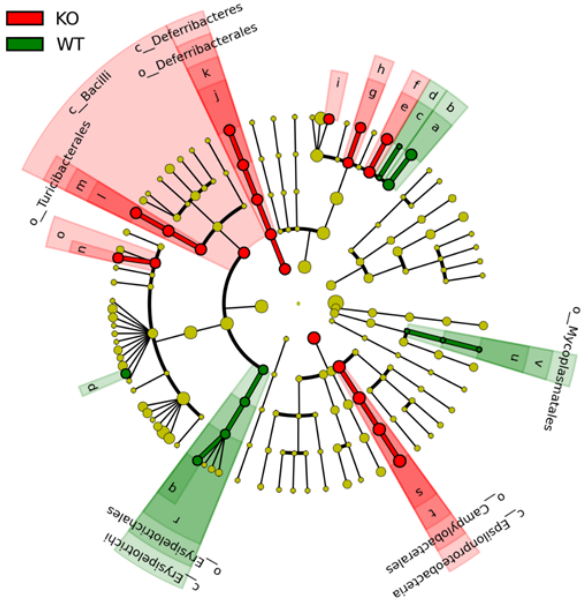
Increasing evidence suggests that gut microbiota play a role in pathogenesis of diseases outside the intestine as well, including multiple sclerosis. We investigated the effects of gut microbiota on the development of murine experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis. C57BL/6J mice were fed with drinking water containing ampicillin, vancomycin, neomycin, or metronidazole for one week, and EAE was induced by immunization with myelin oligodendrocyte

glycoprotein (MOG) and pertussis toxin. Bacterial genomic DNA was isolated from the small intestinal contents, and V4 region of 16S rRNA genes was PCR-amplified and sequenced on Miseq. Ampicillin suppressed EAE symptoms as well as the production of IFN-γ and IL-17A by MOG-specific T cells from the small intestine, and concomitantly suppressed the infiltration of inflammatory T cells into the spinal cord. Interestingly, lamina propria cells from the small intestine produced increased amounts of IFN-γ and IL-17A in response to *in vitro* MOG stimulation, and ampicillin treatment suppressed the induction of these MOG-specific immune responses. The meta-16S rRNA gene analysis from each antibiotic treated mice revealed that an *Erysipelotrichaceae* bacterium (OTU0002) was significantly reduced in the small intestine of ampicillin-treated mice. We found that OTU0002 adhered to intestinal epithelium and induced Th17 responses in the small intestine. Furthermore, the mice mono-associated with OTU0002 showed the increased EAE susceptibility compared to germ-free mice, suggesting that OTU0002 bacterium would play a pathogenic role in the inflammation in the central nerve system.

F57. The NLRP1 Inflammasome Attenuates Inflammatory Bowel Disease Pathogenesis by Modulating the Microbiome Composition in the Gut

Irving Coy Allen[1], Veronica M. Ringel-Scaia[1], Yufeng Qin[2] and Paul Wade[2]. [1]Virginia Tech, Blacksburg, VA; [2]National Institute of Environmental Health Sciences, Research Triangle Park, NC

NLRs maintain immune system homeostasis in the gut and function to attenuate inflammatory bowel disease (IBD) pathogenesis. Previously, our research group reported that NLRP1 attenuates the progression of experimental colitis and colitis associated tumorigenesis. Using *Nlrp1b*$^{-/-}$ and *Asc*$^{-/-}$ mice, we observed a significant increase in disease severity that was associated with attenuated IL-1β and IL-18 production. Subsequent, co-housing studies revealed that the microbiome is strongly associated with disease progression in the absence of the NLRP1 inflammasome. Here, we expand upon these previous findings. Our current data reveal significant differences in the microbiome composition of *Nlrp1*$^{-/-}$, *Asc*$^{-/-}$, and wild type mice. Both *Nlrp1*$^{-/-}$ and *Asc*$^{-/-}$ mice have significant dysbiosis in the gut microflora associated with disease severity. In the *Nlrp1*$^{-/-}$ mice, we found an increased abundance of *Clostridum*, *Mucispirillum*, *Odoribacter*, and *Helicobacter* species compared to the wild type animals. We observed similar changes in the *Asc*$^{-/-}$ mice, albeit a much greater number of species were observed in overabundance. Functionally, metabolic profiling revealed significant microbiome associated changes in the production of short chain fatty acids in the *Nlrp1*$^{-/-}$ and *Asc*$^{-/-}$ animals, with significant increases observed in acetate, proprionate, and heptanoate. Together, our data indicates that the lack of NLRP1 inflammasome signaling creates permissive niches for commensal microbes to exploit and occupy. The expansion of these detrimental microbial elements in areas of the colon where they would otherwise be excluded drives subsequent overzealous inflammation associated with compensatory innate immune system signaling mechanisms creating a microenvironment that drives IBD and cancer pathogenesis.



F58. Effects of Fermented Soybean Paste (Miso) on Immune Response of Human Faecal Flora-Associated (HFA) Mice

Noriko M. Tsuji[1], Takahiro Adachi[2], Tomonori Kamiya[1], Takamasa Miura[3], Yohei Watanabe[1], Shigeru Kakuta[4], Yoshiko Aihara[5], Hiroko Kawasaki[3], Cathryn Nagler[6] and Kazuhiro Hirayama[4]. [1]Biomedical Research Institute, Tsukuba, Ibaraki, Japan; [2]Tokyo Medical and Dental University, Tokyo, Japan; [3]National Institute of Technology and Evaluation, Tokyo, Japan; [4]University of Tokyo, Tokyo, Japan; [5]Kobe University, Kobe, Hyogo, Japan; [6]University of Chicago, Chicago, IL

Lactic acid bacteria (LAB) are a major component of small intestinal microbiota and frequently utilized for food fermentation. We have shown the anti-inflammatory effects of LAB and how they impact the functional maturation of immune cells through Toll-like receptor 3 (TLR3). It is therefore important to understand how food components such as LAB-containing fermented foods modulate immune response as well as gut microbiota of human host. Faecal suspensions from three healthy human donors were inoculated to germ-free mice to produce human flora-associated (HFA) mouse groups. HFA mice were fed diet containing 3% of Miso paste (a fermented soybean paste that contains LAB) for one month and immunized with ovalbumin (OVA). Effects of Miso diet on antigen-specific cytokine production in HFA mice were evaluated by *ex vivo* cell culture assay and ELISA. Profile of intestinal microbiota was analyzed by pyrosequencing. Both immune response and gut microbial profile of HFA derived from three different donors were distinct and differently modulated.

F60. Prescription Opioids Use Disrupts Gut Microbiome Resulting in Gut Barrier Compromise; Implications in Secondary Opportunistic Infection and Wound Healing

Umakant Sharma, Jingjing Meng, Li Zhang, Madhulika Sharma and Sabita Roy. University of Miami, Miami, FL

Morphine is the gold standard for pain management. Chronic morphine treatment disrupts intestinal epithelial barrier and suppress immune system, which contribute to induction of sepsis. In the current study we investigated the consequence of chronic prescription opioids (hydromorphone, fentanyl, oxycodone and buprenorphine) to examine alternative opioids to morphine with equal or better effect and fewer risks or side effects. Their effects were evaluated on gut microbial homeostasis and barrier integrity. The wild type mice were treated with prescription opioids for 72 hours. Our results show that opioids treatment resulted in intestinal barrier disruption and consequently increased bacterial translocation. We next investigated the effect of these opioids on gut microbiome using bacterial 16s rDNA amplification and MiSeq 250 sequencing. Opioids treatment decreased gut microbial richness, and altered bacterial composition when compared to control. Taxonomic profiling of fecal microbiota demonstrated increase in *Firmicutes* and *Verrucomicrobia* with significant enrichment of *Turicibacter* and *Akkermensia* respectively, while a decrease in *Bacteroidetes* with reduction in *Barnesiella* and *Xylanibacter* when compared with control. We also observed the fungal dysbiosis in the gut microbiota. The phylum *Ascomycota* was enriched and *Basidiomycota* was depleted. These data indicate that opioids used for pain management may result in the dysregulation of the gut microbiota leading to sustained inflammation and promote disease severity. Increased intestinal permeability results in the invasion of normal microflora and deregulation of the immune response against indigenous microbiota. The results indicate that opioid treated mice have higher risk of opportunistic infection, which may impact ultimate wound outcome.

F61. Role of Exosomes in Gut Immune Cross Talk

Yue Zhang. University of Miami, Miami, FL

The maintenance of intestinal mucosal homeostasis relies on the balances between tolerance towards normal microbiota and the ability to initiate efficient immune responses to detrimental pathogens. Intercellular communication between intestinal epithelial cells (IECs) and local immune system is critical in determining the appropriate course of immune response. Recent studies reveal that exosomes, the small membrane vesicles that originated from endosomes, could be an effective mediator during the inflammatory response. A number of different cell types in gut could efficiently secrete exosomes, including lymphocyte, dendritic cells (DCs), tumor cells, platelets and IECs. The functional transfer of exosomal cargo between IECs and immune cells are investigated in the current study. Isolated exosomes from IECs were co-cultured with mouse macrophage and DCs to investigate

whether they enhance inflammatory cytokine production, and other immune response. Reciprocally, DC-derived and macrophage-derived exosomes were used to promote epithelial restitution following endotoxin-induced damage. We will further investigate the specific composition of the exosomal cargo secreted from different cell types, including peptides, proteins and microRNAs. These studies will increase our knowledge and identify the role of exosomes as an important mediator in the gut immune system. In addition, the corresponding signaling pathways involved in exosome biogenesis, trafficking, and exocytosis may become feasible targets in the clinical treatment of enteric inflammatory diseases.

F62. Sex Work Impacts Diversity and Abundance of *Lactobacilli* in the Vaginal Microbiome

Jocelyn M. Wessels[1], Danielle Vitali[1], Julie Lajoie[2], Ken Omollo[3], Joshua Kimani[3], Julius Oyugi[3], Juliana Cheruiyot[4], Jennifer C. Stearns[1], Keith R. Fowke[2], Michael G. Surette[1] and Charu Kaushic[1]. [1]McMaster University, Hamilton, ON, Canada; [2] University of Manitoba, Winnipeg, MB, Canada; [3] University of Nairobi, Nairobi, Kenya; [4] Kenyan AIDS Control Program, Nairobi, Kenya

Bacterial vaginosis (BV), is consistently linked with increased risk of sexually transmitted infections (STIs). Recent evidence demonstrates that cervicovaginal bacteria regulate inflammation and inflammatory responses in the female genital tract and highly diverse vaginal microbiomes low in *Lactobacillus* are associated with STIs and increased risk of HIV acquisition. Sex Workers are an established risk group for STIs. Although we know that sexual behaviours change the vaginal microbiome and that sex work can modify inflammatory factors within the female genital tract, there is a paucity of data on how sex work affects diversity and composition of the vaginal microbiome. We conducted a retrospective study to compare the vaginal microbiomes of Kenyan women who are and are not engaged in sex work. Results demonstrate bacterial richness and diversity are greater in Sex Workers (N=58) than Non-Sex Workers (N=19), and New Sex Workers (< 3 years) were significantly less likely to have *Lactobacillus* as the most abundant genus in their vaginal microbiomes versus Non-Sex Workers (17% vs. 58%; $P < 0.001$). Further, the vaginal microbiome of 51% of women who were BV^- (21/41) fell within the high diversity community state type (CST), and Sex Workers in the high diversity CST who were BV^- had significantly greater bacterial diversity than Sex Workers who were *Lactobacillus* dominant and BV^- (2.481±0.2591 vs. 1.388±0.3589; P=0.0188). Thus, microbial diversity exists in the absence of BV. Results suggest diversity of the vaginal microbiome and lack of *Lactobacillus* in Sex Workers contribute to their increased risk of acquiring STIs.

F63. Depot Medroxyprogesterone Acetate is Associated with Increased Diversity of the Vaginal Microbiome

Jocelyn M. Wessels[1], Julie Lajoie[2], Ken Omollo[3], Joshua Kimani[3], Julius Oyugi[3], Juliana Cheruiyot[4], Keith R. Fowke[2], Michael G. Surette[1] and Charu Kaushic[1]. [1]McMaster University, Hamilton, ON, Canada; [2] University of Manitoba, Winnipeg, MB, Canada; [3] University of Nairobi, Nairobi, Kenya; [4] Kenyan AIDS Control Program, Nairobi, Kenya

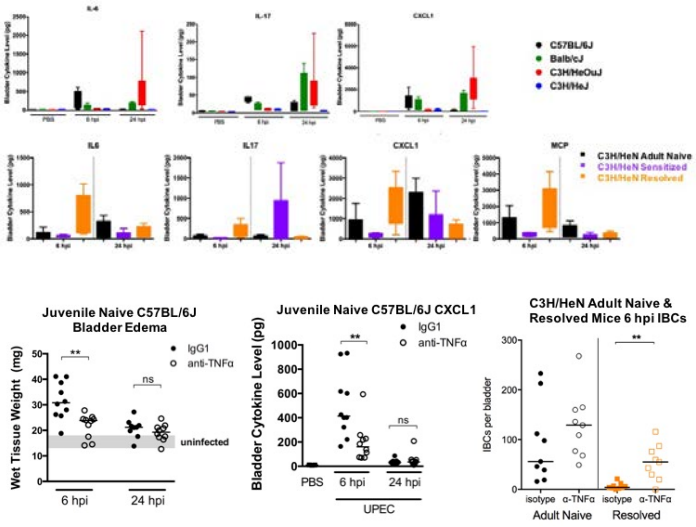
Women are at an increased risk of sexually transmitted infections (STIs) as compared to men. Female sex hormones might regulate the vaginal microbiome and clinical studies support a correlation between use of the injectable hormonal contraceptive (Depo-Provera) and a 2-fold greater risk of acquiring and transmitting Human Immunodeficiency Virus (HIV) as compared to women not on hormonal contraceptives. The aim of this study was to profile the vaginal microbiome of Kenyan women attending the Sex Worker Outreach Program Clinics in Nairobi who were not on hormonal contraceptives (NH, N=21), or were using oral contraceptives (OCP, N=10), or Depot medroxyprogesterone acetate (Depo Provera; DMPA, N=24) to determine if hormones alter the vaginal microbiome. Cervicovaginal lavage was collected and the V3 region of the 16S rRNA was sequenced using the Illumina MiSeq. There was no effect on the number of women who had *Lactobacillus* as the most abundant genus in their vaginal microbiomes (14/21 (66%) NH, 8/10 (80%) OCP, 16/24 (66%) DMPA; P=0.397). However, women on DMPA had significantly greater bacterial diversity in their vaginal microbiomes than women who were not on hormonal contraceptives (1.769±.2315 DMPA, 1.103±0.2465 NH; P=0.0150). This suggests that women on DMPA have a more diverse vaginal microbiome which may put them at increased risk of

contracting STIs like HIV. This is the first study to demonstrate the effect of hormonal contraceptives on bacterial diversity in the vaginal tract, and the first to provide a biological link between the use of DMPA and its increased risk of contracting STIs.

F64. Early Bladder Mucosal TNFα Signaling is a Hallmark of Resistance to Acute Urinary Tract Infection with Uropathogenic *E. coli*

Lu Yu[1], Valerie P. O'Brien[1], Denise A. Dorsey[1], Elisha DO Roberson[1], Jonathan Livny[2], Tom J. Hannan[1] and Scott J. Hultgren[1]. [1]Washington University in St Louis, St Louis, MO; [2]Broad Institute of MIT and Harvard, Cambridge, MA

Urinary tract infections (UTI) caused by uropathogenic *E. coli* (UPEC) are common and highly recurrent. The two leading non-behavioral risk factors for UTI in women are genetics and history of ≥ 2 previous UTI. Concordantly, inbred mice of various genotypes and with different infection histories exhibit different susceptibilities to acute and chronic bladder infection (cystitis) that recapitulate a range of clinical UTI outcomes. Early host-pathogen interactions determine UTI outcomes in mouse models. Thus, we hypothesized that studying the bladder innate immune responses during early infection in different mouse models would reveal patterns of resistance to UTI. We used four strains of juvenile naïve mice (no UTI history; C57BL/6J, Balb/cJ, C3H/HeOuJ, C3H/HeJ), and C3H/HeN mice (two divergent UTI histories: "Resolved," previously spontaneously resolved an infection; "Sensitized," previously suffered from chronic infection). Microscopic and cytokine analysis of infected bladders revealed a multi-phasic pattern. Strains resistant to UTI (C57BL/6J, Balb/cJ) initiated a robust early phase of inflammation (0-6 hpi), while the susceptible strain C3H/HeOuJ experienced muted early phase but robust late phase inflammation (6-24 hpi). The divergent inflammation profiles were phenocopied by C3H/HeN Resolved (resistant) and Sensitized (susceptible) mice. Transcriptomic profiling of whole bladders during early acute infection (3.5 hpi; *E. coli* vs. mock-infected) revealed activation of tumor necrosis factor-α (TNFα) pathways in resistant mice but not in susceptible mice. Inhibition of TNFα signaling in resistant mice reduced inflammation and increased acute bacterial colonization. This suggests that early-phase bladder inflammation is protective while severe late-phase inflammation is detrimental to the host.



F65. Bacterial Communities within the Gingival Tissues of Periodontal Lesions in Comparison with Subgingival Plaque and the Presence of Biofilm within the Tissues

Keumjin Baek[1], Suk Ji[2] and Youngnim Choi[1]. [1]Seoul National University, Seoul, Seoul-t'ukpyolsi, Republic of Korea; [2] Ajou University, Suwon, Kyonggi-do, Republic of Korea

Periodontitis is caused by dysbiosis of subgingival plaque, and bacterial invasion into the gingival tissues plays an important role in the pathogenesis of periodontitis. The presence of bacteria in the lamina propria and deep connective tissues have been shown. Although shifts in subgingival microbiota from health to periodontitis have been well characterized, the characteristics of bacterial communities located within the gingival tissues have not been studied. To characterize microbiota within the tissues of periodontal lesions in comparison with subgingival plaque, the gingival tissues and plaque were obtained from the same tooth of periodontitis patients (*n*=7). Bacterial communities were analyzed by pyrosequencing analysis of 16S rRNA genes. The presence of bacteria within additional gingival tissues were examined by *in situ* hybridization, alcian blue staining, and atomic force microscopy. Although the species richness and diversity were not significantly different between the two communities, the inter-subject variation of the intratissue communities

(0.058±0.003) was smaller than that of plaque communities (0.071±0.004). Compared with the plaque communities, the intratissue communities were characterized by decreased Firmicutes and increased *Fusobacteria* and *Chloroflexi*. Particularly, *Fusobacterium nucleatum* and *Porphyromonas gingivalis* were highly enriched within tissues, constituting 15 to 40% of total bacteria. Interestingly, biofilms formed by bacterial aggregates in the extracellular matrix-free space of gingival tissues were often observed, where degradation of collagen fibers was evident but immune cells were relatively few. Collectively, bacteria form complex communities within the gingival tissues different from subgingival plaque and can serve as the reservoir of persistent infection. These results may provide new insights into the pathogenesis of periodontitis.

F66. Attenuation of Viral-Induced Pulmonary Disease by *Lactobacillus johnsonii* Supplementation: Metabolic Reprogramming and Immune Cell Modulation

Wendy Fonseca[1], Kaitlyn Lucey[2], Andrew Rasky[1], Hung-An Ting[1], Kei Fuimura[2], Susan Lynch[2] and Nicholas Lukacs[1]. [1]University of Michigan, Ann Arbor, MI; [2] University of California, San Francisco, San Francisco, CA

The emerging field of microbiome research has demonstrated the key role of microbial communities in the modulation of host immune responses. Regulation of respiratory mucosal immunity by systemic microbial-derived metabolites has been a proposed mechanism that may provide airway protection. Here we examine the effect of oral *Lactobacillus johnsonii*-supplementation on metabolic and immune response dynamics during respiratory syncytial virus (RSV) infection. *L. johnsonii*-supplementation reduced airway Th2 cytokines, dendritic cell function, increased T-regulatory cells, and was associated with a reprogrammed circulating metabolic environment. RSV-infected bone-marrow derived dendritic cells (BMDC) from *L. johnsonii*-supplemented mice had altered cytokine secretion, reduced expression of co-stimulatory molecules, and modified $CD4^+$ T cell cytokines. This was replicated upon co-incubation of wild-type BMDCs with plasma from *L. johnsonii*-supplemented mice. The airway transfer of BMDCs from *L. johnsonii*-supplemented mice, or with wild-type derived BMDCs pre-treated with plasma from *L. johnsonii*-supplemented mice, reduced airway pathologic responses to infection in recipient animals. Finally, we examine epigenetic changes in BMDC from *L. johnsonni* mice at the pro-inflammatory cytokine IL-1b, IL-6 and TNF-a promoters. Thus, *L. johnsonii*-supplementation could mediate airway mucosal protection via immunomodulatory metabolites by decreasing histone modification and altering immune function

F68. Multi-Omics Comparative Analysis Reveals Host Signaling Pathways Affected by the Gut Microbiota

Nathan P. Manes[1], Natalia Shulzhenko[2], Arthur G. Nuccio[1], Sara Azeem[1], Andrey Morgan[2] and Aleksandra Nita-Lazar[1]. [1]National Institutes of Health, Bethesda, MD; [2] Oregon State University, Corvallis, OR

A systems-wide understanding of the effects that the microbiota has on the host is needed, but proteomics has never before been used to compare the GI tract of conventional and germ-free mice. In this investigation, we quantitatively compared the terminal ileum of conventional and germ-free mice at both the transcriptomic and proteomic levels (female and male, and BALB/c and C57BL/10A strains; five mice per experimental condition). In addition, OMiCC was used to perform a meta-analysis using eight previously published transcriptomics datasets from analyses of conventional versus germ-free mouse whole-tissue intestinal samples. The resulting data were used to perform Ingenuity pathway analyses. We discovered significant transcriptome-proteome discordancy in the adaptation of the host to the microbiota, and we also discovered mouse strain-specific effects. The female and male mice responded similarly to the microbiota, but C57BL/10A mice responded much more strongly than BALB/c mice at both the transcriptome and proteome levels. The microbiota primarily affected two classes of pathways: immunological pathways were upregulated and metabolic pathways were downregulated in response to the microbiome. For example, the microbiota caused the interferon signaling and antigen presentation pathways to be concordantly upregulated, and the serotonin degradation and glutathione-mediated detoxification pathways to be concordantly downregulated in transcriptome and proteome. Discordantly regulated pathways were not principally involved in the immune system. Instead, they were principally involved in metabolism and protein translation and folding. This research was supported by the Intramural Research

Program of the National Institute of Allergy and Infectious Diseases, NIH.

F69. Microbial Fermentation Products Mitigate Autoimmune Arthritis

Daisuke Takahashi, Naomi Yoshida-Hoshina, Hiyori Tanabe, Aiko Egawa and Koji Hase. Keio University, Tokyo, Tokyo, Japan

Gut microbiota and its metabolites have been implicated in several chronic inflammatory disorders. We previously reported that short chain fatty acids (SCFAs) produced by microbiota induce the differentiation of Foxp3-expressing regulatory T (Treg) cells in the colon, which prevents the development of mouse models of IBD. Here we found that SCFA induces the differentiation of follicular regulatory T (Tfr) cells *in vitro* and *in vivo*. Tfr cells are a specialized Treg subset to suppress germinal center reaction and autoantibody production. Feeding of a diet containing SCFA increases Tfr cells in the colonic patches, and markedly improved the clinical and histological scores of collagen-induce autoimmune arthritis (CIA). Furthermore, the collagen-specific IgG level was significantly decreased in the SCFA diet group than in the control group. These data suggest that the microbial metabolite play a key role in preventing the onset of CIA through regulating germinal center B cell reaction and autoantibody production by the induction of Tfr cells. Our data suggest that SCFA regulates not only local inflammation in the gut, but also systemic autoimmune response.

F71. Lacking of Toll-Like Receptor 4 in the Intestinal Epithelial Cells Results in Metabolic Syndrome

Peng Lu[1], Chhinder Sodhi[1], Yukihiro Yamaguchi[1], Hongpeng Jia[1], Thomas Prindle[1], William B. Fulton[1], Amit Vikram[2], Kyle J. Bibby[2], Michael J. Morowitz[3] and David Hackam[1]. [1]Johns Hopkins University School of Medicine; Johns Hopkins Hospital, Baltimore, Baltimore, MD; [2] Department of Civil and Environmental Engineering, University of Pittsburgh, Pittsburgh, PA; [3] Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA

Metabolic syndrome is a group of metabolic abnormalities and the gut microbiota may be involved. We now show that mice lacking the endotoxin receptor Toll-like receptor 4 in the intestinal epithelial cells ($TLR4^{\Delta IEC}$) fed standard rodent chow develop features of metabolic syndrome, including significantly increased body weight, increased adiposity and impaired glucose tolerance, and high fat diet exacerbates the development of metabolic syndrome in $TLR4^{\Delta IEC}$ mice. These metabolic features correlate with the composition and metabolism of gut microbiota, and oral administration of antibiotics prevents the development of metabolic syndrome in $TLR4^{\Delta IEC}$ mice, illustrating the importance of the intestinal microbiota in the process. These results support the pivotal role of interaction between gut microbiota and host metabolism in the development of metabolic syndrome, and we conclude that metabolic syndrome develops in part through abnormal microbial behavior via intestinal epithelial Toll-like receptor 4, suggesting that targeting the mucosal innate immune system may offer novel therapeutic approaches for metabolic syndrome.

F72. Manually Curated 16S rRNA Database and Associated Seamless Updating Platform

Seok-Won Kim and Todd D. Taylor. RIKEN Center for Integrative Medical Sciences, Yokohama, Kanagawa, Japan

Data growth in DDBJ/EMBL-EBI/NCBI is rising exponentially due to the increase of novel bacteria isolation and metagenomic studies. To manipulate these data, the primary data, including its associated metadata and sequences, needs to be checked in the next update stage against its own secondary database. This may result in a bottleneck when updating such massive datasets. Here, we present a massive sequence tracking and management platform for solving this issue. We constructed a manually edited 16S ribosomal RNA (rRNA) gene database called GRD. In GRD, both the 5' regions and 3' regions, including the anti-SD sites, have been carefully checked and contaminating sequences have been removed. Because of this careful manual checking of the 16S rRNA sequences, our database can be considered the most reliable reference source for downstream analyses. In addition, we are including PCR-based sequences which are published in public primary databases. We developed this platform for continuous updating and maintenance of the sequences and taxonomy information in this database. In particular, recently changed taxonomic names are updated according to the NCBI Taxonomy database. As with

the genomic-based sequences, we confirm all amplified sequences which have 5' and 3' regions by manual curation. Our platform can be applied not only for rRNA genes, but also for other marker genes.

F73. Increased Plasma IL-6 and Prevalence of Gram-Negative *Bacteroides xylanisolvens* in Faeces from Type 1 Diabetes Patients

Gislane Lelis Vilela de Oliveira[1], Bruna Stevanato Higuchi[1], Nathalia de Campos Rodrigues[2], Aline Zazeri Leite[3], João Carlos Cicogna Paiolo[3], Euclides Matheucci[2] and Vânia Sammartino Mariano[4]. [1]School of Health Sciences from Barretos, Barretos, Sao Paulo, Brazil; [2]DNA Consult Genetics and Biotechnology, São Carlos, Sao Paulo, Brazil; [3]Board of Health from Barretos, Barretos, Sao Paulo, Brazil; [4]Barretos Cancer Hospital (HCB), Barretos, Sao Paulo, Brazil

Abnormal relationship between host immune system and commensal gut microbiota can contribute to infectious or autoimmune diseases development in humans. The aim of this study was to characterize the intestinal microbiota from type 1 diabetes patients (T1D) and correlate this data with inflammatory cytokines. This study was approved by the Ethics Committees from Barretos Cancer Hospital (903/2014) and subjects signed the informed consent form. Stool samples were required to sequencing V3/V4 regions from bacterial 16S by using Illumina platform. Peripheral blood were used to quantify plasma anti-CVB IgG and cytokines by ELISA and CBA flex, repectively. Statistical analysis was performed by Mann-Whitney and Spearmans' tests. The study included 23 T1D patients (24.13 ± 11.74 years) and 28 healthy controls (25.25 ± 9.79 years). The predominant phyla found in T1D patients were *Firmicutes* and *Bacteroidetes*. The prevalent species were *Bacteroides vulgatus*, *Bacteroides rodentium*, *Blautia coccoides*, *Prevotella copri*, *Akkermansia muciniphila* and *Bacteroides xylanisolvens*. Positive correlation was found (P=0.02; r=0.67) between fasting glucose and glicated hemoglobin A_{1C} percentages (P = 0.03; ; r= 0.74) with *Bacteroides xylanisolvens* reads. Plasma levels of IL-6 were increased in patients (P < 0.05). Negative correlations between TNF (P=0.04; r=-0.57) and IFN-γ (P=0.01; r=-0.65) with *Bacteroides xylanisolvens* reads were observed. In conclusion, we observed dysbiosis in T1D patients, with lower diversity of phyla and species compared with controls. Further studies with the gram-negative *Bacteroides xylanisolvens* are necessary to determine whether it may represent a target for probiotics.

F74. Gut Microbiota as a Source of Signals that Trigger Spontaneous Ocular Autoimmunity

Ryan Salvador[1], Reiko Horai[1], Carlos Zárate-Bladés[1], Kikuji Itoh[2], Yingyos Jittayasothorn, Yoshinori Umesaki[3] and Rachel Caspi[1]. [1]National Eye Institute, NIH, Bethesda, MD; [2]Bio-Technical Center, Hamamatsu, Shizuoka, Japan; [3]Yakult Central Institute, Kunitachi, Tokyo, Japan

Autoimmune uveitis is a blinding disease of unknown etiology. Activated autoreactive T cells specific for unique retinal antigens elicit disease, but where and how they become activated to be able to cross the blood-retinal barrier remains to be elucidated. We developed a retina-specific TCR transgenic (R161H) mouse model of spontaneous uveitis to study natural triggers. Using this model, we demonstrated that elimination of gut commensals by oral broad-spectrum antibiotic treatment (ABX) or by rearing under germ-free conditions (GF) significantly attenuated uveitis and reduced Th17 cells in the gut lamina propria. Bacteria-rich intestinal content extract from specific-pathogen-free (SPF), but not GF or ABX mice, activated retina-specific T cells, several-fold more than nonspecific T cells, suggesting a role for gut microbiota as a source of surrogate antigen. To dissect the importance of antigenic signals vs innate "adjuvant" effects, we colonized GF mice with selected commensals. Colonization of GF-R161H mice with SPF flora restored full development of uveitis. In contrast, monocolonization with segmented filamentous bacteria (SFB) or *Turicibacter* strain H121 (T.H121) partially restored disease, which only in the case of SFB reached statistical significance over GF control. SFB, but not T.H121, monocolonization restored gut Th17-producing cells, indicating that SFB possesses "adjuvant" activity. However, unlike intestinal content extract from SPF mice, the extracts from SFB or T.H121-associated gut contents failed to activate R161H T cells in culture, indicating that both lack "antigen" activity. We conclude that microbial-derived innate "adjuvant" and adaptive "antigen" components are both required to trigger full development of uveitis.

F75. Anti-Commensal IgG1 Antibodies Identify a Group of Immunostimulatory Commensal Bacteria

Eduard Ansaldo Gine. University of California, Berkeley, CA

The signals that govern immune recognition and induction of adaptive immune responses in the intestine are still poorly understood. Anti-commensal immune responses during homeostasis are characterized by a lack of overt pro-inflammatory responses, leading to the induction of T regulatory cells and/or IgA in most instances of antigen-specific responses. The lack of anti-commensal T-dependent IgG was thought to be a general trait of mucosal immunity in the intestine, and only one instance of a CD4 non-Treg cell response against a commensal species, Segmented Filamentous Bacteria, has been described in mice at steady state. Here we identify a subset of commensal species that induce T-dependent IgG1 antibody responses under homeostasis and we discuss how targeted species may share certain features that lead to distinct sampling or signaling to the immune system. Finally, we describe *Akkermansia muciniphila* as one of the main targets of this response in mice from our colony and generate antigen-specific tools to study the effector T cell response.

F76. HLA-B27-Induced Intestinal Dysbiosis is Strongly Influenced by Genetics and Environment Despite Common Immune Dysregulation

Tejpal Gill[1], Mark Asquith[2], Stephen Brooks[1], James Rosenbaum[2] and Robert Colbert[1]. [1]National Institute of Arthritis and Muscuskeletal and Skin Diseases - NIH, Bethesda, MD; [2] Oregon Health & Science University, Portland, OR

HLA-B27, a major risk factor for spondyloarthritis (SpA), expressed with human beta-2-microglobulin (hb_2m) in rats causes SpA-like disease. Gut microbiota has been implicated in the pathogenesis of SpA, and we recently demonstrated that HLA-B27 alters gut microbiota in rats. To determine the effect of host background and environment on HLA-B27-associated dysbiosis, three different backgrounds; Dark Agouti (DA), Lewis and Fischer rats, and HLA-B7/hb_2m transgenic (HLA-B7) rats on the Lewis background were studied. Gut inflammation, microbiota, and host-transcriptome were analyzed. DA rats are resistant to HLA-B27-induced gut inflammation, while it increases with age in Lewis, and is severe in Fischer rats. HLA-B7 Lewis rats remain unaffected. Disease severity in Lewis and Fischer correlates with increased *Prevotella and Akkermansia* respectively at the expense of *Ruminococcus*. Beside some overlaps, dysbiotic microbes in HLA-B27 Lewis and Fischer are predominantly non-overlapping. HLA-B27 DA and HLA-B7 Lewis rats have altered microbiota, yet neither develops disease. Metagenome predictions reveal perturbed LPS and steroid hormone biosynthesis in Lewis and Fischer HLA-B27 rats. Strikingly, Lewis and Fischer rats harbored segmented filamentous bacteria (SFB) in the ileum, while SFB are absent from the DA background. Transcriptomeanalysis revealed robust activation of IL-17, IL-23, IFNg and TNF in cecum and colon of HLA-B27 Lewis and Fischer rats. Inter-omics analysis of microbes and host transcripts on susceptible backgrounds identified disease-associations not found by comparing relative frequency alone. These results show that HLA-B27 induced microbial dysbiosis is profoundly affected by host background and environment.

F77. Mucosal Immune Recognition of Recombinant *Lactobacillus acidophilus* and the Effect on Intestinal Microbial Community Structure

Gregg A. Dean[1], Jonathan S. LeCureux[1], Sara A. Bumgardner[2], Alora S. LaVoy[1], Lin Zhang[1] and Zaid Abdo[1]. [1]Colorado State University, Fort Collins, CO; [2] University of Georgia, Athens, GA

Eliciting protective mucosal immune responses using commensal-based vaccine vectors offers an attractive, inexpensive, and safe strategy to protect against many mucosally transmitted pathogens including HIV-1. We are exploring recombinant *L. acidophilus* (rLA) as a platform to induce mucosal and systemic humoral immune responses. *L. acidophilus* naturally expresses ligands recognized by TLR2 and NOD2 but the role these innate immune receptors play in antigen-specific antibody responses induced by rLA is uncertain. *In vitro* co-culture of rLA with macrophages induced a M2b phenotype. After oral inoculation of mice, we have found that rLA is rapidly taken into Peyer's patches and is found within $CD103^+$ cells. Antigen specific antibody responses against HIV-1 were dependent on NOD2 and the Caspase-1 pathway. Co-expressed IL-1β or the TLR5 agonist FliC (flagellin) acted as adjuvants to enhance the immunogenicity of HIV-1 antigen expressed on the surface of *L. acidophilus*. The antibody response was

abrogated in CD40L$^{-/-}$ mice, indicating an essential role for *T cell*s even when FliC was used as a co-expressed adjuvant. Oral immunization resulted in shifts in the intestinal microbial community structure that varied depending on the *L. acidophilus* construct. Whether the shift in microbiome was due to the vector, per se, or the mucosal immune response, is under investigation.

F78. The Pathobiont *Helicobacter bilis* Induces Targeted Immune Responses Against Other Gut Symbionts but Not Towards Itself

Joao Carlos Gomes-Neto[1], Hatem Kittana[1], Sara Mantz[1], Rafael Ricardo Segura Munoz[1], Laure Bindels[1], Robert Schmaltz[1], Jennifer Clarke[1], Andrew Benson[1], Jens Walter[2], Jesse Hostetter[3] and Amanda Ramer-Tait[1]. [1]University of Nebraska-Lincoln, Lincoln, NE; [2] University of Alberta, Edmonton, AB, Canada; [3] Iowa State University, Ames, IA

Inflammatory bowel diseases (IBD) are likely driven by exaggerated immune responses directed against the gut microbiota and are associated with an altered microbial composition often hypothesized to be enriched in colitogenic pathobionts. Despite major efforts, the etiological bacterial agents of IBD have yet to be identified, and it is unclear if alterations in microbiota composition contribute to disease. The inability to determine the specific pathological role of individual microbes in a complex ecosystem contributes to that lack of progress. Here, we use a pathobiont-induced colitis model with a defined bacterial community to determine the exact immune-microbiota interactions and gut microbial community patterns of disease. We established that the pathobiont *Helicobacter bilis* causes intestinal inflammation of similar severity in both conventional and Altered Schaedler Flora (ASF) mice following a sub-pathological dose (1.5%) of dextran sulfate sodium (DSS). Disease was less severe in *H. bilis*-colonized germ-free mice, demonstrating a contributing role for the resident microbiota in exacerbating disease pathology. Interestingly, ASF-bearing, DSS-treated mice colonized with the *Helicobacter* pathobiont developed a population of CD4$^+$ T cells producing IL-17 in response to specific ASF members but not against itself. Of note, changes in the relative abundance of neither *H. bilis* nor the ASF members were associated with inflammation or the enhanced immunoreactivity to certain resident species. Together, these findings are relevant for our understanding of IBD as they demonstrate the complexity of microbiota-immune interactions in driving colitis and the inability to draw relevant conclusions through the sole analysis of microbial community patterns.

L.04, F59. The Microbial Metabolite-Sensing Gpr43 Receptor and the Microbial Metabolite Acetate are Important to Host Defense and Immune Modulation Against Lung Bacterial Infections

Luciana Tavares[1], Izabela Galvão[1], Cristiana Garcia[2], Mauro Teixeira[1], Flaviano dos Santos Martins[1], Sergio Costa Oliveira[1], Charles Mackay[3] and Angelica Thomaz Vieira[1]. [1]Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; [2]Fundação Oswaldo Cruz- FIOCRUZ, Rio de Janeiro, Rio de Janeiro, Brazil; [3]The University of Sydney, Sydney, New South Wales, Australia

Klebsiella pneumoniae and *Streptococcus pneumoniae* are the mostly common cause of pneumonia and death wordwide. The gut microbiota wires local intestinal mucosal immunity and is increasingly recognized as an important modulator of the systemic immune response although the mechanisms are still poorly understood. Here, we hypothesized that one of these mechanism evolved the intestinal production of the metabolite acetate reaching higher systemic levels and binds to their receptor Gpr43 modulating lung inflammation. We found that in the absence of Gpr43, by using Gpr43-deficient mice, these mice display similar susceptibilities to both bacterial pneumoniae, as previously demonstrated in germ-free mice, indicating that the absence of microbiota or metabolite/Gpr43 activation confers no protection and increased lung injury. However, infected mice acetate-treated presented faster resolution of inflammation with enhancement of the cytokine IL-10, decreased of pro-inflammatory cytokines TNF-a and IL-1b, also neutrophils and macrophages, decreasing lung damage and reduction of pathogen growth contributed to rescue 70% of mice from death. Also, alveolar macrophages showed high production of reactive oxygen species in the presence of acetate. This phenotype correlates with reduction of bacteria in the lung from infected mice acetate-treated. However, alveolar macrophages from Gpr43-deficient mice showed a diminished capacity to phagocytose and reduction of ROS related with higher amounts of

bacteria in the lung. Thus, we propose that the metabolites produced by gut commensal microbiota protects the host against pneumonia-induced death by fine tuning the immune cells in the lung and contributing to faster return to lung homeostasis through activation of Gpr43 receptor.

OR.01, W25. T Follicular Helper Cells Promote a Beneficial Gut Ecosystem for Host Metabolic Homeostasis by Sensing Microbiota-Derived Extracellular ATP

Lisa Perruzza and Fabio Grassi. Institute for Research in Biomedicine, Bellinzona, Ticino, Switzerland

The ATP-gated ionotropic P2X7 receptor regulates T follicular helper (Tfh) cells abundance in the Peyer's patches (PPs) of the small intestine; deletion of *P2rx7,* encoding for P2X7, in Tfh cells results in enhanced IgA secretion and binding to commensal bacteria. Here we show that Tfh cells activity is important for generating a diverse bacterial community in the gut and that sensing of microbiota-derived extracellular ATP via P2X7 promotes the generation of a proficient gut ecosystem for metabolic homeostasis. The results of this study indicate that Tfh cells play a role in host-microbiota mutualism beyond protecting the intestinal mucosa by induction of affinity matured IgA and suggest that extracellular ATP constitutes an inter-kingdom signaling molecule important for selecting a beneficial microbial community for the host via P2X7-mediated regulation of B cell help.

OR.02, W22. Microbial Regulation of Host Vitamin D Metabolism

Stephanie Bora, Andrew Patterson, Philip Smith and Margherita Cantorna. Pennsylvania State University, University Park, PA

Vitamin D deficiency changes the microbiota. We determined how microbial depletion with antibiotics (Abx) affected vitamin D metabolism. Vitamin D is converted to 25(OH)D(25D) which is further metabolized to active vitamin D ($1,25(OH)_2D$, 1,25D). Both 25D and 1,25D are further hydroxylated by a 24-hydroxylase to produce $24,25(OH)_2D$ (24,25D) and $1,24,25(OH)_3D$. Vitamin D, 25D, and 24,25D were measured before and after Abx treatment for 2 wks. Depleting the microbiota increased 25D and 24,25D suggesting the microbiota or Abx were altering vitamin D metabolism. To determine whether microbial signals through toll-like receptors were required, mice with a deletion in the adapter protein (MyD88KO) were treated with Abx. Abx did not increase 25D in MyD88KO, but did increase 24,25D levels perhaps suggesting a difference in the microbial regulation of 25D and 24,25D. Extra-renal production of 1,25D and 24,25D by immune cells has been demonstrated. The Abx effect on vitamin D metabolism was tested in bone marrow chimeras, such that renal vitamin D metabolism was eliminated (VDRKO recipient, wildtype (WT) donors). Abx had no effect on 25D or 24,25D in VDRKO-WT chimeras. If 1,25D was produced by immune cells, then the chimeras should have had detectable 24,25D following Abx treatment. The regulation of the 25hydroxlase by Myd88 and the immune system is novel and suggests that the microbiota may be regulating 25D levels. The increase in 24,25D in the MyD88KO host suggests that the induction of 24hydroxylase may be a direct result of the Abx treatment and not the microbial depletion.

OR.03, W21. A Protective MHC Class II Molecule Prevents Autoimmune Diabetes by Selecting for Specific Intestinal Microbes Early in Life

Lidiya Denu[1], Kua Lindsay[2] and Michael Aaron Silverman[1,3]. [1]Children's Hospital of Philadelphia, Philadelphia, PA; [2]Harvard Medical school, Boston, MA; [3]University of Pennsylvania, Philadelphia, PA

Type 1 diabetes (T1D) affects millions of people worldwide. The incidence has risen significantly over the past few decades. Both genetic and environmental factors contribute to T1D. We recently demonstrated that the protective major histocompatibility complex (MHC) E molecule prevents autoimmune diabetes in NOD mice by shaping the intestinal microbiome early in ontogeny. This finding presents a novel paradigm in which genetic protection from immune system dysregulation is mediated via commensal microbiota. An important gap in knowledge is which intestinal microbes prevent autoimmunity by modulating the immune system. We hypothesize that those microbes that differentially stimulate humoral immune responses in the previously reported MHCII E-expressing NOD (Eα16/NOD) mice may also modulate the immune system to prevent autoimmunity. We address this question by leveraging the exquisite sensitivity and specificity of the humoral immune system to identify a subset of microbes that stimulate mucosal and/or systemic immune responses.

OR.05, W24. Intestinal Microbiota Regulates Peyer's Patch Size via Modulation of Naïve Lymphocyte Homeostasis

Milas Ugur, Anne Kaminski and Oliver Pabst. Uniklinik RWTH Aachen, Aachen, Nordrhein-Westfalen, Germany

Intestinal microbiota affects multiple aspects of intestinal immune responses. Peyer's patches (PPs) are particularly sensitive to changes in intestinal microbiota as they contain active germinal centers and constantly exposed to microbes and microbial products from the intestine. It is well known that germ-free or antibiotics-treated mice have smaller PPs with altered compositions compared to SPF mice. Surprisingly, we observed that antibiotic induced changes in PP size happen much faster that previously assumed and the reduction in size was apparent even after 3 days. Interestingly, these changes were limited to distal PPs and other lymphoid organs, including the proximal PPs, were not affected by the short-term antibiotics treatment. Furthermore, this effect was not due to total bacterial loads and different combinations of antibiotics had different effects on distal PP size, suggesting specific members of the microbiota having different effects on PP size. Decrease in PP size was associated with reduced homing of naive lymphocytes, which represents >60% of total lymphocytes in PPs. We also observed reduced TNF-alpha and IL-22 expression from innate lymphoid cells which might contribute to different aspects of naive lymphocyte homeostasis in PPs. Our results show a previously unrecognized effect of intestinal microbiota on naive lymphocyte circulation in PPs. We speculate that changes in PP size can qualitatively affect adaptive immune responses in the small intestine due to low frequencies of naïve lymphocytes specific for an antigen.

OR.100, F70. Microbiota-Derived GABA Exacerbates Intestinal Inflammation by Modulating Innate Immunity

Sara Dann[1], Alex Peniche[1], Jennifer Spinler[2] and Tor Savidge[2]. [1]University of Texas Medical Branch, Galveston, TX; [2] Baylor College of Medicine, Houston, TX

The intestinal microbiota produces many metabolites that play crucial roles in intestinal homeostasis and inflammatory diseases. Gamma-aminobutyric acid (GABA) is one metabolite produced by several commensal species that has been reported to have anti-inflammatory properties, which has led to speculation that GABA administration may have therapeutic potential for inflammatory bowel disease. The aim of the present study was to determine the effect of microbiota-derived GABA on intestinal inflammation following acute intestinal injury induced by dextran sodium sulfate (DSS) and *Clostridium difficile* infection. C57BL/6 mice were colonized with GABA-producing *Bifidobacterium dentium* or admainistered GABA in the drinking water before induction of colitis (3% DSS or *Clostridium difficile* infection) and for another week thereafter. Intestinal tissue was collected and examined for histopathology, gene expression and cellular responses. In both colitis models, administration of GABA exacerbated intestinal inflammation and damage, whereas inhibition of GABA with the receptor antagonist picrotoxin reduced disease severity. Damage was associated with GABA-A receptor signaling. *In vitro*, GABA impaired IL-6 release by LPS-activated bone marrow derived macrophages, but treatment with GABA-A receptor agonist significantly enhanced IL-6 production and promoted an upward trend in TNF-α secretion, suggesting there are different effects of GABA depending on the receptors being activated. Our data demonstrate that microbial-derived GABA increases host sensitivity to epithelial damage and inflammation. Identifying the effects of GABA on host immune defenses may reveal new approaches to treating and preventing IBD.

OR.101, F55. Antibiotics Induce Persistent Intestinal Th1 Responses by Perturbing Macrophage Homeostasis

Elizabeth Mann[1], Peter Andersen[2], Hannah Wessel[1], Allison Bancroft[3], Alberto Bravo-Blas[1], Carolyn Thomson[1], Verena Kaestele[1], Daniel Peterson[4], Xuhang Li[2], Richard Grencis[3], Allan Mowat[1], Mark Travis[3] and Simon Milling[1]. [1]University of Glasgow, Glasgow, Scotland, United Kingdom; [2]Johns Hopkins Medicine, Baltimore, MD; [3]University of Manchester, Manchester, England, United Kingdom; [4]Eli Lilly, Indianapolis, IN

Intestinal macrophages are specialized to deal with the huge antigenic load in the gut and are critical for local immune homeostasis. However, it is unknown

how intestinal macrophages become conditioned to promote microbial tolerance. Here, we show that the intestinal microbiota shapes the regulatory functions of macrophages in the colon, mediating host responses to infections and inflammation. We provide evidence that broad-spectrum antibiotic use disrupts intestinal macrophage homeostasis, causing macrophages to become hyper-responsive to bacterial stimulation. Thus, re-colonization of antibiotic-treated mice with a normal microbiota induced intestinal macrophages to produce excess amounts of pro-inflammatory cytokines, driving a subsequent, macrophage-dependent long-term increase in Th1 cells in the colon. The consequences of this dysregulated macrophage activity for *T cell* function were demonstrated by enhanced susceptibility of recolonized mice to bacterial infection requiring Th17 responses for clearance (*Citrobacter rodentium)* and helminth infection requiring Th2 responses for clearance (*Trichuris muris),* corresponding with increased inflammation in both cases. Administration *in vivo* of the short-chain fatty acid (SCFA) butyrate (usually generated from dietary fibre by the gut microbiota) partially restored the normal anergy of colonic macrophages to stimulation and abolished the enhanced Th1 responses after re-colonization. Furthermore, butyrate enabled sufficient Th17 responses to be generated during *C.rodentium* infection for bacterial clearance. In summary, the gut microbiota is essential for maintaining macrophage-mediated intestinal immune homeostasis, mediated by a SCFA-dependent pathway. Oral antibiotics can disrupt this process and promote susceptibility to persistent intestinal infections and inflammation, highlighting the potential impact of broad-spectrum antibiotic use in human health.

OR.103, F52. Iron Deficiency Modulates the Intestinal Microbiome Influencing Distal Liver Immunogenecity and alloimmunity.

Lindsey Ann Edwards, Elliot Merritt, Elizabeth H. Gray, Sotiris Mastoridis, Juanjo Lozano, Toni L. Meister, Elisavet Kodela, Marc Martinez-Llordella and Alberto Sanchez-Fueyo. King's College London, London, England, United Kingdom

The liver is in direct contact with the intestinal-microbiota, receiving 70% of blood flow via the portal vein from the intestine; thus, high concentrations of commensal-antigens reach the liver. The liver maintains a state of local-immune-tolerance to commensal-antigens. Indeed, liver grafts are accepted across major histocompatibility barriers *in vitro* without immunosuppressive therapy. In germfree-mice liver transplant tolerance and hepatocyte regeneration is microbiota-dependent. We previously found, one key parameter discriminating tolerant from non-tolerant human liver transplant recipients is iron status. Iron is an element essential for bacterial growth and is important in bacterial pathogenesis. We used pre-clinical models of iron deficiency and conducted ribosomal 16s sequencing, high-dimensional flow cytometry, and transcriptomic analysis. Data indicates that a reduction in iron levels akin to non-tolerant rejecting humans, promotes liver allograft immune damage via intestinal-microbiota modification. We found depleted levels of butyrate producers such as *Roseburia* and out-growth of iron-scavenging pathogenic species. Effects to the epithelial barrier function and differentiation along the “crypt-villus-axis” were observed. Iron levels modified the molecular profile of intestinal epithelial cells; including, Indole-amine-2,3-dioxygenase (IDO) expression. IDO catabolizes tryptophan to kynurenine, a ligand of the aryl-hydrocarbon receptor, affecting APC immunogenicity and *T cell* activation. Indeed, we observe alterations in IL-17 and IL-22 expression in innate cells and modulated Th-17 induction in iron deficiency. We provide a potential link between iron, the intestinal-microbiota, and distal immune responses; studying this interaction further should provide insight into the systemic immune-modulatory effects of the microbiota and may have future clinical implications.

OR.31, W23. Maternal Antibodies Regulate Neonatal Immunity

Meghan Anne Koch. UC Berkeley, Oakland, CA

Following birth, the mammalian intestinal tract is colonized by a complex community of microbes collectively termed the microbiota. To ensure the optimal health of the host, it is critical that the immune system respond to these microbes in non-inflammatory fashion. Although there has been extensive research into the mechanisms by which mucosal homeostasis is maintained in adulthood, little is known about how these immune responses are initially established following microbial colonization. We found that maternal antibodies regulate mucosal immunity following birth. Characterization of this

response revealed the surprising result that healthy animals generate IgG antibodies reactive to the microbiota in addition to antibodies of the IgA isotype. Ultimately these IgG antibodies are transmitted from mother to offspring where they coordinate with IgA to prevent bacterial translocation, limit effector T cell responses in the intestinal mucosa and promote weight gain during this critical developmental window. These results shed important insight into how maternal derive factors influence offspring physiology and help establish appropriate immune responses to the intestinal microbiota.

OR.34, W20. Deciphering How Invariant NKT Cells Establish Tissue Residency in Mucosal Tissue During Neonatal Development

Shankar Iyer[1,2], Thomas Gensollen[2] and Richard Blumberg[2]. [1]Harvard Medical School, Boston, MA; [2]Brigham and Women's Hospital, Boston, MA

The post-natal period represents a critical time for mucosal immune development that coincides with early life colonization of the host by microbial pioneers and inital host exposure to environmental antigens. Consequently, during this timeframe, the neonatal immune system undergoes profound developmental changes that involve dynamic interactions between embryonically derived tissue resident immune cells already entrenched within mucosal tissue, post-natal immune emigrants, and diverse microbial communities that populate mucosal tissue during this period of ecological succession. One week after birth, CD1d restricted invariant Natural Killer T (iNKT) cells emigrate from the thymus and undergo rapid expansion to establish long life residency in peripheral tissues. In some mucosal compartments, like the colon, this process is limited by the presence of the microbiota, as germ free (GF) animals exhibit elevated frequency of iNKTs and consequently increased senstivity to CD1d dependent inflammatory responses such as oxazolone colitis compared to animals raised under specific pathogen free (SPF) conditions. This period of iNKT proliferation modulated by the microbiota is restricted to the neonatal period as conventionalization of GF animals at birth but not after weaning resolves iNKT frequency in the colon to to that of animals raised under SPF conditions. Furthermore, during this period of expansion, iNKTs exhibit hyporesponsiveness to CD1d lipid antigen stimulation suggesting a decoupling of immune response from their proliferative capacity. Here we have defined a kinetic transcriptional program mediated by the AP-1 family of transcription factors that influence microbial dependent regulation of iNKT expansion and function in mucosal tissue during early life.

OR.41, T60. A Disease-Protective Human Commensal Discovered Using Microbial Pedigree Analysis

Neil Surana[1] and Dennis Kasper[2]. [1]Boston Children's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA

Microbiome-wide association studies have established that numerous diseases are associated with changes in the microbiota. These studies typically generate a long list of commensals implicated as biomarkers of disease, with no clear relevance to disease pathogenesis. In order to move the field beyond correlations and to begin to address causation, an effective system is needed for refining this catalog of differentially abundant microbes for subsequent mechanistic studies. Herein, we demonstrate that principles of family pedigree analysis used in genetics can be applied in microbiota studies to reduce the noise inherent in these experiments. We found that gnotobiotic mice harboring different microbial communities exhibited differential survival in a colitis model. Co-housing of these gnotobiotic mice generated “progeny” that had hybrid microbiotas reflective of both “parents” and displayed intermediate susceptibility to colitis. Mapping of microbe–phenotype relationships in parental mouse strains and in mice with hybrid microbiotas identified the bacterial family *Lachnospiraceae* as a correlate for protection from disease. Using directed microbial culture techniques, we discovered *Clostridium immunis*, a previously unknown bacterial species from this family, that—when administered to colitis-prone mice—protected against colitis-associated death. Thus, we have used “microbial pedigree” analysis to move beyond the standard correlative microbiome study and found a hitherto unidentified commensal that causally protects from colitis. More broadly, identifying disease-modulating commensals by means of microbial pedigree analysis may also be applicable to human microbiome studies.

OR.43, T61. Host-Protozoan Interactions Protect from Mucosal Infections through Activation of the Inflammasome.

Arthur Mortha. University of Toronto, Toronto, ON, Canada

While conventional pathogenic protists have been extensively studied, there is an underappreciated constitutive protist microbiota that is an integral part of the vertebrate microbiome. The impact of these species on the host and their potential contributions to mucosal immune homeostasis remain poorly studied. Here, we show that the protozoan *Tritrichomonas musculis* activates the host epithelial inflammasome to induce IL-18 release. Epithelial-derived IL-18 promotes dendritic cell-driven Th1 and Th17 immunity and confers dramatic protection from mucosal bacterial infections. Along with its role as a "protistic" antibiotic, colonization with *T. musculis* exacerbates the development of *T cell*-driven colitis and sporadic colorectal tumors. Our findings demonstrate a novel mutualistic host-protozoan interaction that increases mucosal host defenses at the cost of an increased risk of inflammatory disease.

OR.44, T59. *Clostridia* Mediated Protection from *Entamoeba histolytica*

Stacey L. Burgess and William A. Petri. University of Virginia School of Medicine, Charlottesville, VA

Mice colonized with the *Clostridia* SFB are protected from amebiasis. Neutrophils were increased in the intestine of SFB-colonized mice only after ameba infection. Transfer of BMDCs derived from SFB-positive mice provided protection from *E. histolytica* infection. These data suggested that commensal *Clostridia* could have potentially epigenetic effects on bone-marrow derived cells and susceptibility to infection. We hypothesized that the human commensal *Clostridium scindens* might also provide protection.CBA/J mice were colonized with *C. scindens,* and then infected with *E. histolytica*. Mice were treated with an inhibitor of the epigenetic mediator JMJD3 before and during *C. scindens* colonization but prior to infection with ameba. C*. scindens* colonized mice were protected from *E. histolytica* (culture positive = 18% vs 81%, p=0.01) and had increased intestinal neutrophils (10.3% ± 1.7 vs 5.9% ± 1.2, vs, p=0.04). JMJD3 expression in bone-marrow was increased (ΔΔ s, 113,700 ± 28,600 vs. 28, 400 ± 3, 200 vs, p=0.04). Bone-marrow granulocyte monocyte progenitors (GMPs) as a % of live cells were also increased from 0.63% ± 0.08 to 1.00% ± 0.12, p=0.02. Inhibition of JMJD3 blocked both the increase in GMPs and protection from amebiasis. *C. scindens* altered granulopoiesis and provided intestinal immunity to amebic colitis. JMJD3 inhibitors blocked this phenotype, suggesting that epigenetic pathways may underlie protection from ameba. Future studies utilizing adoptive bone-marrow transfers, flow cytometry for H3K27Me3, ChIP-seq and metabolic profiling will provide a better understanding of how changes in the microbiota may train the innate immune system to respond robustly to subsequent challenges.

OR.55, T58. Low Intestinal IgA Production in CVID Facilitates Parabiont-Mediated IFN Responses and Enteropathy

Natalia Shulzhenko[1], Xiaoxi Dong[1], Dariia Vyshenska[1], Renee Greer[1], Manoj Gurung[1], Stephany Vasquez-Perez[1], Kate Peremyslova[1], Stanislav Sosnovtsev[2], Michael Yao[3], Kim Montgomery-Recht[4], Warren Strober[2], Ivan Fuss[2,5] and Andrey Morgan[1]. [1]Oregon State University, Corvallis, OR; [2] National Institutes of Health, Bethesda, MD; [3] VA Medical Center, Washington, DC; [4] Leidos Biomedical Research, Inc., Frederick, MD; [5]NIAID, Bethesda, MD

Common variable immunodeficiency (CVID), the most common symptomatic primary immunodeficiency state, is characterized by decreased serum immunoglobulin levels and mucosal infections. A subgroup of these patients exhibit enteropathy due to villous atrophy and malabsorption associated with an IL-12-driven Th1 mediated immune response. In this study using a systems biology approach we investigated human immune gene expression and bacterial composition in duodenal biopsies obtained from three groups of individuals: healthy volunteers, CVID patients w/o enteropathy and CVID patients with enteropathy. The data obtained was then used to identify immune and metabolic abnormalities in CVID enteropathy and to define candidate organisms causing the enteropathy. Whereas CVID patients with enteropathy and without enteropathy had equivalent levels of deficiency in serum IgA levels, those with enteropathy had significantly lower levels of intestinal IgA expression. In addition, CVID patients with enteropathy but not those without enteropathy exhibited a gene expression profile in which IFN-related genes were up-

regulated whereas metabolic genes were down-regulated. These two facts define the intestinal conditions underlying the enteropathy. To find microbial drivers of the enteropathy, we first narrowed the universe of possible organisms in the duodenal microbiome capable of inducing IFN responses on the basis of association with low IgA mRNA levels and presence in the enteropathic bowel. We then integrated enteropathy associated gene expression with microbiome results into network called a "transkingdom" network in which organisms amongst those identified by the initial screening are selected by their connection with immune inflammatory genes. This analysis revealed *Acinetobacter baumannii* as a top candidate "pathobiont" capable of causing the enteropathy. Further experiments performed *in vitro* with a monocyte derived THP-1 cell line and in germfree mice supported this idea by showing that *A. baumannii* can induce inflammatory/interferon (IFN-beta and IFN-g) driven genes similar to those upregulated in CVID enteropathy patients. In addition, *A. baumannii* co-cultured with a macrophage cell line led to increased inflammatory cytokine induction. Consequently, interferons induced metabolic defect in cholesterol uptake in epithelial cells. These studies strongly suggest that CVID enteropathy is caused by low intestinal IgA expression that facilitates induction of IFN production by inflammatory macrophages stimulated with a pathobiont such as *A. baumannii*. This, in turn, causes secondary defects in epithelial metabolic function that aggravates the malabsorption syndrome. Studies to determine if this pathologic cycle can be interrupted by treatment with agents that block the Th1 response are underway.

OR.72, T62. Dual Oxidase (DUOX) 2 Mediates Changes in Mucus Composition in Patients with Ulcerative Colitis after Pouch Surgery

Keren Masha Rabinowitz[1], Sarit Cohen-Kedar[1], Karen Chait[2], Eduardo Contijoch[3], Jeremiah Faith[3], Metsada Pasmanik-Shorr[1], Shai Shen-Orr[2] and Iris Dotan[1]. [1]Tel Aviv Medical Center, Tel Aviv, Israel; [2] Technion- Israel Institute of Technology, Haifa, Israel; [3] Mount Sinai School of Medicine, New York, NY

DUOX2 plays a role in protecting mucosal barrier. Its expression is significantly increased in patients with ulcerative colitis after pouch surgery compared to the normal small intestine. Aim: to decipher the interaction between bacteria, DUOX2 and the mucus layer in the small intestine of UC patients with pouchs. Gene expression clustering analysis revealed concordant expression patterns of DUOX2, MUC1 and MUC4. DUOX2, MUC1 and MUC4 expression levels increased in normal pouches compared to the normal small intestine, and the increase was greater in the inflamed pouch. Decrease of key microbial taxa in fecal samples obtained from patients with a normal pouch compared to normal controls was observed ($p < 0.004$), and further decrease in patients with pouchitis ($p < 0.0001$). Interestingly, total microbial biomass was increased in patients with pouchitis compared to patients with a normal pouch. DUOX2 expression in epithelial cell line (IEC) significantly increased in response to inflammatory cytokines, fecal extracts and *Escherichia coli* compared to untreated cells. MUC1 and MUC4 expression increased in IEC in response to inflammatory cytokines as well. In IEC, over-expressing DUOX2: MUC1 and MUC4 mRNA levels were increased compare to control cells (≥ 1000 fold). In conclusion: Bacterial dysbiosis in pouches may cause DUOX2 induced expression. DUOX2 participates in controlling mucin production. Changes in DUOX2 expression and mucus composition lead to disruption of balance between bacteria and mucus lining the intestinal epithelium and may play a role in the development of pouchitis.

OR.99, F42. Genetic Analysis of the Effects of Serum Amyloid A on Zebrafish Innate Immune Function

Caitlin C. Murdoch, Colleen M. McClean, David M. Tobin and John F. Rawls. Duke University, Durham, NC

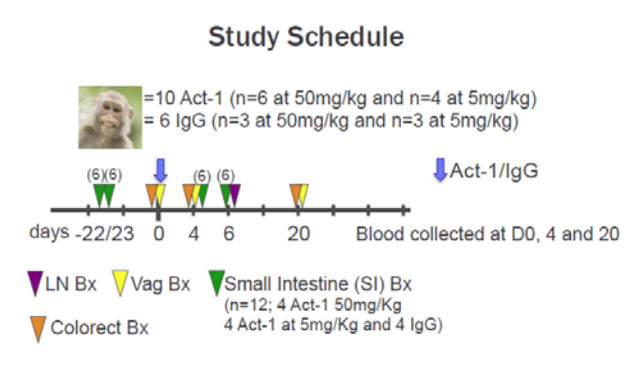
Serum Amyloid A (SAA) is a highly conserved protein secreted by the intestinal mucosa and liver. Induced by commensal and pathogenic microbes and injury, SAA has been identified as a biomarker for inflammatory diseases. Although its potent induction and evolutionary conservation suggest important biological roles, SAA's functions *in vivo* remain poorly understood. This knowledge gap is due in part to redundant paralogs in mammalian genomes, and conflicting reports from rodents and cell culture suggest both pro- and anti-inflammatory activities for SAA on immune cells including neutrophils. Here we use zebrafish, which have a single homolog of *saa*, to specifically elucidate how SAA regulates innate immunity in homeostasis and disease. We generated loss-of-function alleles, creating the first entirely SAA deficient vertebrate model. Using transgenic zebrafish, we provide novel insights into Saa's effects on neutrophil behavior and function. *saa* mutants have impaired neutrophil responses to wounding and differential outcomes following *mycobacterial* infection. Neutrophils in *SAA* deficient zebrafish have increased systemic abundance and altered dissemination at baseline. mRNA profiling of sorted neutrophils reveals SAA suppresses inflammatory transcripts. Using gnotobiotic zebrafish, we observe that Saa's effect on neutrophils depends on microbiota colonization, implicating this protein in potentiating the microbiota's influence on host innate immunity. To complement the phenotypes observed in *SAA* mutants, we over-express Saa in the zebrafish intestinal epithelium and document systemic anti-inflammatory effects. These data support a working model where, following microbiota-dependent induction in the intestine and liver, SAA reduces inflammatory tone while simultaneously priming neutrophils for response to injury or infection.

Immune Cell Migration

OR.53, T65. Impact of the Anti-α4β7 mAb Act-1 on Mucosal Immune Cell Subsets in Naïve Adult Rhesus Macaques.

Giulia Calenda[1], Geraldine Arrode-bruses[1], Ines Frank[1], Brooke Grasperge[2], James Blanchard[2], Agegnehu Gettie[3], Keith Reimann[4] and Elena Martinelli[1]. [1]Population Council, New York, NY; [2]Tulane National Primate Research Center, Covington, LA; [3]ADARC, New York, NY; [4]University of Massachusetts Medical School, Boston, MA

Infusion of a primatized anti-$\alpha_4\beta_7$ mAb (Rh-Act-1) just before and after SIV challenges of rhesus macaques (RM) prevented vaginal SIV acquisition, while RMs that acquired SIV infection (rectally and intravenously) were protected from developing AIDS. Recently, Act-1 in combination with cART to treat SIV infected RMs led to prolonged viral suppression after withdrawal of all therapeutic interventions. The humanized form of Act-1, Vedolizumab, is a highly effective treatment for inflammatory bowel disease. In order to clarify Act-1's mechanism of action (MOA), we infused 10 naïve RMs with Rh-Act-1 (6 at 50mg/Kg, 4 RMs at 5mg/Kg) and 6 RMs with IgG1. Blood, mucosal and lymphoid tissues were sampled at baseline, 4-6 and 20 days post-infusion. Several different immune cell subsets and cytokines/chemokines were monitored. Surprisingly, Rh-Act-1 did not impact α_4^+ cells in any of the mucosal tissues. Instead, Rh-Act-1 decreased Th1-like $CD4^+$ T cells in blood and vaginal tissue, but not in the colorectum, where Rh-Act-1 decreased Th17-like cells. $CCR5^+$ $CD4^+$ T cells decreased in the colorectum, while $CD69^+$ $CD4^+$ T cells decreased in the vaginal tissue. Treg decreased in inguinal lymph nodes and IgA^+ B cells in the small intestine. Notably, in the small intestine, the percentage of memory B cells decreased, while naïve increased. In conclusion, Rh-Act-1 impacts trafficking of selected immune cell subsets in different tissues even in absence of inflammation. Our data help to clarify the requirement for $\alpha_4\beta_7$ in the trafficking of different cell subsets to different tissues and explaining Act-1's MOA in HIV and IBD.



T63. Role of LRRK2 in Immune System Modulation

Juliana Rocha, Sun Chunxiang, Nichole Escalante, Michael Gloucauger and Dana Philpott. University of Toronto, Toronto, ON, Canada;

Variants of the leucine-rich repeat kinase 2 (*LRRK2*) are associated with an increased susceptibility to Parkinson disease but also Crohn's disease (CD). The present research is designed to develop a comprehensive understanding of the role of LRRK2 in immune system modulation, and how dysfunction of this pathway may lead to the development of CD. We found that LRRK2 KO mice have a defect in migration of neutrophils to the peritoneal cavity after injection of different microbial stimuli including FK10565 (NOD1 ligand), MDP (NOD2 ligand) and LPS (TLR4 ligand). Neutrophils from LRRK2 mice were compromised in their ability to transmigrate *in vitro* in a transwell assay using fMLP as a chemoattractant. Chemotaxis was also compromised. In parallel, we designed experiments to examine reactive oxygen species (ROS) produced in response to infection of myeloid cells with bacteria. Neutrophils from LRRK2 KO mice infected with *Listeria monocytogenes* were less able to restrict bacteria growth compared to WT cells. Consistent with these findings, cells from LRRK2 KO mice produced lower levels of ROS following bacterial infection. In order to determine whether myeloid cell migration is compromised *in vivo* during inflammation, we performed experiments in WT and KO mice looking at different models of ileitis/colitis. With this work,we will further characterize the role of LRRK2 in intestinal homeostasis and mucosal barrier maintenance, including how its deficiency may predispose an individual to developing CD.

T64. Human Dendritic Cells Attenuate Retinoic Acid Signalling to Imprint Pro-inflammatory 'Dual Tropic' α4β7^{+}CLA^{+} T cells

Inva Hoti[1], Katherine Wichmann[2], Theodore James Sanders[3], Martha Wildemann[2], Eve Hornsby[1,2], Neil E. McCarthy[1,2], James O. Lindsay[4] and Andrew J. Stagg[1,2]. [1]Blizard Institute, London, England, United Kingdom; [2]Queen Mary University of London, London, England, United Kingdom; [3]University of Oxford, Oxford, England, United Kingdom; [4]Department of Gastroenterology, The Royal London Hospital, Barts Health NHS Trust, London, England, United Kingdom

Intestinal dendritic cells (DC) produce retinoic acid (RA) to imprint gut tropism via induction of α4β7 integrin and suppression of selectin ligands. Altered T cell homing in IBD may contribute to extra-intestinal manifestations and to entry of cells into the intestine by α4β7-independent pathways. However, it is currently unclear how this abnormal homing can be induced by RA-producing intestinal DC. To address this question, we examined the homing profile of human naive CD4^{+} T cells activated with antibodies (anti-CD3/28/2), or with allogeneic colonic or monocyte-derived DC (moDC), using flow cytometry and qRT-PCR. Antibody-activated T cells expressed β7 but did not express the selectin ligand CLA. Inhibition of RA receptor (RAR)α signalling or removal of serum reduced β7 expression and induced expression of CLA suggest the signalling by serum retinoid maintains this phenotype. Unexpectedly, activation with colonic DC or RA-generating moDC also led to the emergence of CLA+ cells as the result of induction of *FUT7*, the fucosyltransferase that generates CLA. No effect of neutralising antibodies against cytokines that induce CLA (eg TGFβ, IL-12) was observed and cell contact, although not absolutely required, enhanced CLA induction. CLA^{+} cells induced by gut DC co-expressed high levels of β7, suggesting a 'dual-tropic' phenotype. Dual tropic β7^{+}CLA^{+} T cells were present in blood at low frequency and were enriched for cells producing pro-inflammatory cytokines (IFNγ, IL-17, TNFα) compared with gut-tropic (β7^{+}CLA^{-}) and skin-tropic (β7^{-}CLA^{+}) cells. Therefore, gut DC generate pro-inflammtory dual tropic T cells that may contribute intestinal and extra-intestinal inflammation.

Immunology of Asthma – Basic

F79. Assessment of IL-33-Expressing Cells in the Airway of Mouse

Hiroki Kabata, Tanel Mahlakoiv and David Artis. Weill Cornell Medicine, New York, NY

IL-33, an epithelial cell-derived cytokine, is constitutively expressed in various tissues all over the body, and cellular stress or death induce the release of IL-33, which causes eosinophilic inflammation largely mediated by group 2 innate lymphoid cells (ILC2). Recent genome wide association studies suggest strong association of asthma and SNPs in IL-33 and its receptor T1/ST2 genes. Furthermore, IL-33 is highly expressed in the airways of asthma patients. Alveolar type 2 (AT2) cells highly express IL-33 in mouse lungs and have been suggested to induce inflammation in mouse models of asthma, however, systematic analysis of IL-33-producing cell types in the airways is lacking. We evaluated the expression of IL-33 in the airway of mouse, and confirmed that airway epithelial cells in the trachea, bronchus, and bronchiole do not express IL-33 at steady state or even after the stimulation with cytokine, papain, or *Alternaria* extract. Although AT2 cells are the dominant IL-33-expressing cell type in the lung at steady state, other type of cells also expresses IL-33, particularly after the stimulation. Furthermore, the distribution of IL-33-expressing cells is different depending on the stimulation. Our preliminary results suggest that IL-33 biology in mouse airway is much more complicated than earlier thought and various type of cells are involved in the IL-33-mediated inflammation in the airway.

F80. Prophylactic Supplementation of *Bifidobacterium longum* 51A Protects Mice from Ovariectomy-Induced Exacerbated Allergic Airway Inflammation and Airway Hyperresponsiveness

Caroline M Ferreira[1], Eduardo Mendes[1], Flaviano dos Santos Martins[2] and Wothan de-Lima[1]. [1]Universidade Federal de São Paulo, São Paulo, Sao Paulo, Brazil; [2]Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil;

Asthma is a chronic inflammatory disease that affects more females than males after puberty, and its symptoms and severity in women change during menstruation and menopause. Recently, evidence has demonstrated that interactions among the microbiota, female sex hormones, and immunity are associated with the development of autoimmune diseases. However, no studies have investigated if therapeutic gut microbiota modulation strategies could affect asthma exacerbation during menstruation and menopause. Here we aimed to examine the preventive effects of a probiotic, *Bifidobacterium longum* 5^{1A}, on airway inflammation exacerbation in allergic ovariectomized mice. We first evaluated the gut microbiota composition and diversity in mice ten days after ovariectomy. Next, we examined whether re-exposure of ovariectomized allergic mice to antigen (ovalbumin) would lead to exacerbation of lung inflammation. Finally, we evaluated the preventive and treatment effect of *B. longum* 5^{1A}on lung inflammation and airway hyperresponsiveness. Our results showed that whereas ovariectomy caused no alterations in the gut microbiota composition and diversity in this animal model, ten days after ovariectomy, preventive use administration of *B. longum* 5^{1A}, rather than its use after surgery was capable of attenuate the exacerbated lung inflammation and hyperresponsiveness in ovariectomized allergic mice. This prophylactic effect of *B. longum* 5^{1A} involves acetate production, which led to increased fecal acetate levels and, consequently, increased Treg cells in ovariectomized allergic mice.

F81. Probiotic Supplementation during Perinatal Period or After Weaning Reduces Allergic Airway Disease in A/J But Not C57BL/6 Mice

Caroline M Ferreira[1], Mateus Casaro[1], Angelica Thomaz Vieira[2], Andrew Thomas[1], Emmanuel Neto[1], Willian Ribeiro[2], Rui Curi[1] and Flaviano dos Santos Martins[2]. [1]Universidade Federal de São Paulo, São Paulo, Sao Paulo, Brazil; [2]Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

Studies have shown that gut microbiota can influence allergic disease. Consequently, strategies that modulate gut microbiota, such as probiotics, may alleviate allergic disease. Interestingly, studies using

the same probiotic have shown inconsistent results for effects on allergic disease; it is possible that the host's genetics and gut microbiota influence probiotics' effects. Our objective was to elucidate if probiotic consumption during the perinatal period or after weaning can reduce Th2 airway inflammation in the inbred mouse strains A/J and C57BL/6 (B6). It is known that A/J are more predisposed to Th2 airway inflammation than B6. We first examined the gut microbiota composition and its importance to allergic inflammation in A/J and B6. Next, we administered *Bifidobacterium longum* 5^{1A} during the whole perinatal period or after weaning as well as during the whole asthma protocol. Experimental asthma was initiated in 6-week-old mice using ovalbumin. Twenty-four hours after the last challenge, pulmonary inflammation and lung function were analyzed. Interestingly, our results show that A/J had lower microbiota diversity than B6. Additionally, when A/J acquired gut microbiota from B6 by embryo transplantation, they showed reduced eosinophil airway infiltration, similar to that in B6, indicating that gut microbiota may have a greater influence on skewing toward the Th2 phenotype than host genes. Subsequently, probiotic treatment reduced allergic inflammation and airway hyperresponsiveness, independently of life stage, only in A/J. These results indicate that when probiotics are used to treat allergic disease, the host's genetics and gut microbiota composition should be considered.

F82. Differential Siganling Through Toll-Like Receptor 4 and Control of Inflammation

Hui-Ying Tung[1], Cameron Landers[1], Wen Lu[2], Nima Baghaei[1], Xuhong Cheng[3], Shao-Cong Sun[3] and David Corry[1]. [1]Baylor College of Medicine, Houston, TX; [2] University of San Francisco, San Francisco, CA; [3] University of Texas MD Anderson Cancer Center, Houston, TX

The immune basis of allergic versus non-allergic diseases differs vastly, however, toll-like receptor (TLR) 4 is involved in both. While the canonical TLR4 ligand, lipopolysaccharide (LPS), induces T helper (Th) 1 immune responses, our laboratory has shown that expression of allergic asthma and antifungal immunity require fibrinogen cleavage product (FCP) that signals through TLR4. Reverse phase protein array of LPS versus FCP treated RAW 264.7 cells demonstrated a distinctive protein activation profile induced by FCP in comparison to LPS. Western blot analyses further showed that LPS and FCP modulate NFκB and MAPKs distinctively. FCP upregulated MUC5AC, Arginase 1 and interleukin-13 receptor alpha gene expressions in the lungs, critical for expression of allergic asthma, are regulated by the transcriptional factor, Signal transducer and activator of transcription 6 (STAT6). Western blot results based on mouse bone marrow derived macrophages showed that STAT6 was clearly induced by FCP, but suppressed by LPS through TLR4. Blocking of Janus kinase 1 (JAK1) inhibited FCP-induced STAT6 phosphorylation, indicating that JAK1 kinase activity was required for FCP-induced STAT6 activation. STAT6 knock-out mice failed to induce airway hyperresponsiveness, eosinophilia, as well as Mucin and Arginase 1 gene expressions in response to FCP. In addition, FCP-induced anti-fungal defense was found to be regulated by NFκB, independent of STAT6. These results indicate that LPS and FCP, both ligands for TLR4, differentially activate STAT6 through TLR4. Further, FCP-induced antifungal immunity is mediated by NFκB signaling. Collectively, LPS and FCP differentially signal through TLR4 to control Th1 and Th2 immune responses, respectively.

F84. Regulatory T Cell Function and Th2 Immunity at Mucosal Barriers

Lucas D. Faustino, Jason W. Griffith, Rod Rahimi, James Moon and Andrew Luster. Massachusetts General Hospital, Harvard Medical School, Charlestown, MA

$Foxp3^+$ regulatory T cells (Tregs) have a central role in maintaining immune tolerance. Tregs have also been shown to directly contribute to tissue repair via the production of amphiregulin, which is induced in Tregs by IL-18 and IL-33. The IL-33 receptor, ST2, is enriched on Tregs residing in non-lymphoid tissues, and IL-33/ST2 engagement promotes their local expansion and tissue protective function during Th1- and Th17-mediated inflammation. Since IL-33 plays an important role in initiating allergic inflammation at mucosal barriers, we sought to determine the role of IL-33 in Tregs in a house dust mite (HDM) model of allergic airway inflammation. We found that $ST2^+$ Tregs accumulated in the lungs following HDM exposure. This was dependent on recruitment from a pool of circulating activated Tregs. $ST2^+$ Tregs in the inflamed lung were allergen non-specific and produced the type-2 cytokines IL-5 and IL-13 in response to IL-33, without any measureable

alterations in their suppressor function. Selective MyD88 deficiency in Tregs impaired lung ST2$^+$ Treg formation and attenuated allergic inflammation while increasing lung monocyte and monocyte-derived dendritic cell infiltration. These results suggest that in allergic settings, allergen non-specific Tregs contribute to tissue eosinophilia in response to innate signals that is distinct from their suppressor function induced by TCR engagement. Thus ST2$^+$ "tissue protective" Tregs are actually "pathogenic" in allergic inflammation as these Tregs release Th2 cytokines, which might contribute to tissue repair in Th1- and Th17-type inflammation, but contribute to disease pathology in Th2-type inflammation.

F85. IL-33 Upregulates Cysteinyl-Leukotriene Receptor Type 1 Expression in Human Peripheral Blood CD4$^+$ T Lymphocytes

Marie Boudaud, Sylvie Turcotte, Jana Stankova and Marek Rola-Pleszczynski. Université de Sherbrooke, Sherbrooke, PQ, Canada

During asthmatic inflammation, IL-33 is released as an alarmin by damaged airway epithelial cells. Following proteolytic cleavage, the cytokine binds to its specific receptor ST2 expressed on leukocytes, leading to the release of cytokines such as IL-5 and IL-13, and initiating the characteristic immune profile and symptoms of asthma. Cysteinyl-leukotrienes (cysLTs) have been described as major contributors to the airway hyperreactivity as strong bronchoconstrictors and also contribute to the Th2 phenotype, stimulating leukocyte chemotaxis and cytokine production. CysLTs are lipid mediators derived from arachidonic acid metabolism and include leukotriene (LT) C_4, D_4 and E_4. They act via, at least, two receptors, CysLT1 and CysLT2, which are expressed on the majority of leukocytes. This study is aimed at exploring the regulation of CysLT1 and CysLT2 expression by IL-33 in human peripheral blood lymphocytes isolated from healthy subjects. Although IL-33 did not affect CysLT2 expression, it increased CysLT1 expression in a concentration-dependent manner, up to 40%, at the protein level, in total lymphocytes. The upregulation was more pronounced in the CD4$^+$ T cell subpopulation with a maximum of 60%. In addition, LTD_4-induced calcium influx and migration was increased in IL-33-stimulated CD4$^+$ T lymphocytes. These results were reproduced in purified CD4$^+$ T cells, demonstrating a direct targeting of this population by IL-33. Finally, CysLT1 upregulation in response to IL-33 was dependent on ST2 in T lymphocytes. These results reveal a potential regulation of peripheral blood lymphocyte responses to cysLTs by IL-33, and reinforce the inflammatory capacity of this alarmin in allergic diseases.

F86. Methilation and Expression of TLRs, IL-28 and IL-33 and Their Role in Asthma

Oxana Svitich[1], Ludmila V. Gankovskaya[2], Leila S. Namazova-Baranova[2], Bella G. Bragvadze[2], Anna A. Alekseeva[3], Lisa Bystrickaya[1] and Viktor A. Gankovskii[3]. [1]Mechnikov Research Institute of Vaccines and Sera, Moscow, Moskva, Russia; [2] Pirogov Russian National Research Medical University, Moscow, Moskva, Russia; [3] Scientific Center of Children's Health, Moscow, Moskva, Russia

Asthma is known to be one of the most prevalent chronic noncommunicable diseases. And epigenitics of this pathology forms a field which has expanded greatly so far. They are immune component, which leads to the allergy, and epigenetic mechanisms, which consist of DNA methylation and regulation of gene expression by miRNA (mainly). We aimed to concentrate on DNA methylation and expression studings in order to find some new marks of asthma. We examined 38 patients with bronchial asthma from the age of 3 to 12 years old and 10 healthy children of the same age. Determinants of miRNA expression in scrapings from the mucous membranes of the respiratory tract and in peripheral leukocytes were carried out by PCR-RT. Mann-Whitney U-test was used to estimate the criteria of trustworthiness. In scrapes from patients with moderate asthma a significant increase was found inthe gene expression of TLR2 and TLR4, 3 times and 10 times respectively that in the control group. In children with severe asthma we also noticed an increase in the gene expression of TLR2 4.8 times more than the same rate in the healthy group ($p \leq 0.05$). TLR2 and TLR4 might be used in early case detection and in further epigenetic discovery of bronchial asthma. Although there were carried many researches only a few have investigated the whole epigenome. It's highly important to know as much epigenetic signatures as possible, as this will help to develop refined predictive algorithms for the purpose of asthma prevention and treatment.

F87. Early Chronic Allergen Exposures Drive Increased Airway Inflammation in Mice

Priya Halvorsen[1], Seddon Y. Thomas[2] and Donald N. Cook[2]. [1]National Institutes of Health, Raleigh-Durham, NC; [2] National Institute of Environmental Health Sciences, Research Triangle Park, NC

A growing body of evidence suggests that exposures to allergens in early life affect responses to allergens in adulthood and might be a contributing factor in allergic asthma. However, conclusions from these studies have been inconsistent, perhaps because of differences in age of first allergen exposures, and nature of the allergen itself. Furthermore, the molecular mechanisms responsible these observations remain obscure. We have therefore developed animal models in which mice are exposed to House Dust Mite (HDM) or House Dust Extract (HDE) as neonates, and after reaching adulthood, sensitized, and challenged with that same allergen, or with a different allergen. We found that mice exposed to HDM or HDE as neonates develop increased airway inflammation after sensitization and challenge compared with adults that were similarly sensitized and challenged, but were not exposed to the allergen as neonates. We hypothesize that this observation is related to long lasting epigenetic changes induced in airway epithelial cells (AECs) by inhaled allergens in early life. To test this, we are using high throughput sequencing methods to identify chromatin accessibility and gene expression changes AECs caused by early life exposures. Correlations between changes in these parameters and allergic responses to inhaled allergens during adulthood will be discussed.

F88. Role of Gut Microbiome in the Pathogenesis of Allergic Airway Disease

Bora Kim[1], Soo-In Kim[1], Min-Jung Kang[1], Ji-Won Choi Choi[1], Jin-Won Kim[1], Hyun Jin Min[2], Joo-Heon Yoon[1] and Chang-Hoon Kim[1]. [1]Yonsei University College of Medicine, Seoul, Republic of Korea; [2] Chung-Ang University College of Medicine, Seoul, Republic of Korea

Gut microbiota and the host have a symbiotic relationship, whereas altered gut microbiota has been associated with immune-mediated diseases, including allergic airway diseases. The first colonizers are derived from the mother before and during delivery and there is emerging evidence of an early-life critical window, when the effects of gut microbial dysbiosis are most influential in immune development at the mucosal surfaces. Here we sought to further clarify the pathophysiology of allergic airway diseases by determining whether perinatal antibiotic treatment has altered of gut microbiota and regulates susceptibility to a TH2 model of allergic asthma. Allergic asthma was induced BALB/c wild-type mice treated perinatally with ampicillin, vancomycin, and metronidazole in drinking water. Disease severity was assessed by measuring lung inflammation, pathology, cytokine response, and serum antibodies. Microbial community analyses were performed on stool samples via Next Generation Sequencing. We found that perinatal triple antibiotics treatment profoundly altered the gut microbial composition and were not observed to develop phenotype of allergic asthma. Perinatal antigen exposure of mice elicited a splenomegaly that was induced by neutrophils in spleen. Interestingly, we showed a decreased in dendritic cells of lung which reside in asthmatic neonatal group. Our findings will inform the development of novel approaches to investigate the relationship lung-gut axis based on modulating the composition of the gut microbiota.

F91. Selective Degradation of Fibrinogen by Diverse Proteinases Promotes TLR4-Dependent Antifungal Immunity

Cameron Landers[1], Hui-Ying Tung[1], Fabrizio Ottoboni[2] and David Corry[1]. [1]Baylor College of Medicine, Houston, TX; [2] Life Science Knowledge Consulting, Milan, Lombardia, Italy

Fibrinogen proteolysis within the airway lumen yields a novel molecule that drives the expression of allergic airway disease and antifungal immunity. Inhibition of airway proteinases or the absence of Toll-like Receptor 4 (TLR4) attenuates these processes both *in vivo* and *in vitro*. However, it remains unclear which proteolytic product of fibrinogen acts through TLR4 and what proteinases can induce this antifungal effect. Fibrinogen was incubated with thrombin, proteinase from *Aspergillus melleus* (PAM), or an extract from the dust mite *Dermatophagoides farinae* to generate three types of fibrinogen cleavage

products (FCPs). FCPs were subsequently analyzed by SDS-PAGE. Fungistasis was assayed by incubating FCPs with murine splenocytes or bone marrow-derived macrophages (BMDMs) for 24 hours, followed by the addition of fungal spores from *Aspergillus niger*. Fungal germination events were then enumerated 18 hours later. TLR4-dependency was confirmed using a TLR4 antagonist (LPS-RS) and $TLR4^{-/-}$ mice. Lipopolysaccharide and proteinase alone showed no fungistatic activity, while the FCP preparations primed murine leukocytes to significantly inhibit fungal growth. Analysis of fibrinogen degradation by proteinases from dust mites, storage mites, ragweed, and fungus indicated that the fibrinogen α chain was preferentially cleaved. Furthermore, d-dimer was a sufficient substrate through which PAM could generate fungistasis-inducing cleavage products. In the presence of diverse proteinases, human fibrinogen degrades to produce similar cytokine-like molecules that potently induce TLR4-dependent antifungal immunity. Given the unique molecular size and degradation patterns of these fragments, we propose the existence of a novel fibrinogen-derived signaling motif with potent immunostimulatory activity.

OR.79, F83. Plasmacytoid DCs or Microbial Metabolites Induce Semaphorin 4a-Mediated Treg Cell Expansion to Confer Protection Against Severe Viral Bronchiolitis and Subsequent Asthma

Jason Lynch[1], Rhiannon Werder[1], Md Al Amin Sikder[1], Bodie F. Curren[1], Zhixuan Loh[2], Vivian Zhang[1], Matthew Rogers[1], Katie Lane[1], Jennifer Simpson[1], Stuart Mazzone[3], Kirsten Spann[4], John Hayball[5], Kerrilyn Diener[5], Mark Everard[6], Christopher Blyth[7], Christian Forstner[1], Paul Dennis[1], Nida Murtaza[1], Mark Morrison[1], Paraic O' Cuiv[1], Ping Zhang[8], Ashraful Haque[8], Geoffrey Hill[9], Peter Sly[1], John Upham[9] and Simon Phipps[1]. [1]University of Queensland, Brisbane, Queensland, Australia; [2]Institute for Molecular Bioscience, Brisbane, Queensland, Australia; [3]University of Melbourne, Melbourne, Victoria, Australia; [4]Queensland University of Technology, Brisbane, Queensland, Australia; [5]The University of South Australia, Adelaide, South Australia, Australia; [6]University of Western Australia, Perth, Western Australia, Australia; [7]Telethon Kids Institute, Perth, Western Australia, Australia; [8]QIMR Berghofer Medical Research, Brisbane, Queensland, Australia; [9]Princess Alexandra Hospital, Brisbane, Queensland, Australia

RSV-bronchiolitis in infancy is a major independent risk factor for subsequent asthma but the causal mechanisms remain obscure. We modelled this association in neonatal mice, and identified that transient plasmacytoid dendritic cell (pDC) depletion during primary Pneumoviral infection alone was sufficient to predispose to severe bronchiolitis in early-life and progression to asthma following re-infection in adulthood. pDC depletion ablated type I and III IFN production and increased viral load, however the heightened immunopathology and induction of type-2 inflammation stemmed from a failure to expand functional neuropilin-1^{+} regulatory T (Treg) cells, which were dependent on pDC-derived semaphorin 4a (Sema4a). In adult mice, pDC depletion perturbed Treg cell expansion and predisposed to severe bronchiolitis but only after antibiotic treatment, implicating a protective role of the microbiome. Conversely, treatment of pDC-depleted neonates with the microbial-derived metabolite propionate restored Treg cell expansion and function in a Sema4a-dependent manner, ameliorating

bronchiolitis severity and subsequent asthma. Nasal propionate levels were also lower in 2-year-old children with viral bronchiolitis and correlated with an IL-6^{high} IL-10^{low} cytokine microenvironment. Our findings highlight a common but age-related Sema4a-mediated pathway by which pDC and microbial colonization induce Treg cell expansion to confer protection against severe bronchiolitis and subsequent asthma.

OR.81, F90. Maternal Exposures and Asthma Severity: Mechanistic Insights from Animal Models.

Ian Lewkowich, Phoebe K. Richgels and Hitesh Deshmuk. Cincinnati Children's Hospital Medical Center, Cincinnati, OH

While there is a clear genetic component to asthma heritability, the rapid rise in incidence is inconsistent with a purely genetic etiology suggesting that environmental factors, particularly those present during early life developmental window(s), increase asthma risk. This “developmental window” includes the prenatal period, as maternal environmental exposures strongly influence a child’s risk for developing asthma. Herein, we explore the capacity of *in utero* exposures to 1) the aeroallergen House Dust Mite (HDM) or 2) Antibiotics, to influence development of allergic asthma. Compared to animals exposed to PBS *in utero*, *in utero* HDM exposure resulted in more severe AHR, airway inflammation, Th2 cytokine production, and immunoglobulin levels following induction of experimental asthma. While transmission of vertical transmission of allergen-specific immunoglobulins was evident, offspring of B cell deficient mothers exposed to HDM during pregnancy demonstrated similarly exacerbated experimental asthma, suggesting that transfer of maternal immunoglobulins was not required. Maternal exposure to antibiotics similarly increased asthma severity, but exacerbated AHR was not associated with an increased magnitude of cytokine production (either Th2 or Th17-associated) from T cells. This suggests that T cell-independent factors regulated the development of more severe experimental asthma in offspring of antibiotic exposed dams. Collectively, these data suggest that maternal exposure to multiple factors known to influence asthma risk have a profound influence on the severity of asthma that develops in offspring. These data lend support to developmental origins of allergic disease hypothesis and suggest that these observations may be successfully modeled in experimental models of allergic asthma.

OR.82, F89. Determining the Role of Airway Macrophages in Immunoregulatory Cytokine Signalling During Pulmonary Homeostasis and Inflammation

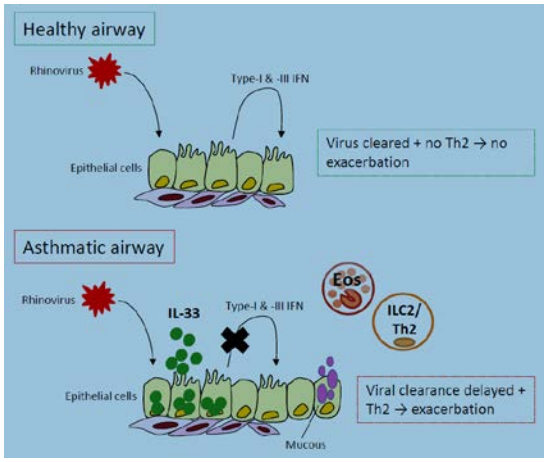
William Branchett[1], Robert Oliver[1], Simone A. Walker[1], Leona Gabryšová[2], Laura Denney[1], Mark S. Wilson[2], Anne O'Garra[1,2] and Clare M. Lloyd[1]. [1]Imperial College London, London, England, United Kingdom; [2] The Francis Crick Institute, London, England, United Kingdom;

Discrimination between harmful and benign inhaled particles is critical for maintenance of healthy airway function. $CD11c^{+}$ Siglec F^{+} Airway macrophages (AM) are thought to regulate pulmonary immune responses during homeostasis and inflammation. However, the role of the key mucosal regulatory cytokines TGF-β1 and IL-10 in these processes is unclear. Murine AM were shown to express high levels of TGF-β1, both *in vitro* and *in vivo*. In contrast, IL-10 was largely undetectable in AM, at baseline, after *in vitro*LPS stimulation and during house dust mite (HDM)-elicited allergic airways disease (AAD). Instead, $FoxP3^{-}$ $CD4^{+}$ T cells were the major IL-10-producing cells in the allergic lung. Accordingly, CD11c-Cre-driven knockout of IL-10 from AM ($CD11c^{Il10}$ mice) did not affect AAD severity, while intranasal IL-10 receptor blockade throughout HDM exposure resulted in an enhanced, Th1-skewed, AAD phenotype. Thus, pulmonary IL-10 from non-AM sources regulates T cell responses to HDM exposure. In contrast to IL-10, AM-conditional TGF-β1 knockout ($CD11c^{Tgfb1}$mice) resulted in marked airway dysfunction. Pulmonary compliance was reduced by 33% compared to llittermate controls in the absence of allergen challenge, and both airway hyperresponsiveness and Th2 cell numbers were increased in $CD11c^{Tgfb1}$ mice during AAD. These data suggest that AM-derived TGF-β1, rather than IL-10, is an essential regulator of airway homeostasis and allergic inflammation. However, IL-10, predominantly derived from $FoxP3^{-}$ $CD4^{+}$ T cells, is crucial for control of T cell subsets in the allergic lung. Thus, regulatory cytokines in the lung operate within cell type-specific niches, further understanding of which may provide novel therapeutic insights into pulmonary inflammation.

OR.83, F92. Chronic IL-33 Expression Predisposes to Viral-induced Exacerbations of Asthma by Increasing Type-2 Inflammation and Dampening Antiviral Immunity

Rhiannon Werder[1], Vivian Zhang[1], Jason Lynch[1], Kirsten Spann[2] and Simon Phipps[1]. [1]University of Queensland, Brisbane, Queensland, Australia; [2] Queensland University of Technology, Brisbane, Queensland, Australia

Rhinovirus infection triggers acute exacerbations of asthma. Expression of IL-33, an instructive cytokine of type 2 inflammation, is upregulated during experimental rhinovirus infection of asthmatic subjects and correlates with the production of type-2 cytokines and eosinophilic inflammation. Using a novel model of virus and allergen exposure, we sought to determine whether targeting IL-33 attenuates a rhinovirus-induced asthma exacerbation in mice. To simulate the synergistic effects of virus infection and allergen exposure on asthma susceptibility, mice were exposed to low dose pneumonia virus of mouse (PVM; 1pfu) and low dose (1 µg) cockroach antigen (CRE) in early life and again in later life. Four weeks after the final CRE exposure, mice were inoculated with rhinovirus (RV-1B, $TCID_{50}$ $5x10^6$). Anti-IL-33 or dexamethasone was administered twice/week between the CRE and RV challenge, then daily until euthanasia. IL-33 levels were elevated immediately following the final CRE exposure and persisted in the airways until the time of RV-1B challenge. Mice co-exposed to PVM/CRE, but not CRE or PVM alone, presented with eosinophilic inflammation, increased numbers of type 2 innate lymphoid cells, mucous hypersecretion and elevated IL-13 levels following RV infection. Treatment with anti-IL-33 or dexamethasone attenuated the RV-1B-induced type 2 inflammation but had no effect on mucous production. Critically, anti-IL-33, but not dexamethasone, promoted the expression of antiviral cytokines, accelerating RV-1B viral clearance. In summary, both anti-IL-33 and dexamethasone suppress the magnitude of type 2 inflammation during a rhinovirus-induced acute exacerbation; however, anti-IL-33 has the added benefit of boosting antiviral immunity and lowering viral burden.



M.04. Pathologic Functions of Dendritic Cells and T Cells in the Small Intestine

Gianna Hammer. Duke University, Durham, North Carolina, United States.

What defines a pathologic immune response to gut microbiota? To answer this question my lab first began by interrogating dendritic cells from the small intestine. We found that DCs lacking the signaling attenuator A20 induce pathologic T cells and that the signals perceived and antigen presenting cell functions are unique for different DC subsets. We identified two DC subsets that expand inflammatory Th17 cells *in vivo*, for disease pathogenesis. Thus, in IBD pathogenesis, specific DCs instruct specific pathologic T cells. To understand the nature of these pathologic Th17 cells, we investigated their localization in small intestine. In health, Th17 localize to ileum. Th17 expansion in this region is known to be driven by the gut microbe, SFB. By contrast, in IBD, Th17 cells are abundant throughout the small intestine, suggesting that microbes that drive IBD pathogenesis are not exclusive to ileum, but are instead present throughout small intestine. Compared to Th17 in health, inflammatory Th17 were distinct not only by localization, but also in "appearance". First, pathologic Th17 made much more IL-17 than do Th17 in health, and did so at all regions of pathology. Second, pathologic Th17 appeared to have a different Vbeta repertoire than do Th17 in health. Taken together, our work suggests that pathologic immune responses in IBD may be directed toward a unique set of gut microbes. Understanding these microbes, pathologic T cells reactive to these microbes, and DCs that expand these T cells is key to understanding the pathologic immune response in IBD.

Immunology of Asthma – Clinical

OR.80, F93. The Asthmatic Airway Metabolome Reveals Novel Mediators of Inflammation

Gerard E. Kaiko and Thaddeus S Stappenbeck.
Washington University in St Louis, St Louis, MO

Asthma is a chronic inflammatory disease of the airways that represents a major health burden both in terms of morbidity and economic costs. Severe asthma represents 10-15% of those suffering the disease and poses an urgent problem due to its resistance to current therapies. In order to understand the pathogenesis of asthma, traditional approaches have employed transcriptomics. However, currently it is unknown how asthma alters the global metabolite profile present in the lungs. We investigated whether severe asthmatics have an altered airway metabolome and whether this also acts as a functional driver of airway inflammation. Bronchoalveolar lavage (BAL) was collected from 150 asthma patients and subjected to global metabolomics profiling. Analysis revealed that ~30 metabolites were highly enriched in severe asthmatic patients compared to all other groups indicating a unique metabolic endotype. Several of these metabolites also showed positive correlations with clinical parameters, such as FEV1. By using metabolites that showed significant differences between asthma and healthy controls we created the first asthma-associated metabolite catalog. We conducted a secondary functional analysis by screening this metabolite catalog on immune cell and human tracheal epithelial cell assays *in vitro*. These secondary screens revealed that two families of molecules, specifically upregulated in severe asthma, were potent inducers of; IFNs, IL-1β, and Th2-associated molecules such as amphiregulin and Trefoil factor 3. This suggests these metabolites may play a role in exacerbating disease. This study demonstrates that functional analysis of the asthma airway metabolome reveals metabolic drivers of inflammation that may offer new therapeutic targets.

Immunology of the Eye

OR.42, T66. An Ocular Commensal Protects from Corneal Infection by Driving an IL-17 Response from Mucosal γδ T cells

Anthony J. St. Leger[1], Jigarkumar V. Desai[2], Rebecca Drummond[2], Abirami Kugadas[3], Fatimah Almaghrabi[1], Phyllis Silver[1], Mihaela Gadjeva[3], Yoichiro Iwakura[4], Michail S. Lionakis[2] and Rachel Caspi[1]. [1]NIH/NEI, Bethesda, MD; [2]NIH/NIAID, Bethesda, MD; [3]Harvard Medical School, Brigham and Women's Hospital, Boston, MA; [4]Tokyo University of Science, Tokyo, Japan

Mucosal sites such as the intestine, oral cavity, nasopharynx, and female reproductive tract all have their associated commensal flora. The surface of the eye (conjunctiva) is also a mucosal site, but existence of a resident microbiome on the ocular surface is highly controversial. We used a mouse model of ocular surface disease to study whether commensal microbes are present in ocular mucosa and modulate immunity. We found that IL-17 is constitutively produced within the conjunctiva and recruits neutrophils to the ocular surface in the steady state and after a bacterial challenge. IL-17 sources in CALT include γδ T cells, αβ T cells and innate lymphoid cells (ILCs), in that order. Notably, a strain of *Corynebacterium* isolated from ocular tissue of mice, and known to occur on the ocular surface of humans, induced conjunctival γδ T cells to secrete IL- 17, which in turn modified the local inflammatory signature and barrier function. Elimination of this putative commensal by antibiotic treatment, or its introduction into non-colonized mice, correlated inversely with severity of an experimental *Candida albicans* infection. Our results thus indicate that a relationship exists between commensals and immune cells at the ocular surface, which is critical for maintenance of homeostasis and host defense within the ocular mucosa.

Immunology of the UG Tract

OR.26, W32. A Primary Human Fallopian Tube Epithelial Cells System Grown in Air-Liquid Interface to Study Chlamydial Pathogenesis

Uma Nagarajan. University of North Carolina at Chapel Hill, Chapel Hill, NC

Chlamydia trachomatis infection can result in immunopathology and severe tissue damage leading to infertility. Our objective was to develop a human Fallopian tube epithelial (FTE) cell culture model to study innate responses promoting immune pathology. Fallopian tube explants obtained via salpingectomy from pre-menopausal women were dissociated and cells were cultured for 3-5 days in basal-epithelial cell growth medium. Cultures were transferred to air-liquid interface (ALI) culture on a PTFE transwell membrane. Cells were characterized by histology, confocal, electron microscopy, transcriptomic and proteomic approaches. Calcein^{AM}-labeled human PMNs were used to study transmigration through inverted FTE. Ciliated cells and mucus were apparent 8-12 days later. Histology and EM revealed polarized secretory and ciliated epithelium that resembled *in vivo* morphology. Infection was established in cells passaged 10-15 days after transfer to ALI (20-40% infection). *C. trachomatis* completed a full developmental cycle, with RB forms observed at 24 h pi and EB by 48 h pi. FTE displayed high basal expression of IL-8. Significantly increased CXCL-10, CXCL-11, CXCL-1, and IL-6 levels were detected in apical secretions post infection. Eotaxin, ENA, CXCL-10, 6Ckine, and MCP-3 were increased basolaterally. Mass spectrometry of apical washes identified 27 differentially-expressed proteins including CXCL1, CXCL8, amino acid transporters and transferrin receptor. PMN transmigration was observed in response to infection and CXCL-11 was sufficient to allow their transmigration across these cells. Our FTE cell culture model recapitulates key morphological features of the *in vivo* epithelium and allows characterization of innate immune responses to infection in a physiologically relevant system.

OR.28, M.06, W30. Tissue-Resident Memory CD4 T Cells in *Chlamydia* Female Reproductive Tract Infection

Sarah E. House, Jessica I. Gann and Lin-Xi Li. University of Arkansas for Medical Sciences, Little Rock, AR

Numerous attempts have been made over the past few decades to develop a vaccine strategy for *Chlamydia*. However, among all immunization strategies tested in mouse models of *Chlamydia* infection, none generates protective immunity any better than a naturally resolved prior *Chlamydia* female reproductive tract (FRT) infection. In this study, we aim to understand how immunological memory shaped by a natural episode of *Chlamydia* primary infection confers such potent protective immunity. Using *Chlamydia*-specific MHC Class II tetramers, we show that *Chlamydia* secondary infection induced massive clonal expansion of antigen-specific CD4 T cells in the FRT seven days post reinfection. In contrast, a robust recall response is not observed in local draining lymph nodes or spleen during the resolution of secondary infection. Surprisingly, although mouse parabiosis experiments reveal that *Chlamydia*-specific memory CD4 T cells in the FRT are exclusively non-circulating tissue-resident memory cells (T_{RM}), these cells only partially express the common T_{RM} marker, CD69. In addition, using bone marrow chimeric mice and *in vivo* cell depletion strategies, we demonstrate that neither $CD11c^+$ cells or $CD19^+$ B cells is required for antigen-specific CD4 T cell clonal expansion in the FRT during Chlamydia secondary infection. Future studies will dissect the activation, effector function, and maintenance of *Chlamydia*-specific CD4 T_{RM} cells in the FRT during *Chlamydia* reinfection.

OR.29, W26. Divergent outcomes for Intra- and Extracellular IgG in Urogenital Chlamydial Infections

Charles W. Armitage[1], Alison Carey[1], Connor P. O'Meara[1], Avinash Kollipara[1], Richard S. Blumberg[2], and Kenneth Beagley[1]. [1]Queensland University of Technology, Brisbane, Queensland, Australia; [2]Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Urogenital *Chlamydia trachomatis* infects over 100 million people per annum and can cause inflammation, scarring, pelvic inflammatory disease and infertility in women. However, infections are often asymptomatic with an unknown underlying trigger for immunopathology. Male IgG serostatus to *C. trachomatis* is also a correlate of the disease outcome of female partners in fertility clinics. Using IgG targeting different antigens expressed during the extracellular and intracellular stages of chlamydial infection, we identified two distinct outcomes of infection where IgG can either exacerbate infection and pathology, or enhance intracellular degradation of *Chlamydia*-infected cells respectively. Enhanced *in vitro* phagocytosis by female reproductive tract columnar epithelia was mediated by neonatal Fc receptor (FcRn) binding and internalization of seminal plasma or serum IgG-coated *Chlamydia*. Infected epithelia then facilitated translocation of viable *Chlamydia* to the lamina propria, infecting macrophages and dendritic cells *in vivo*. APCs infected with IgG-coated *Chlamydia* promoted enhanced pro-inflammatory $CD4^+$ and $CD8^+$ T cell responses, leading to increased incidence of infertility. In the absence of FcRn and CD8 T cells, this enhanced infertility was significantly reduced. These results help to explain the asymptomatic nature of *Chlamydia*-induced pathology and infertility in human females. Conversely, FcRn-mediated transcytosis of IgG specific for intracellular chlamydial protein was able to bind *Chlamydia* within infected epithelial cells, activating lysosomal and autophagosomal pathways normally inhibited by *Chlamydia*, enhanced MHC-I and II presentation within infected DCs, and significantly reduced infertility in immunized mice. Taken together, we demonstrate that IgG can have significantly divergent outcomes, dependent on antigen specificity.

W27. Linking Cervicovaginal Immune Signatures and Microbiota Composition in Cervical Carcinogenesis

Pawel Laniewski[1], Alison Goulder[1], Dominique Barnes[2], Haiyan Cui[3], Denise Roe[3], Dana Chase[2] and Melissa Herbst-Kralovetz[1]. [1]University of Arizona, Phoenix, AZ; [2]Maricopa Integrated Health Systems and Dignity Health St. Joseph's Hospital and Medical Center, Phoenix, AZ; [3]University of Arizona Cancer Center, Tucson, AZ

Infection with high-risk types of human papilloma virus (HPV) are the primary cause of invasive cervical cancer (ICC). However, the underlying cause of persistent HPV infection and progression to invasive cervical carcinoma (ICC) remains unclear. Mucosal inflammation and a dysbiotic vaginal microbiome (VMB), which is characterized as a depletion of *Lactobacilli* and overgrowth of diverse microaerophiles and anaerobes, can cause epithelial barrier breach and promote infection. We hypothesize that VMB regulates mucosal inflammation that could impact progression to invasive disease at this site. In our study, 100 women with histologically confirmed low grade (LGD) and high grade cervical dysplasia (HGD), ICC, and healthy controls were enrolled. Vaginal pH, vaginal swabs and cervicovaginal lavages (CVL) were collected for microbiome and immune analyses, respectively. CVLs were quantified for 52 secreted immune mediators. VMB analysis indicated significant changes in microbiome structure in patients at different stages of cervical cancer. *Lactobacilli*-dominant community state types significantly decreased, whereas dysbiotic VMB, increased. Feature selection analysis also revealed operational taxonomic units that significantly differentiated HGD and ICC versus control patients. Moreover, VMB analysis revealed that vaginal pH, ethnicity, and age were significantly correlated with beta-diversity differences. *Analysis* of immune mediators demonstrated significantly increased levels of proinflammatory mediators across groups as dysplasia progressed to ICC. In conclusion, our study revealed local, host immune and microbial drivers associated with cervical carcinogenesis and provides an initial step to understanding the complex interplay between mucosal inflammation, epithelial barrier function and the vaginal microbiome in these women. Funding: The Flinn Foundation (#1974).

W28. IL-36γ Induces a HSV-2 Resistant Environment in Human Vaginal Epithelial Cell and Mouse Models

Jameson K. Gardner and Melissa Herbst-Kralovetz.
University of Arizona, Phoenix, AZ

Genital herpes simplex virus 2 (HSV-2) infections remain one of the most common sexually transmitted infections affecting women. Recently, the IL-36 cytokines have been implicated as important inflammatory mediators in response to infections at mucosal epithelia. We hypothesize that IL-36γ is a key regulator of mucosal inflammation to HSV-2 infection in the vaginal epithelium. Here, we utilized a three-dimensional (3-D) human vaginal epithelial cell (VEC) model and an intravaginal infection mouse model to identify the impact of IL-36γ exposure on HSV-2 infection and disease pathogenesis. We found that viral replication was significantly ($P < 0.01$) decreased in 3-D VEC exposed to a range of IL-36γ doses. To evaluate impact of IL-36γ treatment on viral pathogenesis, we utilized a lethal intravaginal HSV-2 challenge model. Mice exposed to IL-36γ prior to lethal HSV-2 challenge survived significantly ($P < 0.005$) longer and disease development was significantly ($P < 0.0001$) delayed. In addition, vaginal viral replication was significantly ($P < 0.0001$) reduced, which most likely limited primary infection and establishment of disease. Similar to *in vitro* findings, cervicovaginal lavages from mice exhibited increased levels of IL-6, KC/IL-8 and other immune mediators as measured by BioPlex analysis and quantitative RT-PCR. Together, using *in vitro* and *in vivo* models, we demonstrate that IL-36γ exposure prior to infection increases resistance to genital HSV-2 infections in a dose- and time-specific manner. Our data provides evidence that IL-36γ is functioning as a key regulator of downstream signaling molecules and limits acute HSV-2 infection, disease development and progression. Acknowledgement: Funding support from NIH NIAID 1R15AI113457-01A1

W29. The Presence of Dairy Fats in a High Fat Diet Decreases Blood Inflammatory Markers and Modulates Gene Expression in Intestine, Liver, and Adipose Tissues of Growing Pigs

Mylene Blais[1], Jose Luis Martinez Gonzalez[2], Yves Pouliot[2], Sylvie Gauthier[2], Yvan Boutin[3], Denis Roy[2], Andre Marette[2], Claude Asselin[4] and Martin Lessard[1].
[1]Agriculture and Agri-Food Canada, Sherbrooke, PQ, Canada; [2] Laval University, Quebec, PQ, Canada; [3] TransbioTech, Quebec, PQ, Canada; [4] Sherbrooke University, Sherbrooke, PQ, Canada

While high fat diets have been associated with obesity, new evidences show that the source of fat plays an important role on its impact on metabolic health. In order to investigate the specific role of dairy fats, a model of growing pigs fed high fat diet (17.5% lard) was used. Dairy fats from either butter or cheddar were incorporated in experimental diets to provide 4.5% of fats and to replace part of the lard. Forty pigs of 6 weeks of age were *randomly* allocated to 4 different treatments, including a low fat diet, and fed for 10 weeks. After 6 and 10 weeks, blood samples were collected to determine inflammatory markers levels by ELISA and pigs were euthanized to measure expression of genes involved in inflammation, oxidative stress and energy metabolism in intestinal, hepatic and adipose tissues by qPCR. Finally, fecal samples were collected to analyze gut microbiota using DNA sequencing approaches. Blood levels of IL-2, IL-6, IL-1β and TNFα were decreased in pigs fed either butter or cheddar compared to the control lard high fat diet. Butter and cheddar affected gene expression profile in jejunum, while butter specifically modulated the expression profile in liver and mesenteric fat, and cheddar changed the profile in colon. Microbial populations from feces collected from pigs fed the high fat diets were not affected. In conclusion, replacement of lard for dairy fats in a high fat diet protects from systemic inflammation and changes the expression profile in pig intestinal, hepatic and adipose tissues.

W31. IL-17 Plays a Critical Role in Mediating Efficient Th1 Anti-Viral Memory Responses in the Female Genital Tract

Puja Bagri, Varun C. Anipindi, Philip V. Nguyen, Danielle Vitali and Charu Kaushic. McMaster University, Hamilton, ON, Canada

Although it is well established that IFN-γ plays a protective role during vaginal HSV-2 infection, the relevance of IL-17 in anti-viral immunity remains elusive. We recently published a study suggesting Th17 responses augment Th1 anti-viral immunity in the female genital tract (FGT), however, the underlying mechanism is unknown. The aim of this study was to delineate the *in vivo* mechanism by which IL-17 influences anti-viral immune responses in the FGT. Ovariectomized C57BL/6 (WT) and IL-17 knockout (KO) mice were immunized either intravaginally or intranasally with HSV-2, and then challenged intravaginally with a lethal dose of HSV-2. Survival, pathology, and viral shedding were monitored, and differences in susceptibility were further investigated by examining $CD4^+$ T cell phenotype and functional responses using flow cytometry. Regardless of the route of immunization, IL-17 KO mice demonstrated significantly higher mortality and greater disease severity compared to WT mice post-challenge. This indicated increased susceptibility, and corresponded with significantly lower proportions of protective, vaginal IFN-γ^+ Th1 cells in IL-17 KO mice post-challenge. Finally, there was a *smaller* population of vaginal IFN-γ^+ $CD4^+$ tissue-resident memory (TRM) T cells ($CD44^+$ $CD69^+$ $CD62L^-$) in IL-17 KO mice post-immunization, which coincided with overall decreased protection post-challenge. These results indicate that IL-17 plays an important role in establishing proficient IFN-γ^+ $CD4^+$ TRM cell populations in the FGT post-immunization, and consequently, IL-17 is critical for mediating efficient HSV-2 anti-viral Th1 responses post-challenge. Better understanding the role of IL-17 in the FGT can assist in the development of more effective vaccines against STIs.

Inflammatory Bowel Disease – Basic

L.01, F94. Signaling Pathway of Alarmin IL-33/ST2 During Mucosa Inflammation

David Diaz-Jimenez[1], Karen Dubois[1], Marjorie De la Fuente Lopez[1], Glauben Landskron[1], Daniela Simian[1,2], Maripaz Martinez[2], Rodrigo Quera[2] and Marcela A. Hermoso-R[1]. [1]Universidad de Chile, Santiago, Region Metropolitana, Chile; [2]Clinica Las Condes, Santiago, Region Metropolitana, Chile;

The Interleukin-33 (IL-33)/ST2 axis has been implicated in numerous disease states, including asthma, rheumatoid arthritis, and inflammatory bowel diseases and, more recently, in cancer and Alzheimer's disease. IL-33, a member of the IL1 cytokine family is mainly associated with the induction of T-helper type 2 (Th2) immune response through its receptor, ST2. ST2, encoded by the *IL1RL1* gene, is expressed as both a membrane-anchored receptor (ST2L) activated by IL33 and as a soluble receptor (sST2) with anti-inflammatory properties that increase during inflammation. During mucosa inflammation such as in ulcerative colitis (UC), IL-33 and sST2 are increased and regulated by pro- and anti-inflammatory stimuli; however, molecular regulation of their expression remains unknown. Moreover, single-nucleotide polymorphisms (SNPs) in *IL1RL1* have been associated with gene expression regulation. We explored the role of IL-33/ST2 system in innate and adaptive immunity and determine novel molecular mechanisms that impact on mucosal inflammatory disorder treatment and prognosis. Fondecyt 1120577, 1110381 and 1170648

M.03, F95. Tryptophan-Derived Metabolite, Indole-3-Propionic Acid, Promotes Homeostasis, and Prevents Inflammation of the Intestinal Mucosa

Pablo Alberto Romagnoli[1], Gregory K. Shenk[2], Quynh-Mai Pham[3], Leigh Maher[3] and Kamal M Khanna[3]. [1]Instituto Universitario de Ciencias Biomédicas de Córdoba, Córdoba, Argentina; [2]Academy of Aerospace and Engineering (CREC), Windsor, CT; [3]UConn Health, Farmington, CT

Signals provided by microbiota-derived metabolites can promote the homeostasis of the epithelial cell layer of the intestinal mucosa. These signals can shape immune responses to bacterial pathogens as well as to prevent dysregulated responses to microbiota that result in inflammatory bowel disease (IBD). We tested the hypothesis that a metabolite arising from the tryptophan metabolism of commensal bacteria, indole-3-propionic acid (IPA), provides signals that promote intestinal mucosa homeostasis and in turn control inflammation to prevent IBD. We investigated the effect of IPA in the intestinal mucosa using a mouse model of colitis caused by oral infection with *Citrobacter rodentium.* Remarkably, we found that IPA treatment prevents colitis in mice. Although most active indoles derived from the tryptophan metabolism act as ligands of the aryl hydrocarbon receptor (AHR), previous reports have suggested that IPA acts through the nuclear receptor pregnane X receptor (PXR), independently of AHR. Here, we investigated if the effect of IPA is mediated by the activation of PXR promoting the integrity of the epithelial barrier present in the gut mucosa. Indeed, we found that IPA did not protect against colitis in PXR deficient mice. Moreover, the absolute numbers of lymphocytes producing IL-17A and IFNg are increased in the intraepithelial lymphocyte compartment of the colon after IPA treatment and that this effect is also dependent on the presence of PXR. In conclusion, we believe the discovery of metabolites like IPA that promote the maintenance of the intestinal mucosa will provide exciting new strategies to prevent and treat IBD.

OR.04, W44. Microbial Colonization at Weaning Period Impacts Colitis Severity in Adult Mice

Ziad Al Nabhani, Sophie DuLauroy, Bernadette Polomack and Gérard Eberl. Institut Pasteur, Paris, Ile-de-France, France

The etiology of inflammatory bowel diseases (IBD) is not fully established. Increasing evidence supports that perturbation of the immune system before weaning may imprint the intestine with an increased susceptibility to IBD in adulthood. It remains enigmatic how perturbations in host–microbial symbiosis in childhood impact the intestinal immunity later during adulthood. We demonstrate that the production of pro-inflammatory cytokines is increased in colon during weaning period under specific pathogen-free (SPF) but not in germ-free conditions. Moreover, we show that gut microbiota induces the production of short chain fatty acids (SCFA) which in turn are inducing the observed pro-inflammatory response during the weaning period. Exposition of germ-free mice to SPF conditions only at weaning, but not later, protects the intestine of adult mice from dextran sodium sulfate (DSS) induced colitis. On the other hand, antibiotics treatment during weaning results in higher sensitivity to DSS-induced colitis at adult age. Treatment with selective antibiotics revealed that the protective effect of gut microbiota is linked to the presence of Gram-positive bacteria. Furthermore, administration of SCFAs at the weaning period to antibiotics treated mice ameliorates colitis severity at adult age. This protective effect of SCFAs early in life is dependent of regulatory T cells expressing the transcription factor RORγt (Retinoid-Acid Receptor-related Orphan Receptor gamma t). Our study reveals how host-microbial symbiosis early in life impacts the long-term health.

OR.102, F96. Gut Microbiota Fermentation Metabolite-Inflammasome Axis Regulates Intestinal Inflammation During Chronic Colitis in Mice

Vishal Singh, Beng San Yeoh, Rachel Walker, Jingwei Cai, Xia Xiao, Gregory Shearer, Andrew Patterson, and Matam Vijay-Kumar. Pennsylvania State University, University Park, PA

The microbial metabolites, particularly short-chain fatty acids (SCFAs) derived from bacterial fermentation of dietary soluble fibers, play a key role in maintaining mucosal barrier function and immune responses. We hypothesized that soluble fiber (inulin and pectin) which are more accessible for microbiota fermentation may offer more pronounced mucoprotection than insoluble fiber (cellulose) in a murine model of chronic colitis. Dietary cellulose exhibited robust colitis upon IL-10R neutralization as examined by serological, biochemical, histological, and immunological parameters in WT mice, and to a greater extent in colitis-prone toll-like receptor 5 knockout (*Tlr5*KO) mice. Surprisingly, prebiotic fiber inulin failed to protect against colitis in both WT and *Tlr5*KO mice. Remarkably, pectin ameliorates colitis development in these mice. GC-MS analysis of cecal content revealed that cecal butyrate—an inflammasome (NLRP3) response modulator in the gut—was augmented in inulin-fed colitic mice, whereas, cecal acetate was elevated in pectin-fed mice. Serum cytokine analysis showed that dietary inulin diminished the IL-1 receptor antagonist (sIL-1Ra) in the colitic mice. On the flip side, dietary pectin augmented the sIL-1Ra. More importantly, suppressing butyrate production by metronidazole, which specifically depletes the butyrate producers in the gut, protected the mice from chronic colitis, suggesting that SCFAs-inflammasome axis shapes the beneficial effects of dietary fiber on the gut health. Collectively, mitigation of chronic colitis by dietary pectin can be partly explained by reduced cecal butyrate and elevated IL-1β inhibitor, sIL-1Ra. A detailed mechanistic study on how pectin-derived metabolites modulate inflammasome signaling may yield development of novel therapeutics to treat intestinal inflammation.

OR.16, W70. Function and Regulation of AhR Ligand-Driven AhRR Expression in Intestinal Immune Cell Subsets

Oliver Schanz[1], Olga Brandstätter[1], Tetsushi Mori[2], Toru Maruyama[2], Haruko Takeyama[2], Heike Weighardt[1] and Irmgard Förster[1]. [1]Life & Medical Sciences Institute, Nordrhein-Westfalen, Germany; [2]Waseda University, Tokyo, Japan

The aryl hydrocarbon receptor (AhR) is an important sensor for environmental polyaromatic chemicals and plays an essential role in immune regulatory processes, including maintenance of intestinal barrier integrity. AhR activity is regulated through the AhR repressor (AhRR), which is encoded by an AhR target

gene. Regulation and interplay of AhR and AhRR, however, remain elusive. Here, we used AhRR/EGFP-reporter mice to show that the AhRR is specifically expressed in immune cells of barrier organs but not in intestinal epithelial cells, different from the more widespread expression of the AhR. Interestingly, similar to AhR-knockout mice, AhRR-deficient mice were highly susceptible to dextran sodium sulfate-induced colitis. This was accompanied by enhanced Th17/Tc17 and reduced Th1/Tc1 frequencies in the gut of AhRR-knockout mice. In contrast, AhR-deficiency resulted in accumulation of both Th1/Tc1 and Th17/Tc17 cells in the intestine. Further, significantly elevated IL-1β levels could be detected in steady state colon tissue of AhRR-deficient mice, indicating enhanced activation of the innate immune system. We also demonstrate that cell type-specific AhRR expression is driven by dietary AhR-ligands, whereas depletion of microbiota by treatment with broad-spectrum antibiotics had no effect on AhRR expression. Accordingly, the composition of intestinal microbiota under homeostatic conditions, analyzed by next generation sequencing of bacterial 16S rDNA, was unchanged in AhRR-deficient mice. Using conditional AhRR-knockout mice, we currently determine the consequences of the AhR/AhRR interplay in specific immune cell subsets. Our findings highlight the physiologic importance of cell type-specific balancing of AhR/AhRR expression in response to microbial, nutritional, and inflammatory stimuli.
The aryl hydrocarbon receptor (AhR) is an important sensor for environmental polyaromatic chemicals and plays an essential role in immune regulatory processes, including maintenance of intestinal barrier integrity. AhR activity is regulated through the AhR repressor (AhRR), which is encoded by an AhR target gene. Regulation and interplay of AhR and AhRR, however, remain elusive. Here, we used AhRR/EGFP-reporter mice to show that the AhRR is specifically expressed in immune cells of barrier organs but not in intestinal epithelial cells, different from the more widespread expression of the AhR. Interestingly, similar to AhR-knockout mice, AhRR-deficient mice were highly susceptible to dextran sodium sulfate-induced colitis. This was accompanied by enhanced T_h17/T_c17 and reduced T_h1/T_c1 frequencies in the gut of AhRR-knockout mice. In contrast, AhR-deficiency resulted in accumulation of both T_h1/T_c1 and T_h17/T_c17 cells in the intestine. Further, significantly elevated IL-1β levels could be detected in steady state colon tissue of AhRR-deficient mice, indicating enhanced activation of the innate immune system. We also demonstrate that cell type-specific AhRR expression is driven by dietary AhR-ligands, whereas depletion of microbiota by treatment with broad-spectrum antibiotics had no effect on AhRR expression. Accordingly, the composition of intestinal microbiota under homeostatic conditions, analyzed by next generation sequencing of bacterial 16S rDNA, was unchanged in AhRR-deficient mice. Using conditional AhRR-knockout mice, we currently determine the consequences of the AhR/AhRR interplay in specific immune cell subsets. Our findings highlight the physiologic importance of cell type-specific balancing of AhR/AhRR expression in response to microbial, nutritional, and inflammatory stimuli.

OR.17, W53. The Plasminogen Activation Pathway Plays a Critical Role in Driving Colitis

Gerard E. Kaiko and Thaddeus S Stappenbeck.
Washington University in St Louis, St Louis, MO

Despite the critical role of IL-17 in autoimmunity and inflammatory bowel disease (IBD) its function in the intestinal mucosa during disease is still unclear. In IBD, IL-17 is produced by mucosal pro-inflammatory immune cells. However, multiple clinical trials using monoclonal therapy blocking IL-17 suggest that this cytokine actually plays a protective role in IBD. To investigate this possibility, we treated primary cultured intestinal epithelial cells with IL-17 and conducted transcriptomic analysis. Comparison of this IL-17-induced epithelial signature with transcriptomic analysis of biopsies from active versus inactive ulcerative colitis (UC) patients revealed a potential dysregulation of the coagulation pathway during active disease. We found that IL-17 induced epithelial cells to produce tissue plasminogen activator (tPA), and most UC patients had a marked upregulation of the direct tPA inhibitor, known as plasminogen activator inhibitor-1 (PAI-1). Based on these findings, we used both genetic and chemical inhibitor models to show that tPA was protective against damage by dextran sodium sulfate and *Citrobacter* infection, whereas PAI-1 exacerbated damage responses. We found that tPA inhibited inflammation through activation of the immunosuppressive molecule TGF-β. tPA cleaved the ubiquitous blood factor plasminogen, which in turn activated latent TGF-β to its mature form. This process was inhibited by PAI-1. Finally, we demonstrated that the level of colon PAI-1 in UC patients was predictive of both disease activity and

response to biologic therapy. This study identifies a new pathway in UC whereby dysregulated PAI-1 leads to exacerbated inflammation and disease activity by blocking an IL-17-tPA-TGF-β axis.

OR.18, W81. Retinoic Acid Signalling is Required for the Pathogenicity of Effector CD4$^+$ T cells During the Development of Intestinal Inflammation

Aymeric Rivollier[1], Lieneke Pool[1], Ulrika Frising[2], Kerstin Wendland[2] and William Agace[2]. [1]Danish Technical University, Frederiksberg C, Hovedstaden, Denmark; [2] Lund University, Lund, Skane Lan, Sweden

The regulation of CD4$^+$ T cell lineage (T helper (Th) and T regulatory (Treg) cells) differentiation and plasticity is critical for appropriate immune responses and for the prevention of autoimmunity, however factors that regulate this plasticity remain largely unknown. Here we explored the role of retinoic acid (RA) signalling in CD4$^+$ T cells during the development of intestinal inflammation in the T cell transfer colitis model. We found that RA signalling-deficient CD4$^+$ T cells induced attenuated intestinal inflammation compared to their RA signalling-proficient counterparts, upon transfer into RAG$^{-/-}$ mice. This was associated with increased numbers of IL-17$^+$ and foxp3$^+$ cells and decreased numbers of IFNγ$^+$ cells, while the total numbers of colonic T cells remained similar. Mechanistically, RA signalling-deficient CD4$^+$ T cells exhibited a colon-homing defect, which was compensated for by increased proliferation. RA signalling-deficient naïve CD4$^+$ T cells were impaired in their capacity to generate *bona fide* pathogenic Th1, while their Th17 differentiation remained unaffected. Interestingly, RA signalling-deficient Th17 cells also inefficiently converted into pathogenic Th1-like cells. Finally, RA signalling-deficient and -proficient Tregs were equally competent to inhibit colitis development. Together our results indicate that RA, through its receptor RARα, negatively regulates the lymphopenia-induced T cell proliferation in RAG$^{-/-}$ mice during colitis and is necessary for the generation of colitogenic bona fide Th1 and Th17-derived Th1 cells. In contrast, RA is dispensable for the protective function of Treg cells in this model.

OR.19, W84. Enhanced NLRP3 Inflammasome Signaling Remodels Intestinal Microbiota to Mitigate Gut Inflammation

Xiaomin Yao[1], Yue Xing[1], Chenhong Zhang[2], Liping Zhao[2] and Guangxun Meng[1]. [1]Institut Pasteur of Shanghai, Shanghai, China; [2]Shanghai Jiaotong University, Shanghai, China

A balance between commensal microbiota and host inflammatory signals plays a critical role in maintaining gut homeostasis, loss of which may lead to inflammatory bowel disease (IBD). However, the mechanism underlying this balance remains elusive. Here, we discovered that mice carrying an gain-of-function mutation in an inflammasome component gene *Nlrp3* (*Nlrp3*^{R258W}), not only maintained gut homeostasis but also acquired a greater resistance to colitis and colorectal cancer via remodelling the intestinal microbiota. The reshaped microbiota in the *Nlrp3*^{R258W} mice exhibited a sparingly interconnected co-abundance network, and nourished functional bacteria capable of inducing T_{regs} to combat inflammation. This anti-inflammatory phenotype was attenuated during cohousing with wild-type, and transplantable to germ-free mice. Mechanistically, the microbiota was reshaped via increased local antimicrobial peptides boosted by the enhanced IL-1β production from lamina propria mononuclear phagocytes containing mutated NLRP3. Thus, elevated NLRP3 inflammasome signaling can remodel the gut microbiota into an anti-inflammatory structure, which implies new strategies for intervention of IBD in the future.

OR.20, W80. Mice with Inflammatory Bowel Disease are Susceptible to *Clostridium difficile* Infection with Severe Disease outcomes

Fenfen Zhou[1], Therwa Hamza[1], Ashley Fleur[1], Yongrong Zhang[1], Hua Yu[1], Kevin Chen[1], Jonathon Heath[3], Ye Chen[4], Haihui Huang[5] and Hanping Feng[2]. [1]University of Maryland School of Dentistry, Baltimore, MD; [2]University of Maryland School of Medicine, Baltimore, MD; [3]Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong, China; [4]Huashan Hospital, Fudan University, Shanghai, China

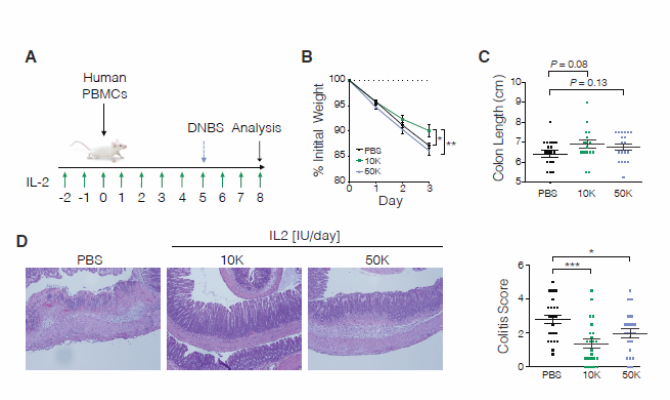
Over the past several decades, there has been a significant increase in the incidence of *Clostridium difficile* infection (CDI) in patients suffering from inflammatory bowel disease (IBD). However, the mechanisms of increasing incidence and prevalence of the IBD patients infected with *C. difficile* remain unclear. We evaluated the susceptibility to CDI of mice with dextran sulfate sodium salt (DSS)-induced colitis (IBD mice) with or without antibiotic exposure and examined the severity of the concomitant diseases. No CDI occurs in healthy control mice, while the incidence of CDI in IBD mice is 40%; however, in IBD mice that received antibiotics the incidence of CDI is 100% and the disease is accompanied by high levels of toxins in the mouse feces and sera. Compared to IBD and CDI alone, those IBD mice infected with *C. difficile* have more severe symptoms, toxemia, histopathological damage and higher mortality. Moreover, several pro-inflammatory cytokines and chemokines are significantly elevated in the colon tissues from IBD mice infected with *C. difficile*. We, for the first time, demonstrate in an animal model that mice with DSS induced-inflammatory bowel disease are significantly more susceptible to *C. difficile* infection, and that the bacterial infection led to more severe disease and death. These findings are consistent with clinical observations; thus the animal model permits us to study the pathogenesis of these concurrent diseases and to develop therapeutic strategies against the comorbidity of IBD and CDI.

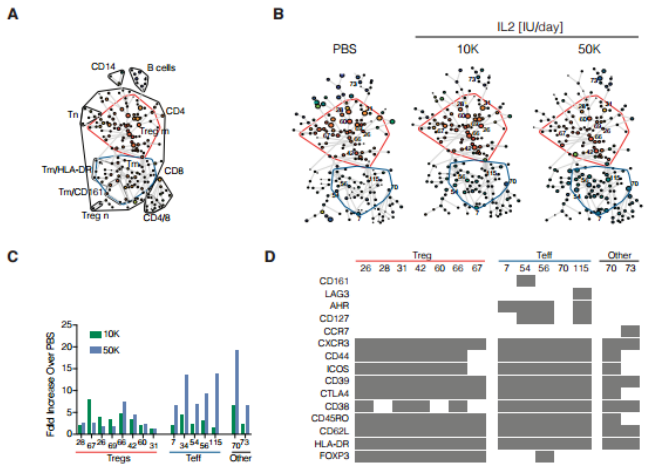
OR.36, W55. Low-Dose IL-2 Administration Expands Human Regulatory T Cells and Protects Against Colitis in Humanized Mice and Patients with UC

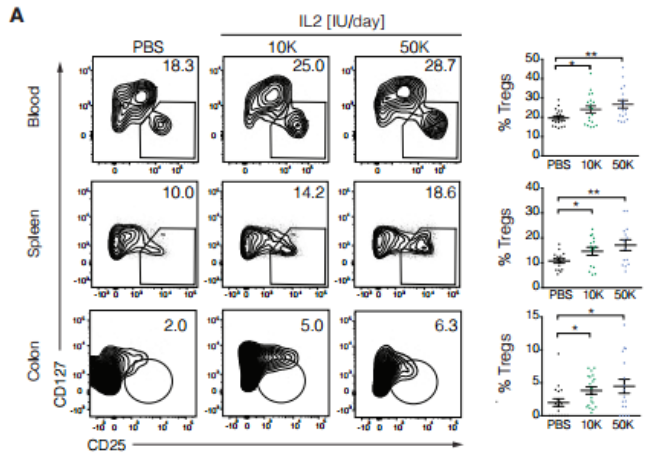
Jeremy A. Goettel[1,2], Daniel Kotlarz[2], Matthew J. Hamilton[1,3], James B. Canavan[1,2], Liza Konnikova[1,2] David Illig[2], Ryan Kelly[2], Sandra M. Frei[1,2], Madeline Carrellas[3], Alexandra E. Griffith[2], Anastasia Nelina[2], Jerome Ritz[1,4], Robert Soiffer[1,4], John Koreth[1,4], Athos Bousvaros[1,2], Joshua R. Korzenik[1,3], Jessica R. Allegretti[1,3] and Scott B. Snapper[1,2,3]. [1]Harvard Medical School, Boston, MA; [2] Boston Children's Hospital, Boston, MA; [3]Brigham & Women's Hospital, Boston, MA; [4]Dana-Farber Cancer Institute Boston, MA

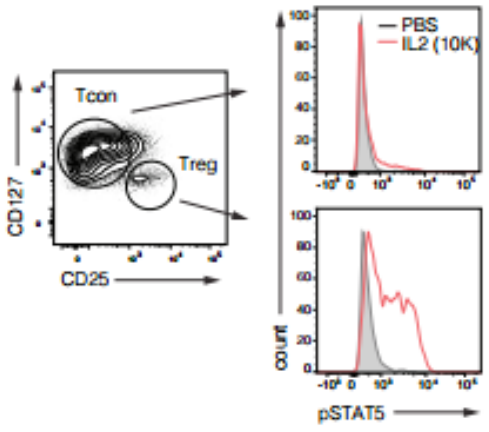
Low-dose IL2 has been shown to preferentially expand Tregs and ameliorate manifestations of graft-versus-host disease, HCV-associated vasculitis, and SLE. We investigated low-dose IL2 as a therapeutic for IBD using humanized mice and in a Phase 1b clinical trial in patients with moderate to severe ulcerative colitis (UC). NOD.*Prkdc*^{scid}*Il2rg*$^{-/-}$ mice were injected with healthy human donor PBMCs and treated with PBS, or two different doses of IL2 (10K or 50K IU) daily for 5 days followed by DNBS-induced colitis. Immunophenotype of treated mice was assessed using FACS and CyToF. Development of colitis was monitored by body weight and histology. Four UC patients were administered daily subcutaneous injections of low-dose IL2 (0.3×10^6 IU/m^2) for 8 weeks. Endoscopic, histologic findings, and complete Mayo scores were assessed at baseline and at 8 weeks with immunophenotyping and partial Mayo scores monitored weekly. In humanized mice, low-dose IL2 selectively activated STAT5 in Tregs and increased Tregs in blood (10K=32% and 50K=41% vs. PBS=22%), spleen (16% and 31% vs. 11%), and colon (6% and 10% vs. 5%), which correlated with protection against DNBS-induced colitis. In patients with UC, low-dose IL2 treatment upregulated FOXP3 in CD127$^{lo/-}$ CD25$^+$ Tregs in all patients. SPADE analysis from CyToF on both humanized mice and patients receiving low-dose IL2 showed expansion of HLADR$^+$CD45RO$^+$CCR4$^+$ subpopulations of Tregs. Three patients completed the 8-week study with one achieving a clinical response manifested by a four-point reduction in Mayo Score. Clinical response correlated with increased peripheral blood Tregs over baseline (15% from 5%), increased Treg:Tcon ratio, and improved histologic findings. Conclusion: Low-dose IL2 was protective against colitis in humanized

mice and is a promising therapeutic in human UC patients.









OR.37, W49. Enhanced Th17 Polarization and Reduced B-Cell Frequency and Maturity in Patients with IL-10 Receptor Deficiency

Liza Konnikova[1,2], Sarah Wall[2], Michael Field[2], Alex Griffith[2], Dror Shouval[3], Sarah Glover[4], Anthony Loizides[5], Jefferey Goldsmith[2] and Scott B. Snapper[1,2,6]. [1]Harvard Medical School, Boston, MA; [2]Boston Children's Hospital, Boston, MA; [3]Safra Children's Hospital, Tel Hashomer, Israel; [4]University of Florida, Gainesville, FL; [5]Children's Hospital of Montefiore, New York, NY; [6]Brigham & Women's Hospital, Boston, MA

IL-10 receptor (IL-10R) deficiency cause early-onset IBD and is associated with increased risk for B-cell malignancies. Intact IL-10R-dependent signals are important for murine immune cell function. We have reported a key role of IL-10 in innate immunity. The aim of this study was to determine the role of IL-10R signaling in adaptive immunity in humans. We examined peripheral blood mononuclear cells (PBMC) and intestinal lamina propria (LP) cells from IL-10R-deficient patients and controls by (1) flow cytometry for frequencies of $CD4^+$ T cell and Treg/ Th17 generation, (2) NanoString for transcriptional profiling, (3) *in situ* hybridization of LP for Tregs and Th17 cells and (4) CyTOF for immunoprofilimg and cytokine production. Although there were no detectable differences in frequencies of Tregs, they exhibited an increased responsiveness to IL1β. Additionally, transcriptional profiling showed that IL-10R-deficient Tnaïve cells exhibited a strong Th17 signature and *in situ* hybridization of LP showed an increase in Th17 cells. Similarly, using CyTOF, we

show an increase in IL-17 production. We show a concomitant decrease in IL-21 production by *T cell*s in IL-10R-deficient patients. Furthermore, we show a decrease in B-cell frequency and maturation status in IL-10R-deficient patients. IL-10R signaling regulates Th17 polarization in humans, but is not required for Treg generation. However, IL-10R-deficient Tregs might be more sensitive to environmental pro-inflammatory signals. Therapies targeting the Th-17 axis might be beneficial for IL-10R-deficient patients as a bridge to transplantation. Moreover, IL10R-deficient patients show B-cell abnormalities that might be a contributing factor to their risk to develop B-cell lymphomas.

OR.38, W64. Antigen-Presenting Cell-Derived IL-1β is Controlled by IL-10 and Promotes T Cell-Responses in a Subgroup of Pediatric Inflammatory Bowel Disease Patients

Sharon Veenbergen[1], Peng Li[2], Rolien Raatgeep[1], Dicky Lindenbergh-Kortleve[1], Ytje Simons-Oosterhuis[1], Lisette van Berkel[1], Maria Joosse[1], Lilian de Ruiter[1], Marieke van Leeuwen[1], Dwight Winter[3], Yoshiyuki Wakabayashi[2], Jun Zhu[2], Lissy de Ridder[3], Gertjan Driessen[1], Hankje Escher[3], Warren Leonard[2] and Janneke Samsom[1]. [1]Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands; [2]National Institutes of Health, Bethesda, MD; [3]Sophia Children's Hospital-Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands

Chronic gastrointestinal inflammation, as seen in inflammatory bowel disease (IBD), is driven by uncontrolled *T cell* responses to commensal microbiota. Interleukin-10 (IL-10) plays a crucial role in suppressing microbiota-specific *T cell* responses. However, it is unknown how IL-10 prevents *T cell* reactivation in human intestine. Using cells of an *IL10RA*-deficient patient, we revealed that IL-10R expression on dendritic cells (DC), but not T cells, is essential for controlling IFNγ-secreting $CD4^+$ T cells. To identify DC-derived factors that are under IL-10 control and drive *T cell* activation, we conducted a full transcriptomic analysis of *IL10RA*-deficient and control DC. *IL1B* was amongst the genes showing the greatest fold-change increase after bacterial lipopolysaccharide stimulation. Moreover, blockade of IL-1β significantly reduced IFNγ secretion by $CD4^+$ T cells in a DC-T cell co-culture. In a subgroup of our pediatric IBD patient cohort, IL-1β plasma levels were increased at time of diagnosis, and high IL-1β protein was observed in macroscopically affected intestinal tissue compared to unaffected regions. We next questioned whether high IL-1β expression related to altered IL-10 responsiveness. Concomitant analysis of both parameters within one patient group established that high IL-1β mRNA expression in intestinal tissue correlated with reduced IL-10 responsiveness of activated peripheral blood mononuclear cells. By comparison, IBD patients with low intestinal IL-1β mRNA expression showed normal IL-10 responsiveness. Altogether, our study demonstrates that IL-10-driven control of DC is essential for suppressing IFNγ-secreting T cells, and suggests that DC-derived IL-1β may drive T cell-driven inflammation in a subgroup of IBD patients showing suboptimal IL-10 function.

OR.39, W68. One $CD14^+$ Mononuclear Phagocyte Population Accumulates in Inflamed Clons and Favors Th17/Th1 Responses in an IL-1β-Dependent Manner in Crohn's Disease and Ulcerative Colitis

Laurence Chapuy, Marwa Bsat, Amélie Therrien, Manuel Rubio Trujillo and Marie Sarfati. University of Montreal, Montreal, Canada, Montreal, PQ, Canada

Crohn's disease (CD) and Ulcerative colitis (UC) are distinct chronic inflammatory bowel diseases (IBD). We previously showed that pro-inflammatory mononuclear phagocytes ($SIRP\alpha^+$MNPs) that include macrophages and dendritic cells accumulated in inflamed colon of CD patients. We here showed that: 1) The percentage of $SIRP\alpha^+$MNPs also increased in inflamed UC mucosa. 2) $SIRP\alpha^+$MNPs comprised at least two $CD14^+$ sub-populations, $CD64^+CD163^+$ (L3) and $CD64^+CD163^-$ (L4) in both diseases. 3) L4 and not L3 MNPs accumulated in the inflamed colon of patients with CD, as well as UC, compared to non-inflamed mucosa or non-IBD control, and decreased in patients in endoscopic remission. 4) L4 and not L3 promoted the emergence of $IL\text{-}17^+IFN\text{-}\gamma^+$ (Th17/Th1) and $IL\text{-}17^+IFN\text{-}\gamma^-$ (Th17) cells in autologous stimulated colonic $CD4^+$ T cells, in an IL-1β dependent manner, in CD and UC. Accordingly, IL-1β augmented Th17/Th1 and Th17 responses in $CD4^+$ T cells, as well as IL-17 and IFN-γ in the culture supernatant. Furthermore, L4 as well as IL-1β appeared to favor a pathogenic

signature by increasing the level of TNF-α, IL-6 and GM-CSF expression in CD $CD4^+$ T cells, corroborating murine studies. Remarkably, the frequency of L4 but not L3 MNPs positively correlated with the endoscopic severity, evaluated by the SES-CD score. In conclusion, our data provide a link between one $CD14^+$ MNP sub-population (L4) that accumulates in inflamed colons, IL-1β-expressing L4 cells and pathogenic mucosal Th17/Th1 responses in IBD. Since the percentage of L4 correlates with endoscopic disease severity in CD, these cells might contribute to disease pathogenesis.

OR.40, W72. NLRP3 Inflammasome Activation Due to a CARD8 V441 Mutation Leads to Crohn's Disease Response to IL-1β Blockade

Liming Mao[1,2], Atsushi Kitani[2], Morgan Similuk[1], Andrew Oler[1], Lindsey Albenberg[3], Nur Aktay[4], Kim Montgomery-Recht[5], Ivan Fuss[1,2] and Warren Strober[2]. [1]National Institute of Allergy and Infectious Diseases, Bethesda, MD; [2]National Institutes of Health, Bethesda, MD; [3]Children's Hospital of Philadelphia, Philadelphia, PA; [4]Cleveland Clinic, Cleveland, OH; [5]Leidos Biomedical Research, Inc., Frederick, MD

The role of the NLRP3 inflammasome in the development of Crohn's disease remains poorly understood. In this study, we evaluated the contribution of the NLRP3 inflammasome in the development of Crohn's disease-like intestinal inflammation in a patient with a missense mutation in Card8, a protein known to inhibit inflammasome activation. DNA from a patient with Crohn's disease-like intestinal inflammation unresponsive to anti-biologic monoclonal therapy was subjected to whole exome sequencing. The patient's peripheral monocytes were stimulated with NLRP3, NLRC4 or AIM2 activators to evaluate inflammasome activity and the interaction between mutant Card8 and NLRP3 inflammasome components. NLRP3 phosphorylation in the patient's monocytes were examined by immunoprecipitation and Western blot analysis. The patient was administered anakinra and subsequently IL-1β neutralizing antibody to evaluate their capacity to treat the patient's gut inflammation. Whole exome sequencing identified that the patient had an amino acid substitution (V44I) in a single allele of Card8. The serum concentration of IL-1β and IL-6 in the patient was increased compared with that in a healthy control. In immunoblot studies, the mutant Card8 did not bind to NLRP3 or caspase-1. NLRP3 serine phosphorylation, a marker of inflammasome inactivity, was reduced in patient monocytes. Finally, the administration of the IL-1β receptor antagonist anakinra or subsequently IL-1β-neutralizing monoclonal antibody remarkably reduced the intestinal inflammation. These data demonstrated that a Card8 V44I mutation causing excessive activation of the NLRP3 inflammasome and production of IL-1β gives rise to a Crohn's-like intestinal inflammatory disease.

OR.62, T71. Pro-Inflammatory Human $CD11c^+$ Macrophages, but Not Tolerogenic $CD11c^-$ Macrophages, are Expanded in the Inflamed Mucosa from Patients with Inflammatory Bowel Disease

David Bernardo Ordiz[1,2], Irene Mora-Gutierrez[2], Alicia C Marin[1,2], Alba Díaz-Guerra[2], Ana Montalban-Arques[1,2], Ruth Caminero-Fernández[2], Pablo Miranda[2], Fernando Casals[2], María Caldas[2], Mirella Jiménez[2], Felipe De la Morena[2], Cecilio Santander[2], María Chaparro[1,2] and Javier P Gisbert[1,2]. [1]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas, Madrid, Spain; [2]Hospital Universitario de La Princesa e Instituto de Investigación Sanitaria Princesa, Madrid, Spain

Macrophages (MΦ), the most abundant intestinal mononuclear phagocytes, are critical at shaping immune responses. However, there is not much information about their role in human inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC). Hence, we have characterized human intestinal MΦ subsets phenotype and function both in healthy controls and IBD patients. Human intestinal MΦ were identified within singlet viable cells as $CD45^+$HLA-$DR^+CD14^+CD64^+$ and further divided into $CD11c^{high}$, $CD11c^{dim}$ and $CD11c^-$ subsets. While the $CD11c^{high}$ subset resembled the phenotype of circulating phagocytic ($Dextran^{high}$) pro-inflammatory $CD14^+$ monocytes ($CX3CR1^+$, $SIRP\alpha^{high}$, $CCR2^+$, $CD40^+$), the $CD11c^{dim}$ subset displayed an intermediate phenotype towards the $CD11c^-$ MΦ subset, which was not phagocytic ($Dextran^{low}$), had higher expression of HLA-DR and CD64 and lower expression of CX3CR1, SIRPα, CCR2 and CD40. $CD11c^+$ MΦ displayed a higher

production of IL-1β, both in resting conditions and after LPS stimulation, compared with the $CD11c^-$ subset, which produced larger amounts of IL-10. Total MΦ numbers were increased in the inflamed tissue from IBD patients (both UC and CrD), although not on the non-inflamed tissue or on quiescent patients, due to specifically higher numbers of the $CD11c^{high}$ MΦ subset but not the others. Finally, differentiation of monocyte-derived MΦ with intestinal secretomes enhanced the differentiation of $CD11c^-$ tolerogenic MΦ while pro-inflammatory secretomes abrogated it. MΦ subsets are therefore likely to represent transition stages from newly arrived pro-inflammatory monocytes ($CD11c^{high}$) into transient ($CD11c^{dim}$) and resident ($CD11c^-$) tolerogenic MΦ, while the pro-inflammatory cytokine milieu in IBD patients would abrogate that differentiation.

OR.70, T67. Identifying Microbiota Antigen-Specific Antibody Responses and $CD154^+$ T Cells in Inflammatory Bowel Disease

Katie L. Alexander, Wayne Duck and Charles O. Elson. University of Alabama at Birmingham, Birmingham, AL

Over half of the individuals with Crohn's Disease (CD) have serum IgG antibody responses to the microbiota flagellin CBir1. Our lab has constructed a novel antigen microarray consisting of over 65 different bacterial lysates or recombinant proteins in order to further elucidate the pattern of microbiota reactivity in individuals with CD or Ulcerative Colitis (UC) compared to healthy controls (HC). Flagellins emerged as an immunodominant antigen for CD, with approximately 70% of CD patients (n=178) responding to at least one flagellin, though the majority of patients respond to multiple. Interestingly, a hierarchy of reactivity among flagellins was established, with the percent of serum IgG reactivity among flagellin responders ranging from 14.6%-47.8% for specific flagellins. UC patient serum IgG reactivity was similar to HC, although serum IgA reactivity to microbiota flagellins was significantly increased. To correlate the antibody responses to T cell reactivity, our lab has used the ARTE (antigen reactive T cell enrichment) method to enumerate and analyze the associated microbiota-specific T cells. Interestingly, the frequency of antigen-specific $CD154^+CD4^+$ T cells in both CD patients (0.037%, n=11) and HC (0.026%, n=10) responding to bacterial lysates was equivalent despite elevated antibody responses in CD patients. Due to the low frequency of these cells, we have expanded these cells in culture in order to analyze phenotypic differences between IBD patients and healthy controls, differences that may play a role in IBD pathogenesis.

OR.71, T68. Naïve Regulatory T Cells are Dysfunctional in CD Patients Possessing the TNFSF15 Risk SNPs Associated with High TL1A Expression

Maninder Sidhu-Varma and Stephan Targan. Cedars Sinai Medical Center, Los Angeles, CA

CD patients carrying SNPs in *TNFSF15* (TL1A), genotyped as homozygous or heterozygous risk, express increased levels of TL1A and are at an increased risk for IBD. Using TL1a transgenic mice that constitutively produce TL1a, we have recently shown that high levels of TL1a are inhibitory towards Treg generation and functional capability of Tregs. We hypothesize that high levels of TL1A produced in *TNFSF15* genotyped CD patients alters the phenotypic and functional characteristics of Tregs. To study the Treg activity, we analyzed the frequency and functional capacity of naïve $CD4^+CD25^+CD127^-CD45RA^{high}$ (nTregs) and memory $CD4^+CD25^+CD127^-CD45RA^-$ (mTregs) Tregs in TL1A genotyped CD patients and healthy controls. Compared to healthy controls, CD patients producing high TL1A had decreased numbers of nTregs in the peripheral blood and their *ex vivo* suppressive ability was abrogated. It has been reported that CD patients have an increased frequency of FOXP3 exon 7 splicing and this isoform is nonsuppressive. In order to study if TL1A promotes the splicing event in differentiated Tregs, nTregs and mTregs were FACS sorted from healthy human donors and exposed to rTL1A *in vitro*. Our results show that the FOXP3Δ2Δ7 isoform mRNA expression was 5-fold higher in the nTregs suggesting that alternative splicing may be one of the mechanisms by which the function of nTregs is directly regulated by TL1A. This study will help understand how TL1A associated genetic risk may negatively influence Treg function and identify patient populations that would benefit from interventions targeting these pathways that restore function.

OR.73, T69. Gut Microbiota Induce Local and Systemic CD4 T Cell Responses in Healthy Individuals that are Altered in Inflammatory Bowel Diseases

Ahmed N. Hegazy[1], Nathaniel West[1], Michael J. T. Stubbington[2], Sarah Teichmann[2], Satish Keshav[1] and Fiona Powrie[1]. [1]University of Oxford, Oxford, England, United Kingdom; [2] Wellcome Trust Sanger Institute, Cambridge, England, United Kingdom

Sensing of commensal microbes and their metabolites by the host immune system plays an important homeostatic role, but active systemic responses to the same commensals are thought to be rare and tightly regulated. Here we report that $CD4^+$ T cells with reactivity towards intestinal microbiota are abundant in the circulation and enriched in the intestinal mucosa of healthy adults. Microbiota-specific memory T cells have a diverse TCRVβ repertoire and are a functionally heterogeneous population with Th17 and Th1 characteristics. These cells accumulate in the intestine of patients with inflammatory bowel disease and show enhanced interleukin-17A production driven by the altered cytokine milieu. Thus, gut microbiota-specific $CD4^+$ T cells are part of the normal human T cell repertoire and do not necessarily indicate disturbed host-microbiota interactions. T cell responses to commensals may therefore support local and systemic immune responses by generating a plethora of memory T cells reactive to pathogens.

T128. LRRK2 Inhibitor Attenuates Intestinal Inflammation and Becomes a Therapeutic Strategy in Inflammatory Bowel Diseases

Tetsuya Takagawa[1], Masaki Tajima[2], Atsushi Kitani[2], Akihiro Nishio[1], Toshiyuki Sato[1], Mikio Kawai[1], Yoko Yokoyama[1], Yuko Kita[1], Takako Miyazaki[1], Nobuyuki Hida[1], Kazutoshi Hori[1], Shiro Nakamura[1], Ivan Fuss[2,3] and Warren Strober[2]. [1]Hyogo College of Medicine, Nishinomiya, Hyogo, Japan; [2]National Institutes of Health, Bethesda, MD; [3]National Institute of Allergy and Infectious Diseases, Bethesda, MD

The SNP at *LRRK2/MUC19* locus identified by genome-wide association studies has the second strongest association in inflammatory bowel diseases (IBD). Recent evidence indicates that LRRK2 is a proinflammatory molecule which positively regulates NF-κB signaling. However, recently developed LRRK2 inhibitors for the treatment of Parkinson's disease have not been tested yet for IBD. In this study, we investigated the effect of LRRK2 inhibitors for intestinal inflammation. We used bone marrow-derived dendritic cells (BMDCs) from C57BL/6J background mice and human dendritic cells (DCs) from patients with Crohn's disease. We obtained 12 LRRK2 inhibitors (CZC-54252, GNE-7915, GNE0877, GSK2578215A, HG-10-102-01, JH-II-127, LRRK2-IN-1, PF-06447475, PF-06454589, FX2149, MLi-2 and GNE-9605). BMDCs and human DCs were pretreated with DMSO or LRRK2 inhibitor and then stimulated with Zymosan depleted. TNFα was detected in the supernatant by ELISA. C57BL/6J mice treated with DMSO or LRRK2-IN-1 were subjected to 2% Dextran sulfate sodium (DSS). The severity of DSS colitis was assessed by the percentage of body weight loss and histological score. Among 12 LRRK2 inhibitors, 8 inhibitors (CZC-54252, GNE-7915, JH-II-127, LRRK2-IN-1, PF-06447475, PF-06454589, MLi-2 and GNE-9605) significantly and dose-dependently suppressed TNFα production in BMDCs. 3 inhibitors (GNE0877, GSK2578215A and HG-10-102-01) had a partial effect and only FX2149 did not have suppressive effect in TNFα level. For human DCs, we tested CZC-54252 and LRRK2-IN-1. Both inhibitors significantly suppressed TNFα. In concert with these findings, LRRK2-IN-1 significantly ameliorated DSS colitis. Our results indicate that normalization of LRRK2 activation by inhibitor may become an attractive therapeutic strategy in IBD.

W35. MiR-378a-3p a Potential Molecular Regulator of IL-33 in Ulcerative Colitis

Karen Dubois-Camacho[1], David Diaz-Jimenez[1], Rodrigo Quera[2], Daniela Simian[2], Maripaz Martinez[2], Alejandro Torres-Riquelme[1], Mauricio Olivares-Morales[1], Marjorie De la Fuente Lopez[1], John A Cidlowski[3], Guangping Gao[4], Jun Xie[4], Jonas Chnaiderman[1], Ricardo Soto[1] and Marcela A. Hermoso-R[1]. [1]Universidad de Chile, Santiago de Chile, Region Metropolitana, Chile; [2]Clinica Las Condes, Santiago de Chile, Region Metropolitana, Chile; [3]National Institute of Environmental Health Sciences, National Institute of Health, Research Triangle Park, NC; [4]University of Massachusetts Medical School, Worcester, MA

IL-33, a member of IL-1 family, is produced by epithelial cells and its binding to the ST2 receptor induces secretion of pro-inflammatory cytokines by

immune cells. Intestinal mucosa of active ulcerative colitis (aUC) patients presents increased levels of IL-33. MicroRNAs are deregulated in mucosa of UC patients and are candidates to control IL-33-mRNA expression. Aim: to study the association of IL-33-mRNA levels with microRNAs. MicroRNAs and mRNAs that were differentially expressed according to inflamed vs. non-inflamed intestinal mucosa by microarray analysis (from 8 UC patients) were selected. MicroRNAs and mRNAs with a high fold-change (FC) in microarrays were detected by TaqMan and RT-PCR, respectively, in 20 UC patients, 13 inactive (iUC) patients, and 12 controls. Colonic epithelial cells (HT-29) were exposed to inflammatory stimuli (TNFα) to evaluate the expression of selected microRNAs and mRNAs. Inflamed mucosa was associated with increased expression of 26-microRNAs^{+}432^{-}mRNAs, and decreased expression of 22-microRNAs^{+}314-mRNAs. IL-33-mRNA was increased (3.7 FC) and miR-378a-3p, a microRNA coded into an oxidative energy metabolism gene and concomitantly with a target sequence in the IL-33-mRNA 3'UTR, was decreased (-3.4 FC). aUC patients showed decreased expression of miR-378a-3p and increased IL-33-mRNA levels, compared to iUC and controls (Mann-Whitney test), and the two molecules were inversely correlated (*Pearson-r*=-0.35, *P*=0.04). miR-378a-3p and IL-33-mRNA were also inversely correlated (*Pearson-r*=-0.49*, P*=0.0093) in HT-29 cell assays. Over-expression of miR-378a-3p decreased the β-gal levels in an IL-33-target-sequence reporter gene. These results suggest a novel regulatory mechanism of alarmin IL-33 by a metabolism-controlled miRNA.

W36. The Cation Channel TRPM8 Regulates Human Macrophage Differentiation and is Upregulated in the Inflamed Intestinal Mucosa of Crohn's Disease Patients

Eve Hornsby, Madusha Peiris, Ashley Blackshaw and Andrew J. Stagg. Queen Mary University of London, London, England, United Kingdom

Expression of TRPM8, a member of the TRP (transient receptor potential) cation channel superfamily, was once thought to be largely restricted to sensory neurons. However, murine myeloid cells express TRPM8 and have a hyper-inflammatory phenotype in TRPM8 knockout mice which are more susceptible to experimentally-induced colitis. TRPM8 expression is increased in inflamed colonic tissue in mice and humans. Here, the aim is to test the hypothesis that regulation of human macrophages by TRPM8 contributes to immune regulation in the intestine. TRPM8 expression was detectable by qRT-PCR in human blood T cells from healthy donors, but not in B cells or monocytes. However, expression was significantly upregulated upon differentiation of monocytes to macrophages (MO-M) *in vitro.* Exposure to a TRPM8 antagonist during differentiation resulted in a ~2-fold increase (p= < 0.05) in lipopolysaccharide-induced TNFα production by MO-M. Antagonist exposure also affected MO-M morphology and gene expression profile, indicative of effects on differentiation fate rather that TNFα production alone. TRPM8 protein was detectable by immunofluorescence staining on CD45^{+} immune cells in colonic tissues from Crohn's disease patients but not healthy controls. Staining localised to CD11c^{+} myeloid cells. These data show for the first time that functional TRPM8 is expressed in the human immune system and that expression by myeloid cells is upregulated within the inflamed intestinal mucosa. Constitutive activity of TRPM8 influences MO-M differentiation and limits lipopolysaccharide-induced TNFα production. TRPM8-regulated myeloid cell activity is likely to contribute to immune regulation in the human intestine.

W37. A20 and Atg16L1 Cooperate to Protect the Intestine from the Development of Chronic Inflammatory Pathology

Karolina Slowicka[1], Lars Vereecke[1], Emilio Boada-Romero[2], Mozes Sze[1], Hanna K Vikkula[1], Savvas Savvides[1], Felipe X Pimentel Muiños[2] and Geert van Loo[1]. [1]Ghent University and VIB Center for Inflammation Research, Gent, Oost-Vlaanderen, Belgium; [2] Centro de Investigacion del Cancer, Salamanca, Spain

Inflammatory bowel disease (IBD) is a chronic inflammatory pathology of the gastrointestinal tract. Multiple loci have genetically been linked to IBD, among which *A20* and *ATG16L1*. A20 is a ubiquitin editing enzyme crucial for the control on NF-κB signaling upon cytokine and pattern recognition receptor stimulation. Mice deficient for A20 in intestinal epithelial cells (IECs) do not develop a spontaneous pathology but are more susceptible to experimental colitis and to TNF-mediated IEC apoptosis. The autophagy protein Atg16L1, on the other hand, is a central adaptor required for the

formation of the autophagosome, a process which involves the lipidation of LC3. Also, IEC-specific Atg16L1 knockout mice in our lab do not develop spontaneous pathology nor show enhanced susceptibility to experimental colitis. However, mice deficient for both A20 and Atg16L1 in the intestinal epithelium develop spontaneous IBD-like pathology characterized by severe inflammation in both the small and large intestine, crypt abscesses and villi erosion. Through *in silico* prediction models, we could identify an Atg16L1-interaction motif in A20, and confirmed the direct interaction between both proteins *in vitro*. Together our data suggest that A20 and Atg16L1 interact and cooperatively control intestinal homeostasis, and also show that disruption of A20-Atg16L1 signalling can induce the development of chronic intestinal inflammation.

W38. Stratification of Ulcerative Colitis Patients According to Distinctive Tissue Cytokine/Microbiota Profiles

Alessia Butera[1], Monica Di Paola[2], Daniela De Nitto[3], Bernardo Ciamberlano[4], Alessandro Giuliani[1], Roberta Pica[3], Annamaria Pronio[4], Carlotta De Filippo[6] and Monica Boirivant[1]. [1]Istituto Superiore di Sanità, Roma, Lazio, Italy; [2]University of Florence, Firenze, Toscana, Italy; [3] S. Pertini Hospital, Roma, Lazio, Italy; [4]Sapienza University, Roma, Lazio, Italy; [5]National Research Council, Pisa, Toscana, Italy

Ulcerative Colitis (UC) has been associated with an atypical Th2 cell response mediated inflammation. However, administration of anti-human IL-13 neutralizing MAb, did not significantly improve clinical response vs placebo in UC patients but significantly increased the proportion of patients who achieved clinical remission, suggesting that UC patients' subgroups might exist. We hypothesized that UC patients might be stratified according to distinctive cytokine profiles and/or microbiota composition. We collected in RNA later multiple mucosal biopsies from 40 endoscopically active (Mayo endoscopic score ≥ 1) UC patients. TNF-α, IFN-γ, IL-13 and IL-17A mRNA tissue content was quantified by RTqPCR and results analyzed by r square of the k means for cluster solution. Bacterial gDNA was extracted from biopsies and metagenomic analysis was performed on dataset of 16SrRNA gene sequences by Illumina MiSeq platform. Only IL-13 and IL-17 mRNA tissue content showed discriminatory ability. Optimal partition of data resulted in four different clusters. The majority of patients (82%) was roughly equally distributed in two different clusters characterized by high (cluster 3) and low (cluster 4) IL-13 mRNA expression in the context of high IL-17 mRNA expression. Microbiota analysis revealed enrichment in patients belonging to cluster 3 of *Ruminococcus gnavus*, a microbial commensal that has been reported to have the ability to modulate mucin expression and degradation and of *Bifidobacterium* in patients belonging to the cluster. The majority of UC patients might be classified into two different groups according to the tissue content of IL-13 and microbial profiles.

W39. Antibiotics-Mediated Increase in Large Intestinal Protease Activity Confers the Risk to Colitis Susceptibility

Hongsup Yoon[1], Monika Schaubeck[2], Patrizia Kump[3], Andreas Blesl[3], Chaysavanh Manichanh[4], Bernhard Küster[1], Dirk Haller[1] and Gabriele Hörmannsperger[1]. [1]Technical University of Munich, Freising-Weihenstephan, Germany; [2]Max Planck Institute of Neurobiology, Munich, Germany; [3]Medical University of Graz Medical University of Graz, Graz, Austria; [4]Vall d'Hebron Research Institute, Barcelona, Spain

Specific antibiotics (ABX) treatment is associated with increased risk for later development of Crohn´s disease but the causal relevance and pathophysiological mechanisms are not understood. We hypothesize that ABX-mediated increase in large intestinal luminal protease activity (liPA) affects the intestinal barrier and immune homeostasis and confers risk to the development of chronic inflammation. Analysis of patient stool before and during ABX treatment revealed a more than 5-fold increase in liPA in 25% of the patients. Interestingly, 44.4% of the patients treated with fluoroquinolones showed a more than 5-fold increase in liPA, in contrast to only 14.3% of the patients treated with β-lactam/makrolid. In mice, vancomycin/metronidazole (V/M) treatment resulted in a rapid rise in liPA (5-10X), which was mostly due to pancreatic trypsin. The abnormally high liPA was found to impair the intestinal epithelial barrier in transwell cultures and Ussing chamber analyses. In WT and IL10$^{-/-}$ mice, the acute V/M-mediated rise in liPA resulted in increased permeability of the intestinal barrier *in vivo* and impaired large intestinal barrier functions *ex vivo*. Co-administration of a serine protease inhibitor (AEBSF)

reversed these detrimental effects. Repetitive V/M treatment of IL10$^{-/-}$ mice resulted in long lasting increase in liPA, acceleration of colitis development and large intestinal tumor formation. Co-administration of AEBSF partially reversed these adverse effects. The V/M-mediated rapid increase in liPA impairs the intestinal barrier and accelerates the development of chronic inflammation in colitis susceptible mice. These findings suggest that the ABX-mediated rise in liPA may contribute to the development of IBD in susceptible individuals.

W40. GSK583, 6-(tert-Butylsulfonyl)-N-(5-fluoro-1H-Indazol-3-yl)Quinolin-4-Amine, is a Highly Potent and Selective Inhibitor of RIP2 Kinase with Efficacy in Models of IBD

Anna Vossenkämper[1], Bartholomew Votta[2], Linda Casillas[2], Robert Marquis[2], John Bertin[2], Thomas T. MacDonald[1,3] and Pamela Haile[2]. [1]Blizard Institute, London, England, United Kingdom; [2]GSK, Collegeville, PA; [3]Queen Mary University of London, London, England, United Kingdom

Crohn's disease (CD) and ulcerative colitis (UC) are characterised by a breakdown in intestinal immune homeostasis and disruption of epithelial barrier integrity. The resulting inappropriate interaction between commensal bacteria and mucosal immune cells leads to aberrant activation of pattern recognition receptors (PRRs) and inflammation. The cytoplasmic PRRs NOD1 and NOD2 serve as sensors for bacterial peptidoglycans. Activation of NOD receptors leads to recruitment of receptor-interacting protein 2 (RIP2) resulting in pro-inflammatory cytokine production via activation of NF-κB and MAPKs. Activated RIP2 kinase is highly expressed in intestinal biopsies obtained from IBD patients, suggesting a role in pathogenesis. Here we characterize a new and highly selective RIP2 kinase inhibitor, GSK583. Its effectiveness was studied in human explant cultures of inflamed mucosa from IBD patients. GSK583 was highly effective in reducing the levels of activated RIP2 kinase in tissue and reduced the spontaneous production of inflammatory cytokines IL-1β, IL-6 and TNFα to a level achieved with prednisolone. Using GSK583, we aim to further investigate the role of RIP2 in the pathogenesis of IBD and explore the potential therapeutic benefit of RIP2 kinase inhibition in patients.

W41. Preclinical Assessment of a Novel Anti-TNFα Domain Antibody (Vorabody) in Development as an Oral Therapy for Crohn's Disease

Anna Vossenkämper[1], Scott Crowe[2], Mike West[2], Kevin Roberts[2], Tim Carlton[2], Luana Maggiore[2], Gareth Parkes[3], Thomas T. MacDonald[1,4], Marion Cubitt[2] and Keith Ray[2]. [1]Blizard Institute, London, England, United Kingdom; [2] VHsquared Ltd, Babraham, Cambridge, England, United Kingdom; [3]Royal London Hospital, Barts Health, London, England, United Kingdom; [4]Queen Mary University of London, London, England, United Kingdom

Crohn's disease (CD) is an inflammatory disease of the intestine that can be difficult to control with conventional therapies. Neutralisation of TNFα is an established and effective treatment. Anti-TNFα antibodies such as infliximab, adalimumab and certolizumab-pegol are being used clinically for the treatment of CD. However, they require injection or infusion which is inconvenient and painful for the patient. VHsquared Ltd. has developed llama engineer domain antibodies (Vorabodies) against human TNF-alpha for oral delivery. The lead Vorabody, V565, has potent neutralising activity against soluble and membrane forms of human TNFα comparable to adalimumab, and engineered resistance to intestinal proteases. Efficacy of V565 was investigated in 24h-*ex vivo* cultures of CD biopsies from patients with active disease. Samples were lysed and supernatants analysed for cytokine levels. Lysates were subjected to an array specific for phosphorylated receptor tyrosine kinases and signalling molecules. V565 showed potent inhibition of the phosphorylation of most of the proteins included on the array compared to the control. Amongst the inhibited phosphoproteins were Ron, M-CSFR, ERK1/2, Src and Lck, i.e. molecules implicated in macrophage and T cell activation. V565 also potently inhibited the production of inflammatory cytokines IL-1β, IL-6, IL-8, IL-17A and TNFα in *ex vivo* CDbiopsy cultures. We demonstrate V565's potent ability to suppress phosphorylation of signalling molecules and inflammatory cytokines in CD mucosa *ex vivo*. These results suggest that V565 might be an effective oral treatment for CD patients. Such a delivery would be more convenient and offer reduced systemic drug exposure compared to currently available anti-TNFα antibodies.

W42. Probiotics and IBD: Selecting Strains Able to Trigger Paneth Cells-Derived Antimicrobial Responses

Jiri Hrdy[1], Carmen Lapadatescu[2], Aurelie Maillard[3], Bernhard Ryffel[3], Mathias Chamaillard[4], Bruno Pot[4] and Corinne Grangette[4]. [1]Charles University, and General University Hospital, Prague, Stredocesky kraj, Czech Republic; [2] Bioprox, Paris, Ile-de-France, France; [3]University and CNRS, Orleans, Centre, France; [4]Institut Pasteur de Lille, Lille, Nord-Pas-de-Calais, France

In genetically susceptible individuals, inappropriate mucosal immune responses against the intestinal microbiota including impaired antimicrobial response against potential pathogens, appear to be one of the principal mechanism leading to inflammatory bowel disease (IBD). The capacity of three selected probiotic strains (*L. reuteri, L. acidophilus, B. animalis* spp *lactis*) to induce antimicrobial protein expression was tested *in vitro*. Their capacity to activate bone marrow derived dendritic cells (BMDC) and to promote either Th17 or Tregs was evaluated by coculturing naive $CD4^{+}CD25^{-}$ cells with probiotic-primed BMDCs. Anti-inflammatory properties of probiotics and capacity to promote antimicrobial peptides (AMP) expression were confirmed *in vivo*. The expression of the AMP was strain-dependent. *L. acidophilus* was the most potent inducer of both BMDC activation and IL-17 secretion, while *L. reuteri* was found to be good inducer of Treg. *In vivo*, the three strains were able to induce IL-22 and anti-microbial peptides in naive mice, to protect mice against TNBS-induced colitis and to dampen inflammation during *Clostridium rodentium* infection. Finally, oral administration of the 3 strains in a mixture was able to promote AMP gene expression and ILC3 induction in naïve mice. In conclusion, we have selected promising probiotic strains in the context of IBD able to stimulate defensin induction and to dampen the inflammatory tone. This work was supported by ANR BioPaneX and AZV CR 15-26877A.

W45. Novel *Escherichia coli*-Derived Therapeutic Immunotherapies Reduce Innate Immune Responses to Protect MUC-2 Deficient Mice from Spontaneous Colitis

Ho Pan Sham[1], Mark Bazett[1], Momir Bosiljcic[1], Caixia Ma[2], Rebecca Anderson[1], Hyungjun Yang[2], Vijay Morampudi[2], Hongbing Yu[2], Hal Gunn[1], Bruce Vallance[2] and Shirin Kaylan[1]. [1]Qu Biologics, Vancouver, BC, Canada; [2] University of British Columbia/ British Columbia Children and Family Research Institute, Vancouver, BC, Canada

Ulcerative Colitis (UC) is a form of inflammatory bowel disease (IBD) in which the intestinal mucosal barrier, including the mucus layer, is compromised -leading to ulceration. Mice that lack the major mucin found in the intestinal mucus, mucin 2 (Muc2), develop spontaneous colitis that mimics UC. QBECO, an immunomodulator derived from an inactivated strain of *Escherichia coli* that holds potential as a novel immunotherapeutic agent for UC by restoring normal innate immune function, was tested in Muc-2 deficient ($Muc2^{-/-}$) mice. QBECO administered subcutaneously every 2nd day for 30 days reduced spontaneous colitis in the $Muc2^{-/-}$ mice. Specifically, QBECO treatment markedly improved the overall histological score, reduced T cell infiltration, and decreased neutrophil numbers in the colonic tissues. These observations were accompanied with a reduction in pro-inflammatory mediators IL-17A in the colon and keratinocyte-derived chemokine (KC) in serum. QBECO treatment did not impact regulatory T cell marker (FOXP3) and anti-inflammatory growth factor (TGF-β) expressions in affected tissues. Additionally, QBECO treated mice attenuated levels of the antimicrobial lectins, RegIII-β and RegIII-γ, which favorably affected the gut microbiome by limiting the growth of gamma-proteobacteria and increasing the probiotic lactobacilli. These data demonstrate QBECO treatment ameliorates spontaneous colitis in aged $Muc2^{-/-}$ mice. Together, these findings may have broader implications for our understanding of IBD pathology and aid in the development of novel immunotherapeutics focused on reconstituting normal immune function in the context of IBD.

W46. Europium-Doped Cerium Oxide Nanoparticles Limit Reactive Oxygen Species Formation and Ameliorate Intestinal Ischemia-Reperfusion Injury

Ekaterina O. Gubernatorova[1], Xiaobo Liu[2], Ali Othman[2], Wayne T. Muraoka[3], Ekaterina Koroleva[3], Silvana Andreescu[2] and Alexei V. Tumanov[3]. [1]Lomonosov Moscow State University, Moscow, Russia; [2]Clarkson University, Potsdam, NY; [3]University of Texas Health San Antonio, San Antonio, TX

Ischemia-reperfusion induced intestinal injury is associated with a wide range of clinical conditions, including necrotizing enterocolitis in neonates, intestinal transplantation rejection, and sepsis. Accumulating evidence suggests that reactive oxygen species (ROS) contribute to the pathogenesis of intestinal ischemia-reperfusion induced injury. In this study, we demonstrate the therapeutic effectiveness of novel europium-doped cerium oxide nanoparticles (Eu-doped Ceria NPs) as ROS scavengers in a mouse model of intestinal ischemia-reperfusion-induced injury. Ischemia-reperfusion injury of the small intestine was achieved by inducing ischemia in the region of the distal ileum by temporally occluding the peripheral and terminal collateral branches of the superior mesenteric artery using microvascular clips followed by reperfusion. Kinetics of superoxide concentrations in intestinal tissue during the ischemia-reperfusion were measured electrochemically using microelectrode biosensors. An increased production of superoxide radicals was detected in the intestine throughout the ischemia stage and again after initiating reperfusion. These changes in superoxide radical formation were associated with the induction of inflammatory cytokines in the intestine. We further show that Eu-Ceria NPs exhibited superoxide scavenging activity *in vitro*. Importantly, administration of Eu-Ceria NPs into the intestinal lumen during the onset of ischemia effectively blocked superoxide accumulation, reduced the expression of IL-1b, and ameliorated the intestinal pathology. These results suggest that early increased production of ROS during the ischemia-reperfusion promotes intestinal pathology and that mucosal delivery of Eu-Ceria NPs may be a potential therapeutic approach to block ROS accumulation and ameliorate the severity of intestinal disease.

W47. Impact of Dietary Soybean Oil on the Intestinal Epithelium

Poonamjot Deol[1], Jane Evans[1], Eiwen Yang[1], Christophe Morisseau[2], Amol Trivedi[1], Tao Jiang[1], Bruce D. Hammock[2] and Frances Sladek[1]. [1]University of California Riverside, Riverside, CA; [2]University of California Davis, Davis, CA

The incidence of inflammatory bowel disease (IBD) in the U.S. has increased significantly since 1980, preceded by a remarkable increase in the prevalence of soybean oil (SO) in the American diet. Although the role of SO in the etiology of IBD is ambiguous, one of its major components, linoleic acid (LA), is associated with an increased risk of ulcerative colitis (UC). We have established a diet-based model to examine the effect of soybean oil on intestinal health. Isocaloric diets, moderately high in saturated fats from coconut oil (CO) were supplemented, or not, with conventional soybean oil (SO+CO) or the genetically modified, low-LA Plenish (PL+CO) soybean oil. Male C57/Bl6N mice were fed vivarium (Viv), CO, SO+CO or PL+CO diets for up to 35 weeks. Luminex analysis shows increased levels of the eosinophil-recruiting and -activating cytokine IL-5 in serum of mice fed SO+CO diet. Intestinal RNAseq analysis shows dysregulation of IBD related genes such as *Muc2*, *Irf8*, and *Prkce*. Treating mice on the Viv, SO+CO or PL+CO diets with Dextran Sodium Sulfate (DSS) shows increased inflammation and crypt damage in colons of mice fed SO+CO diet as compared to those fed the PL+CO diet, suggesting an involvement of LA or its metabolites. LA is metabolized into oxylipins (also referred to as leukotoxins) via a two-step reaction involving cytochrome P450 enzymes and the enzyme soluble epoxide hydrolase (sEH). Experiments with an inhibitor of soluble epoxide hydrolase are underway to elucidate their role in the pathogenesis of IBD.

W48. Inhibition of IL-13Rα2 Protects Mice from Acute Inflammatory Bowel Disease Pathogenesis

Erik P. Karmele[1], Kevin M. Vannella[1], Marion T. Kasaian[2] and Thomas A. Wynn[1]. [1]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD; [2] Pfizer, Cambridge, MA

Patients with inflammatory bowel disease (IBD) are commonly administered anti-TNFα agents for treatment, however up to 40% of patients are non-

responders for unknown reasons. Previous studies identified elevated *IL13RA2* mRNA transcripts in mucosal biopsy samples of patients with active IBD who are non-responders compared to responders, serving as a possible predictive marker for non-responsiveness. IL-13Rα2 is a high affinity "decoy receptor" for IL-13, a cytokine with both anti-inflammatory and wound healing functions. In this study, we hypothesized that TNFα and IL-17 produced during the initiation of IBD induces IL-13Rα2 production that neutralizes the endogenous anti-inflammatory activity of IL-13, promoting IBD pathogenesis. Using an acute dextran sodium sulfate (DSS) model of mouse colitis with a 7-day recovery period, we show that DSS increases the production of both systemic and colonic IL-13Rα2 compared to untreated controls. DSS-induced colitis was less severe in *Il13ra2*$^{-/-}$ mice compared to wild-type controls as decreased shortening of colon length was observed. Additionally, histological analysis of the distal colon revealed less goblet cell depletion, inflammatory cell infiltration, and submucosal inflammation in DSS-administered *Il13ra2*$^{-/-}$ mice compared to DSS-administered wild-type mice. Gene expression, measured by Nanostring, revealed decreased expression of pro-inflammatory genes, such as *Il17ra, Ccl5, Csf1,* and *Il1r1* in colon tissue of DSS-administered *Il13ra2*$^{-/-}$ mice compared to DSS-administered wild-type mice following 7 days of recovery. Together, these findings suggest that IL-13Rα2 functions as an important regulator of IBD pathogenesis and the absence of IL-13Rα2 increases endogenous IL-13 bioactivity, which promotes recovery from acute IBD.

W50. Mouse Colonic Stromal Cells Induce a Protective ILC3 Phenotype in the Absence of NKX2-3 Transcription Factor

Zoltán Kellermayer[1], Dora Vojkovics[1], Bela Kajtar[1], Gergely Berta[1], Istvan Nag[2,3], Eva Boros[2], Balazs Balint[3], Angela Schippers[4], Nobert Wagner[4] and Peter Balogh[1]. [1]University of Pecs, Pécs, Baranya, Hungary; [2]Biological Research Centre of the Hungarian Academy of Sciences; Szeged, Csongrad, Hungary; [3]SeqOmics Biotechnology Ltd, Morahalom, Csongrad, Hungary; [4]RWTH Aachen University, Aachen, Nordrhein-Westfalen, Germany

Single nucleotide polymorphisms within the homeodomain transcription factor NKX2-3 have been associated with human Crohn's disease and ulcerative colitis. In mice, lack of NKX2-3 leads to absence of endothelial MAdCAM-1 and consequently to an altered lymphocyte homing to intestinal lymphoid tissues. We wished to characterize how the absence of NKX2-3 influences the course of colitis in mice. 2.5% DSS was used to induce colitis in BALB/c, NKX2-3$^{-/-}$, C57BL/6 and MAdCAM-1$^{-/-}$ mice. In contrast with the other three genotypes, NKX2-3$^{-/-}$ mice did not develop colitis. We found a significantly higher absolute number of Th17 and ILC3s among NKX2-3$^{-/-}$ colonic lamina propria lymphocytes compared to controls. RT-PCR from distal colons revealed that on day 7 mRNA for IL-22, IL-23, Reg3β and Reg3γ, but not IL-17a, was significantly higher in NKX2-3$^{-/-}$ colons. Histology and RT-PCR results indicated a less-pronounced lymphangiogenesis in the absence of Nkx2.3 while dendritic cell migration from colons was significantly slower, revealed by photoconverting colons of [*NKX2-3* $^{-/-}$ *x Kikume*$^{+}$] mice. NKX2-3$^{-/-}$ -> BALB/c bone marrow chimeras did not develop colitis, indicating a role of Nkx2.3$^{-/-}$ stromal cells in protection. We also analyzed the whole transcriptome profile of untreated and DSS-treated BALB/c and NKX2-3$^{-/-}$ colonic stromal cells (depleted of CD45^{+} cells) to identify factors that influence ILC3 function in the absence of NKX2-3. Our results indicate that the protection observed in NKX2-3$^{-/-}$ mice is not caused by the endothelial absence of MAdCAM-1; instead, NKX2-3$^{-/-}$ stroma promotes ILC3s to exert protective functions. Identification of downstream factors may thus have therapeutic potentials.

W51. CD206 Positive Intestinal Macrophages Accelerate the Colonic Epithelial Wound Healing

Shusaku Hayashi, Donald Zinsou, Kazuyuki Tobe and Makoto Kadowaki. University of Toyama, Toyama, Japan

Recent studies have revealed that the resident macrophages in the intestinal mucosa show M2 macrophage-like properties such as high production of IL-10 and expression of M2 macrophage marker. CD206 is one of the common M2 macrophage markers and a part of intestinal macrophages actually express CD206. However, the precise role of CD206 positive intestinal macrophages in the intestinal inflammation still remains unclear. In the present study, we investigated the role of CD206 positive intestinal macrophages in the acute colitis and wound healing after colonic epithelial damage. To elucidate the role

of CD206 positive intestinal macrophages, we used transgenic mice expressing human diphtheria toxin receptor (DTR) under the control of CD206 gene promoter. For ablation of CD206 positive macrophages, CD206-DTR transgenic mice were injected intraperitoneally with diphtheria toxin. The expression of IL-10 mRNA in the intestinal macrophages was markedly decreased in CD206 positive cell-depleted mice compared with WT mice. There was no difference in the development of acute colitis between WT and CD206-DTR mice. Next, we evaluated the repairing ability of CD206 positive intestinal macrophages on the colonic epithelial damage using CMT-93, a murine colonic epithelial cell line. The intestinal macrophages isolated from WT mice promoted the wound healing of CMT-93 after the scratch damage. We found that the CD206 positive cell-depleted intestinal macrophages did not affect the wound healing. The present findings suggest that CD206 positive intestinal macrophages play an important role in the wound healing following inflammatory damage to the intestinal mucosal such as inflammatory bowel disease

W52. Enhancer of IL-10 Production in Intestinal Macrophages Suppresses the Development of an Experimental Colitis in Mice

Nonoka Wakabayashi, Shusaku Hayashi and Makoto Kadowaki. Institute of Natural Medicine, University of Toyama, Toyama, Japan

Inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis are chronic inflammatory disorders. Abnormalities of intestinal innate immune functions have been regarded as key properties in immunogenetic profile of IBD. Intestinal macrophages play pivotal roles in the regulation of immune homeostasis in the intestine. We have recently demonstrated that the enhancement of IL-10 production in the intestinal macrophages has the potential to be a novel therapeutic mechanism against IBD. Thus, to address the development of new therapeutic medicines for IBD, we have screened compounds derived from medicinal herbs for the ability to enhance IL-10 production in the intestinal macrophages. Firstly, we performed an *in vitro* screening of 96 compounds derived from medicinal herbs that are frequently used in Japan using bone marrow-derived macrophages (BMDMs). BMDMs were prepared from BALB/c mice and cultured with M-CSF (100 ng/ml) for 7 days. Then, compounds (10 μM) were added to BMDMs 1 h before LPS stimulation (100 ng/ml) for 24 h. Among the 96 samples, we found that 3 compounds significantly increased the IL-10 production in BMDMs. To evaluate the effect of these compounds in the intestinal inflammation, a murine colitis model was induced by treatment with 3% dextran sulfate sodium (DSS) in the drinking water for 7 days. The administration of compound X (10 mg/kg) significantly attenuated the severity of DSS-induced colitis. These results can lead to the hypothesis that enhancers of IL-10 production in intestinal macrophages suppress the development of colitis.

W54. Improvement of Oxazolone-Induced Colitis by Vagus Nerve Stimulation

Elisa Meroni[1], Simon Verheijden[1], Nathalie Stakenborg[1], Pedro J. Gomez-Pinilla[1], Gera Goverse[1], Gianluca Matteoli[2] and Guy Boeckxstaens[1]. [1]University of Leuven, Leuven, Vlaams-Brabant, Belgium; [2]KU Leuven, Vlaams-Brabant, Belgium

The cholinergic anti-inflammatory pathway (CAIP), acting through the vagus nerve (VN), represents a novel mechanism that modulates the immune system, mainly by dampening the activation of the innate immune system, in particular macrophages. To what extent manipulation of the CAIP also affects the adaptive immune system remains however to be elucidated. In the present study therefore, we investigated the effect of VN stimulation (VNS) in a Th2-mediated model of colitis, i.e. oxazolone-induced colitis. Colitis was induced in Balb/c mice by cutaneous sensitization with 3% oxazolone followed by intrarectal administration of 1% oxazolone 7 days later. Intrarectal oxazolone administration resulted in a rapid and severe destruction of the colonic mucosa finally leading to 65% death rate at day 5. To evaluate the effect of CAIP in this model, VNS or sham stimulation was performed in mice with intact splenic innervation and mice that underwent splenic denervation. Two weeks prior to rectal application of oxazolone. In both groups, VNS significantly improved survival rate and resulted in a reduction in colonic (*Il6* and *Cxcl1* mRNA) and serum cytokines levels (IL-6, TNFα and KC) (at 6h) compared to sham mice. Surprisingly, however, no improvement in colonic morphology (H&E) was observed. Our results indicate that oxazolone-induced colitis is a rather severe model of colitis associated with a high mortality rate and a severe destruction of the colonic architecture.

Nevertheless, our data indicate that VNS is beneficial in this model, likely by dampening both colonic and systemic immune responses. The underlying mechanism however requires further study.

W56. Wnt5b Potentiates TGF-β-Dependent Epithelial Mesenchymal Transition in Human Intestinal Epithelium

Isabella Dotti[1], Elena Ferrer-Picón[1], Núria Planell[1], Egbert H van Herk[2], Míriam Cuatrecasas[3], Julián Panés[3] and Azucena Salas[1]. [1]IDIBAPS, Barcelona, Catalonia, Spain; [2]Tytgat Institute for Liver and Intestinal Research, Amsterdam, Netherlands; [3]Hospital Clinic de Barcelona, Barcelona, Spain

Epithelial mesenchymal transition (EMT) has been postulated to contribute to regeneration of the intestinal epithelium. Emerging evidence indicates that Wnt5b could play a role in EMT. We hypothesize that Wnt5b is expressed in the intestine and contributes to epithelial regeneration through EMT. To test this, we: 1) evaluated Wnt5b expression in the intestine of patients with inflammatory bowel disease (IBD) and 2) investigated the effect of Wnt5b on human epithelial organoid cultures (EpOCs). Human intestinal samples were used for *in situ* hybridization and RNAseq to characterize Wnt5b expression in controls and IBD patients. Intestinal crypts were used to generate EpOCs for subsequent incubation with Wnt5b, TGF-β or LY2157299 (TGF-βR1 inhibitor). Total RNA was isolated for transcriptional analysis. Migration assays were performed to check Wnt5b-induced mesenchymal changes in EpOCs. *WNT5B* mRNA was expressed in both the lamina propria and the epithelium of healthy gut. Its transcription was greater in inflamed, versus non-inflamed, IBD samples. In EpOCs, Wnt5b promoted changes resembling the TGF-β-induced phenotype. These included a significant up-regulation of EMT markers, TGF-β targets and TGF-β itself. Indeed, LY2157299 fully suppressed the Wnt5b-mediated changes. Finally, Wnt5b-treated EpOCs showed higher migratory potential compared to un-stimulated cultures. In conclusion, we show that Wnt5b acts on intestinal epithelium by potentiating a TGF-β -dependent EMT response. Its up-regulation in IBD mucosa could promote epithelial cell migration, thus contributing to a regenerative response in the context of tissue injury.

W57. Impact of Microbiota on Immune Cell Composition and Barrier Function in the Gut

Franziska Schmidt[1], Katja Dahlke[2], Arvind Batra[3], Christiane Ring[2], Jacqueline Keye[1], Gunnar Loh[4], Michael Schumann[1], Anja Kühl[1], Michael Blaut[2] and Britta Siegmund[1]. [1]Charité-Universitätsmedizin Berlin, Berlin, Germany; [2] German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Brandenburg, Germany; [3]Takeda PharmaVertrieb GmbH & Co. KG, Berlin, Germany; [4]Max Rubner-Institute, Karlsruhe, Baden-Wurttemberg, Germany

To understand the consequences of changes in the intestinal microbiota for immune cell homeostasis the immune cell phenotype in the gut of germ-free (GF-), specific pathogen-free (SPF-) mice and GF-mice colonized at the age of 5 weeks with SPF-microbiota (COL-) in health and intestinal inflammation was assessed. Acute colitis was induced by dextran sodium sulfate (DSS). Barrier function of colon was analyzed by electrophysiology using the Ussing-Chamber. Local cytokine production was assessed in supernatants of cultures *ex vivo* isolated colon by Cytometric-Bead-Array. To confirm successful colonization cecal content was sequenced for 16S ribosomal RNA. In the terminal ileum of GF-mice the number of *T cell*s, monocytes and macrophages was decreased. The immune cell composition in the healthy colon was similar in GF- and SPF-mice. Colonic macrophages from GF-mice showed an increased TNFα-expression after LPS-stimulation compared to macrophages from SPF-mice. Furthermore, in GF-mice the total resistance of the colon was decreased accompanied by an increased ^{3}H-Mannitol flux suggesting a barrier dysfunction. GF-mice died following exposure to DSS, whereas SPF-mice survived and showed signs of colitis. Colonization rescued GF-mice from death. The inflammation score was higher in SPF- than in COL-mice whereas the immune cell composition as well as diversity of intestinal microbiota was similar in these mice. In conclusion, the microbiota is essential for the development of the colonic integrity and local immune cell composition. The dysbiosis might play a role in the pathogenesis of inflammatory bowel disease and could provide a potential target for therapeutic intervention.

W58. Reduced Frequencies of Circulating TIGIT^{+}CD38^{+} Effector T Cells in Active Inflammatory Bowel Disease

Maria Joosse[1], Celia Menckeberg[1], Lilian de Ruiter[1], Rolien Raatgeep[1], Lisette van Berkel[1], Ytje Simons-Oosterhuis[1], Femke Muskens[1], Rudi Hendriks[1], Remco Hoogenboezem[1], Tom Cupedo[1], Lissy de Ridder[2], Johanna Escher[1], Sharon Veenbergen[1,2] and Janneke Samsom[1]. [1]Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands; [2]Sophia Children's Hospital, Rotterdam, Zuid-Holland, Netherlands

Monitoring the total CD4^{+} T cell population in peripheral blood is not sensitive enough to detect inflammatory intestinal T cell responses. Recently, we demonstrated that CD38 expression on circulating effector T cells (CD4^{+}CD62L^{neg}) enriches for T cells with specificity for mucosal antigens. We hypothesize that the composition and activational state of the circulating CD38^{+} effector *T cell* population reflects ongoing intestinal immune responses. Indeed, in healthy individuals circulating CD38^{+} effector T cells had a non-inflammatory phenotype as evidenced by lower frequencies of interferon-g (IFNg)$^{+}$, but higher frequencies of interleukin-10 (IL-10)$^{+}$ T cells when compared to CD38^{neg} effector T cells in an allogeneic mixed lymphocyte reaction. To identify surface proteins that account for this phenotype, we performed comparative RNA sequencing on circulating CD38^{+} and CD38^{neg} effector T cells. Transcripts associated with immune regulation were among the differentially expressed genes. In particular, at protein level T cell immunoglobulin and ITIM domain (TIGIT) was expressed by 40% of CD38^{+} effector T cells versus 20% of the CD38^{neg} effector T cells. In agreement with its inhibitory function, TIGIT expression on CD38^{+} effector T cells correlated with high IL-10 mRNA at baseline and low IFNg secretion after stimulation. In patients with active inflammatory bowel disease, the phenotype of this circulating CD38^{+} *T cell* population was altered, with increased frequencies of activated CD25^{+} and CD45RA^{neg} cells. Such activation was however not detectable in the total CD4^{+} T cell population. Strikingly, frequencies of TIGIT^{+}CD38^{+} effector T cells were significantly reduced in patients with active inflammatory bowel disease but normalized during immunosuppressive treatment.

W59. Hepcidin Regulates Inflammation and Repair in the Mammalian Intestine

Nicholas Bessman, Thomas Fung, Keith Fernandez, and Gregory Sonnenberg. Weill Cornell Medicine, New York, NY

Hepcidin is a liver-derived acute-phase protein that controls systemic iron levels in mammals and is a potential therapeutic target to limit anemia associated with inflammatory bowel disease (IBD). However, it remains unknown whether hepcidin also influences inflammation, repair, or host-microbe interactions in the intestine. To begin to interrogate this, we exposed hepcidin-deficient mice to an experimental model of intestinal damage and observed a substantial impairment in tissue repair and enhanced pro-inflammatory immune responses. Strikingly, levels of hepcidin were reduced in the liver upon intestinal damage, but upregulated in intestinal tissues, suggesting a potential biological role for local hepcidin production. We observed that dendritic cells are one local source of hepcidin that is dynamically upregulated following stimulation with live microbes. Ongoing studies in the laboratory are actively interrogating the role and regulation of dendritic cell-derived hepcidin during intestinal homeostasis, inflammation and repair.

W60. MET-Neutrophils Induce Intestinal Inflammation and Th17 Cells Differentiation

Michelle Stakenborg[1], Gera Goverse[1], Mario Di Matteo[1], Veronica De Simone[1], Massimiliano Mazzone[1] and Gianluca Matteoli[2]. [1]University of Leuven, Belgium; [2]KU Leuven, Vlaams-Brabant, Belgium

Neutrophils play an essential role in the maintenance of intestinal homeostasis as they provide a first line of defense against invading pathogens. However, during chronic inflammatory conditions, such as Inflammatory Bowel Disease, excessive neutrophil accumulation can lead to loss of homeostasis and delayed tissue repair. In the current study, we aim to identify the role of a novel pathway responsible for recruitment and activation of neutrophils in inflamed tissues, the tyrosine-protein kinase MET and its ligand hepatocyte growth factor (HGF). To study the role of MET on neutrophils, we used the neutrophil-specific (Mrp8)-Cre line backcrossed with Met$^{fl/fl}$. MRP8$^{Cre/WT}$ MET$^{fl/fl}$ (KO) mice and MRP8$^{WT/WT}$ MET$^{fl/fl}$ (WT) controls

were subjected to chronic DSS-induced colitis. During DSS-colitis, blood neutrophils upregulated MET and were recruited to the mucosa under the influence of HGF. Under chronic DSS-induced colitis KO mice displayed a decreased disease activity index compared to WT mice with a reduction of ROS^+ neutrophils, implying a protective effect of MET deletion in neutrophils. Strikingly, analysis of $CD4^+$ T cells showed a decrease of Th17 in KO mice compared to WT mice, while no differences were observed in Th1 cells. In line, we could observed increase expression of MET and of its ligand HGF in biopsies collected from patients affected by ulcerative colitis. Overall our data showed that MET deletion in neutrophils is limiting intestinal inflammation with a specific reduction of Th17 cells. Further understanding the mechanisms underlying neutrophil function during IBD will aid in the development of novel therapies to treat IBD patients.

W61.: IL36 Receptor Antagonism as a Novel Therapeutic Approach Against Inflammatory Bowel Diseases

Ernest Raymond, Gary Caviness, Rajkumar Ganesan, Jennifer Ahlberg, Christine Grimaldi, Joseph Woska and Lamine Mbow. Boehringer Ingelheim, Ridgefield, CT

IL-36 receptor is a member of the IL-1R family associated with skin and gut inflammation. Binding of IL-36 cytokine to IL-36R results in recruitment of IL-1RAcp to form a heterotrimeric complex leading to activation of NF-κb and MAP-Kinase and subsequent production of several pro-inflammatory cytokines/chemokines. IL-36R is linked to inflammatory skin diseases (e.g. generalized pustular psoriasis) based on genetic loss of function mutations associated with IL-36 receptor antagonist (IL-36 Ra). Here we present experimental evidence linking IL36R signalling to IBD based on a) expression of IL36R and its ligands in diseased tissue; b) findings demonstrating that IL36 activation of primary human intestinal epithelial cells and myofibroblasts leads to disruption in epithelial barrier integrity as well as up-regulation of pro-inflammatory mediators linked to IBD pathogenesis such as S100A8/9, LCN2 and pro-fibrotic genes; c) *in vivo* findings demonstrating that treatment with anti-IL-36R antagonist antibody reduces mucosal inflammation in mouse models of colitis. Taken together, these data support the therapeutic potential for IL-36R antagonism in IBD.

W62. IL-36R Signaling in Notch2-Dependent DCs Induces IL-23-Dependent Rsolution of Acute Colonic Damage

Vu L. Ngo[1], Hirohito Abo[1], Akihito Harusato[1], Asma Nusrat[2], Timothy Denning[1] and Estera Maxim[1]. [1]Georgia State University, Atlanta, GA; [2] University of Michigan, Ann Arbor, MI

The contribution of cytokines to inflammatory bowel disease (IBD) is under intensive investigate. Interleukin (IL)-36 ligands, members of the IL-1 family, are expressed in both experimental colitis and human IBD, and believed to play both pathogenic and barrier protective roles depending on the context. IL-36 ligands (α, β, γ) have been shown to induce pro-inflammatory pathways and we and others have implicated the IL-36/IL-36R axis in IL-22 production and the resolution of acute colonic damage. Here, we demonstrate that IL-36γ is a potent inducer of IL-23 production both *in vitro* and *in vivo*. IL-36γ-induced IL-23 was highly dependent upon Notch2-dependent (CD11b+CD103+) DCs, but not CSFR1-dependent macrophages or Batf3-dependent (CD11b-CD103+) DCs. The intracellular signaling cascade required for IL-36γ-induced IL-23 from DCs involved MyD88 and the NF-kB subunits c-Rel and p50. Consistent with *in vitro* observations, IL-36R-deficient mice exhibited dramatically reduced colonic IL-23 and IL-22 expression, and consequently, mice failed to recover from acute intestinal damage. Interestingly, treatment of mice deficient in IL-36R and IL-36γ with exogenous IL-23 induced complete recovery from colonic damage. This recovery was accompanied by a restoration of IL-22 and antimicrobial peptides (RegIII family and S100A8) expression in the colon. In summary, we provide evidence for that the IL-36/IL-36R axis potently induces IL-23-dependent resolution of acute colonic damage.

W63. Role of IFNs in Gastro-Intestinal Mucosal Inflammation

Constance McElrath[1], Jian-Da Lin[2], Jianya Peng[1], Raghavendra Sridhar[1], Vanessa Espinosa[1], Orchi Dutta[1], Hsiang-Chi Tseng[1], Heidi Risman[1], Marvin Sandoval[2], Mark Galan[1], Amariliz Rivera[1], Joan Durbin[1] and Sergei Kotenko[1]. [1]Rutgers University, Newark, NJ; [2]New York University, New York, NY

Both type I and type III interferons (IFNs) are well recognized for their non-redundant importance in limiting viral infection; however, their roles as inflammatory mediators are less well characterized. Inflammatory bowel disease (IBD), such as ulcerative colitis, involves perturbation of the complex interactions between the mucosal immune system and the commensal bacteria of the gut, with cytokines acting as important cross-regulators of these multi-layered interactions. To characterize the contributions of type I and type III IFNs to the formation, progression, and resolution of UC, we used mice deficient in type I IFN, type III IFN, and type I/III IFN signaling and a murine model of acute UC, which follows exposure of the mice to dextran sodium sulfate (DSS)-containing water. **T**he importance of IFN signaling in the acute phase of inflammation was demonstrated by the onset of enhanced pathology and weight loss concurrent with the peak of IFN-induced Mx2 promoter-driven luciferase expression in DSS-treated transgenic Mx2-Luciferase reporter mice. Accordingly, experiments revealed enhanced sensitivity of double type I and type III IFN receptor-deficient mice to DSS-induced disease as measured by decreased body weight, colon length, diffuse destruction of the colonic epithelium and increased mortality. Furthermore, aberrant immune cell infiltrates and decreased regenerative capacity were observed in IFN receptor-deficient mice in comparison with wild type mice. These data suggest that both type I and type III IFNs play important roles in modulating the inflammatory response, tissue homeostasis and repair during DSS-induced colitis.

W65. The NLRP3 Inflammasome is Differentially Activated During Active Ulcerative Colitis and Crohn's Disease.

Nicole Ranson[1], Dale Kunde[1], Marcello Chieppa[2] and Rajaraman Eri[1]. [1]University of Tasmania, Launceston, Tasmania, Australia; [2] IRCCS de Bellis, Castellana Grotte, Puglia, Italy

A contributing factor in development of inflammatory bowel disease (IBD) is thought to be inappropriate activation of the inflammasome complex, enhancing the maturation of interleukin (IL)-1β and IL-18 by providing a signalling platform for the activation of caspase-1. The NLRP3 inflammasome, unlike the other inflammasomes, activates when exposed to a gamut of environmental irritants, cellular stressors or pathogenic insults and is pivotal to directing downstream proinflammatory pathways. This study describes the gene expression, cellular localisation and co-localisation of NLRP3 and IL-1β in human IBD. Targeted RNA-Seq analysis was conducted on active and quiescent biopsy samples from 9 IBD patients. mRNA-Seq data was validated using qRT-PCR on colon biopsies from 44 ulcerative colitis (UC) patients, 21 Crohn's disease (CD) patients and non-IBD controls. The cellular localisation of NLRP3 and IL-1β was determined using immunohistochemistry, immunofluorescence, and confocal imagery. Significant upregulation of inflammasome markers, notably NLRP3, NLRP6, IL-1β and IL6 were observed in IBD biopsies compared to controls and confirmed with qRT-PCR. The upregulation of NLRP3 and IL-1b was also seen in active inflammation biopsy compared to quiescent tissue from IBD patients. Increased NLRP3 and IL-1β protein expression appears to be focussed in non-resident monocytes within the laminar propria monocuclear cell population. However, there was a decreased co-localisation seen in active disease and interestingly differences observed between active UC and CD tissue. Given the broad ligand activating capabilities of the NLRP3 inflammasome, specific targeting this inflammasome activation pathway may provide an alternative to the current options for treating inflammatory diseases.

W66. TTC7A Mutation in a Patient with Crohn's Disease and Recurrent Sinus Infections

Snehdeep Hanspal[1], Connor Alexander[2], Michelle Tseng[2] and Oral Alpan[2]. [1]VCUSOM, Potomac, MD; [2]Amerimmune, LLC., Fairfax, VA

Biallelic TTC7A mutation has been implicated in Multiple Intestinal Atresia - Severe Combined Immunodeficiency (MIA-SCID), as well as Very Early Onset Inflammatory Bowel Disease (VEO-IBD). We present a patient with TTC7A mutation (R163W) presenting with Crohn's disease at 9 years of age. Whole blood was obtained from the patient, PBMCs were then isolated and stimulated with pokeweed, PBS, CD79b, or IL-4/CD40. The samples were then stained and for CXCR4 and CCR7. In the second Stimulation Assay, CD3/28 was used in addition to the previous stimulants, and the cells were stimulated overnight. For the Proliferation Assay, PBMCs were labeled with CFSE, stimulated with CD3/28, and incubated for 3 days at 37ºC. The Stimulation Assay indicated that the patient exhibits lower levels of baseline CXCR4 expression compared to controls. In the healthy control, stimulation resulted in down-regulation of CXCR4, while in the TTC7A patient, CXCR4 was up-regulated. CCR7 expression does not change much in response to stimulation in a healthy control, but down-regulates in the TTC7A patient and up-regulates in the TTC7A carrier mother. The proliferation assay revealed that the TTC7A patient had increased proliferation of T cells compared to the healthy control and mother. Conclusions: TTC7A plays a role in apicobasal polarity of enterocytes, as well as the migration and adhesion capabilities of lymphocytes. This case study shows that carrying one mutant TTC7A allele result in immunologic abnormalities, especially in the molecules that play a role in lymphocyte migration.

W67. MUC2 Mucin Deficiency Disrupts Iron Homeostasis and Elicits an Aberrant Immune Response Towards Lipopolysaccharide Induced Sepsis

Manish Kumar, France Moreau and Kris Chadee. University of Calgary, Calgary, AB, Canada

The colonic mucus bilayers formed by MUC2 mucin allows microbiota to colonize and function as a food source to maintain a healthy gut. In inflammatory bowel diseases (IBD) the mucus layers are severely altered allowing increased microbial penetrants that initiate/perpetuates inflammation rendering IBD patients high risk for developing sepsis-induced mortality. In this study, we quantified the innate immune responses in *Muc2*$^{-/-}$ mice that phenocopied MUC2 deficiency in IBD. *Muc2*$^{-/-}$ mice constitutively exhibit increased intestinal permeability with high numbers of splenic B, CD8^{+} and NK cells than WT littermates. Notably, *Muc2*$^{-/-}$ showed increased apoptosis of splenic B-cells at basal levels suggestive of ongoing systemic inflammation and immunosuppression. Surprisingly, *Muc2*$^{-/-}$ animals had 2-fold higher levels of total serum iron and 50% less iron levels in the liver indicating dysregulated iron homeostasis. LPS-induced sepsis induced 90% mortality in *Muc2*$^{-/-}$ mice characterized by increased bacterial penetrance through the distal ileum and colon with a corresponding 5-fold increase in serum TNF-α and IL-1β that was inhibited with the iron chelator, deferoxamine. This was accompanied by elevated splenic T- and B-cell apoptosis and delayed hypoferremic response in *Muc2*$^{-/-}$ as compared to WT littermate. In WT littermates, LPS treatment led to massive accumulation and secretion of mucus (mucus plug) in the lumen that impaired bacterial translocation. This study suggests that an altered and/or depleted barrier is a predisposing factor for increase bacterial penetrant that leads to aberrant host immune responses in IBD.

W69. Transcription Factor BATF2 in Intestinal Innate Myeloid Cells Controls Th1/Th17 Responses by Regulating Expression of IL-12 Family Genes.

Hisako Kayama. Osaka University, Suita, Osaka, Japan

In the intestine, Th1 and Th17 immune responses are important for host defense against invading pathogens through intensifying barrier functions, whereas inadequate inflammatory responses cause development of intestinal inflammation. IL-12 family proteins, such as IL-12, IL-23, and IL-27, produced by innate immune cells are implicated in the control of Th1 and Th17 responses. However, the mechanisms underlying transcriptional regulation of IL-12 family-related genes in intestinal innate immune cells are poorly understood. In this study, we demonstrate that the transcription factor BATF2 in macrophages is essential for proper Th1/Th17 responses in the intestine by regulating expression of IL-12 family-related genes. The number of IL-17- and IFN-g-producing CD4^{+} T cells in the colon of Batf2$^{-/-}$ mice

was higher than that in wild-type mice. In Batf2$^{-/-}$ bone marrow-derived macrophages pre-treated with IFN-g, LPS-induced expression of IL-23a, which encodes a subunit of IL-23 that enhances Th17 responses, was greatly increased compared to that in wild-type cells. In contrast, expression of IL-27p28, encoding a component of IL-27 that suppresses Th1/Th17 cell development, was markedly reduced in Batf2$^{-/-}$ cells. Furthermore, Batf2$^{-/-}$ mice were resistant to dextran sodium sulfate-induced colitis. In this context, epithelial antimicrobial defense characterized by Reg3b and Reg3g production was reinforced in the colon of Batf2$^{-/-}$ mice. These results suggest that BATF2 in M negatively regulates Th1/Th17 responses by suppressing IL-23a expression while promoting IL-27p28 expression and thereby controls intestinal barrier functions.

W71. Exposure to a Western Diet Induces Microbiota-Driven Colonic Mucus Layer Disruption and Epithelial Hyperplasia

George MH Birchenough, Björn Schröder, Fredrik Bäckhed and Gunnar C. Hansson. Gothenburg Univeristy, Gothenburg, Vastra Gotaland, Sweden

The colonic mucosa regulates the passage of material from the gut into the systemic circulation. Colonic goblet cells secrete polymeric MUC2 that forms the mucus layers protecting the mucosal epithelium. These act as physical barriers preventing direct microbial interaction with the epithelial surface that would otherwise result in inflammatory bowel disease (IBD) and colonic tumorigenesis. A Western diet (WD) has been linked to IBD and colitis associated cancer (CAC) caused in part by alterations in the intestinal microbiota. As the mucus layers control host-microbiota interactions, it is possible that diet-induced alterations in mucus layer function play a role in IBD development and progression to CAC. Using *ex vivo* methods to functionally assess the mucus layer we have demonstrated that WD-fed mice had a disrupted, penetrable mucus layer that was heavily colonized by bacteri and epithelial hyperplasia. Fecal transplantation of microbiota from control mice into WD mice rescued mucus layer disruption. Mucus layer disruption kinetics demonstrated that WD driven disruption was rapid and preceded epithelial hyperplasia. A low fibre diet drives bacteria to target the O-glycans that protect the MUC2 peptide backbone from degradation. The colonic mucus layer is structurally dependent on MUC2 and our data suggests that a WD results in rapid bacterial driven disruption of the mucus layer. IBD and CAC rates are high in Western countries and increasing in regions that have adopted a Western lifestyle. The impact of a WD on the microbiota may be the causative link between a Western lifestyle and inflammation driven colonic disease.

W73. Impaired Epithelial Autophagy Exacerbates Chronic Intestinal Inflammation

Johanna Pott, Agnieszka M. Kabat and Kevin Maloy. University of Oxford, Oxford, England, United Kingdom

Inflammatory bowel disease (IBD) is a chronic disorder of the gastrointestinal tract, thought to arise from an imbalanced immune response to the microflora. Intestinal epithelial cells (IECs) form the physical and chemical barrier separating the microflora from the mucosal immune cells and are a key player in maintaining homeostasis as well as modulating the immune response. Recent genome-wide association studies linked polymorphisms in the autophagy genes *ATG16L1* and *IRGM* with susceptibility to inflammatory bowel disease (IBD). We sought to investigate the role of Atg16l1/ autophagy within the intestinal epithelium during chronic colitis. We employed a tissue-specific Atg16l1-deficient transgenic mouse strain in a *Helicobacter hepaticus* driven model of chronic colitis. In parallel, we studied the role of autophagy during the response of IECs towards inflammatory mediators in an *ex vivo* organoid system. Mice lacking Atg16l1 in IECs (Atg16l1$^{\Delta villin}$) were more susceptible to chronic colitis. Elevated levels of epithelial apoptosis accompanied severely aggravated pathology in Atg16l1$^{\Delta villin}$ mice. *Ex vivo* analysis of IECs revealed increased sensitivity towards cytokine induced apoptosis when autophagy was impaired. These findings suggest that the IBD susceptibility gene *ATG16L1* and more generally the process of autophagy protects the epithelium from inflammation induced cell death and therby controls chronic intestinal inflammation.

W74. Chronic TNFα-driven Inflammation Supresses Epithelial Cell Dynamics in the Small Intestine in a Region-Specific Manner

Aimée Parker[1], Daniele Muraro[2], Laura Vaux[1], Angela M. Patterson[1], Amisha Modasia[3], Alexander G. Fletcher[4], Helen M. Byrne[2], Philip K. Maini[2], Alastair Watson[1] and Carmen Pin[1]. [1]Institute of Food Research, Norwich, England, United Kingdom; [2]University of Oxford, England, United Kingdom; [3]University of East Anglia, Norwich, England, United Kingdom; [4]University of Sheffield, Sheffield, England, United Kingdom

The functional integrity of the intestinal epithelial barrier relies on tight coordination of cell proliferation and migration, with failure to regulate these processes resulting in disease. Increased shedding of villus tip cells is particularly associated with coeliac disease and inflammatory bowel diseases, while altered crypt cell proliferation can drive carcinogenesis. Previously, we have shown that villus epithelial migration is primarily governed by crypt proliferation rates, both during homeostasis and in mouse models of impaired or blocked proliferation. However, it is unclear how epithelial turnover is altered in long-term inflammatory conditions. Using fluorescent reporter 'confetti' mice and thymidine-analog cell labelling, combined with tailored mathematical models, we have quantified the proliferative and migratory responses to both acute and chronic TNFα-driven intestinal inflammation. Acute inflammation was induced by single delivery of LPS or TNFa, with chronic long-term inflammation achieved by hydrodynamic delivery of TNFa-expressing plasmid or subcutaneous osmotic minipump. While acute, short-term inflammation resulted in intense cell destruction at villus tips, followed by rapid recovery, longer-term chronic inflammation resulted in increased cell death along the crypt-villus axis, leading tor educed net crypt cell production and migration rates. Villus atrophy, seen as an overall reduction in villus height and epithelial cell number, was maintained overtime during the chronic injury. These effects on epithelial dynamics were most pronounced in the proximal rather than distal small intestine, reflecting a role for region-specific environmental factors in regulating epithelial responses to inflammation.

W75. Effects of Butyrate on Human Intestinal Epithelium; Influence of Inflammation

Elena Ferrer-Picón[1], Isabella Dotti[1], Ana Corraliza[1], Miriam Esteller[1], Anna Carrasco[2], Maria Carme Masamunt[2], Maria Esteve[2], Julián Panés[3] and Azucena Salas[1]. [1]IDIBAPS, Barcelona, Catalonia, Spain; [2]Hospital Universitari Mutua Terrassa, Barcelona, Catalonia, Spain; [3]Hospital Clinic de Barcelona, Barcelona, Catalonia, Spain

Crohn's Disease and Ulcerative Colitis are chronic inflammatory bowel diseases (IBD) of unknown etiology. In IBD a decrease in the abundance of bacteria involved in short-chain fatty acid (SCFA) metabolism is observed. Besides acting as energy sources for colonocytes, SCFAs (mostly butyrate, acetate and propionate) impact cell cycle and immune function. Our aim was to investigate the effects of butyrate on primary human intestinal epithelium in the context of inflammation. Epithelial organoid cultures (EpOCs) were expanded from human colonic crypts. EpOCs were then induced to differentiate (d-EpOCs). d-EpOCs were stimulated with 5mM butyrate for 24h and total RNA was extracted for transcriptional analysis. The impact of intestinal inflammation on the expression of the butyrate receptors SLC16A1 and ACADS, involved in butyrate metabolism, was explored in d-EpOCs stimulated with TNFα and IBD derived d-EpOCs. Butyrate significantly regulated 4,102 genes in d-EpOCs. Butyrate negatively regulated proliferation and the cell cycle, induced a protective response to oxidative stress (i.e., upregulation of MT1X and CAT) and regulated genes related to the immune response (i.e., CXCL10, CXCL1). TNFα reduced transcription of SLC16A1. Its expression was also decreased in IBD-derived d-EpOCs compared to controls. We demonstrated that butyrate stimulation affects several signaling pathways in human epithelium. However, EpOCs generated from IBD patients, as well as EpOCs that have been exposed to TNFα, present decreased expression of SLC16A1. This suggests that regardless of the availability of butyrate, IBD patients may suffer an impaired ability to uptake and metabolize it.

W76. Human Neutrophil Elastase Degrades the Therapeutic Monoclonal Antibodies Effective in IBD

Renata Curciarello[1,2,3], Paolo Giuffrida[2], Klaartje Kok[1], Guillermo H. Docena[3], Antonio Di Sabatino[5] and Thomas T. MacDonald[1]. [1]Queen Mary University of London [2]San Matteo Hospital, University of Pavia, Pavia, Lombardia, Italy; [3]CONICET y Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina

Therapeutic monoclonal antibodies which are effective in other inflammatory diseases such as rheumatoid arthritis are less effective in inflammatory bowel disease (IBD). Human Neutrophil Elastase (HNE) is highly expressed in IBD mucosa, especially ulcerative colitis (UC). The aim of this study was to determine if HNE degrades biologics, rendering them ineffective, and whether its action can be reversed by its natural inhibitor, elafin. Biologics (Infliximab, Adalumimab, Etanercept) were digested using different concentrations of recombinant HNE in the absence or presence of elafin overnight. Neutrophils where isolated from human blood (UC patients and HC patients) by gradient centrifugation with Na-Dextran solution. Neutrophils where lysed and elastase activity was quantified. Antibody integrity after recombinant or natural HNE digestion was then analysed by western blot, and the functional capacity of the antibodies to neutralise TNF-alpha was tested using recombinant TNF-alpha and a TNFR reporter cell line. Recombinant and HNE from blood cells fully degrades all anti-TNF-alpha agents in a dose-dependent manner. This activity is inhibited by recombinant elafin. Treatment with HNE also partially prevented the ability of the biologicals to inhibit TNF-alpha bioactivity. These results may explain some of the reasons for primary non-responsiveness to anti-TNF-alpha therapy in IBD.

W77. Isolation and Modulation of Pathogen-Specific T cells from Patients with Inflammatory Bowel Diseases

Cecilia Isabel Muglia[1], Renata Curciarello[1,2], Karina Canziani[1], María de los Angeles Serradell[3], Agustina Errea[1], Martín Rumbo[1], Ayelén Hugo[1], Andrés Rocca[4], Santiago Brayer[4], Alicia Sambuelli[4], Martín Yantorno[5], Gustavo Correa[5], Néstor Chopita[5] and Guillermo H. Docena[1]. [1]CONICET y Universidad Nacional de La Plata, Tolosa- La Plata, Buenos Aires, Argentina; [2]Queen Mary University of London [3]Universidad Nacional de La Plata, La Plata, Argentina [4]Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina; [5]Hospital Interzonal General de Agudos General José de San Martín, La Plata, Buenos Aires, Argentina

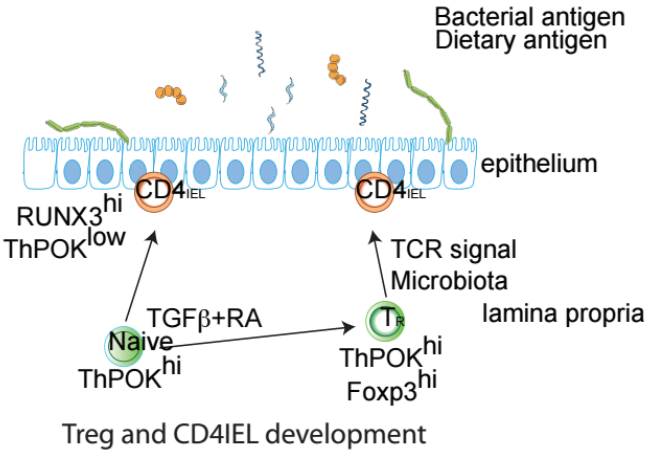
Lamina propria T cells (LPTC) are key cells in inflammatory Bowel Disease (IBD) pathogenesis, contributing to mucosal inflammation by pro-inflammatory cytokines secretion and apoptosis resistance. Kefir is a fermented milk with health-promoting properties. We have isolated and established primary cultures of LPTC from IBD patients and modulated their pro-inflammatory response using microorganisms from kefir. Colonic biopsies or colonic mucosa from surgical specimens from IBD patients (N=12, 5 CD and 7 CU) were washed in HBSS buffer with EDTA and DTT, and digested with collagenase and DNAse. In order to enrich pathogen-specific LPTC, cells were cultured with extracts of enteroadhesive (EA) *Escherichia coli* and IL-2 for 10 days. Microorganisms from kefir (*Lactobacillus kefiri* and *Enterococcus durans*) were evaluated for their modulation capacity on LPTC, by proliferation assays (CFSE) and cytokine secretion (TNF, IL-6, IL-8 and IL-10 by ELISA) of stimulated LPTC (anti-CD3/anti-CD28 and probiotic bacteria, conditioned media or 10 µM lactate stimuli). LPTC lines specific for EA *E. coli* were developed for all patients. Cell proliferation of activated lymphocytes decreased with *L. kefiri* and *E. durans* (proliferation index: 3.0±0.5 vs 0.9±0.3 and 0.56±0.3 respectively; unstimulated control: 1.0±0.1). TNF, IL-6 and IL-8 secretion was decreased in activated LPTC incubated with *L. kefiri* compared to medium while IL-6 and IL-8 diminished with *E. durans* ($P<0.05$). Intermediate results were found for lactate and conditioned media. No significant differences were observed for IL-10. Our results show that probiotic strains modulate

pathogen specific activated T cells from IBD patients. These findings could contribute to future therapies for IBD.

W78. Tissue Adaptation of Regulatory and Intraepithelial CD4$^+$ T Cell Lineages Controls Gut Inflammation

Tomohisa Sujino[1], Maria London[2], David Hoytema van Konijnenburg[2], Tomiko Rendon[2], Bernardo Reis[2] and Daniel Mucida[2]. [1]Keio University, School of Medicine, Shinjyuku, Tokyo, Japan; [2]Rockefeller University, New York, NY

Foxp3-expressing regulatory T cells (T_{regs}) are instrumental in the control of inflammatory responses. Within the intestinal wall, constantly exposed to dietary and microbial stimulation, CD4$^+$ CD8aa$^+$ intraepithelial lymphocytes ($CD4_{IELs}$) also exhibit anti-inflammatory properties. Both peripheral T_{regs} (pT_{regs}) and $CD4_{IELs}$, depend on similar environmental cues for differentiation, namely TGF-β, retinoic acid (RA) and microbiota-derived signals. However, pTregs are found primarily in the intestinal *lamina propria*, while CD4$^+$ CD8aa$^+$ T cells are located within the epithelial layer. pT_{regs} highly express Thpok (CD4 transcriptional factor). On the other hand, $CD4_{IELs}$ express low level of Thpok but express Runx3 (CD8 transcriptional factor). We asked how the intestinal environment segregates pT_{regs} and $CD4_{IELs}$, the transcriptional factor involved in this regulation and how this balance affects gut inflammation. Using IVM and a combination of fate-mapping, we investigated the fate decision between Tregs and CD4-IELs and the inflammatory outcomes. We also used induced diarrhea models by feeding dietary antigen to T_{regs} and $CD4_{IELs}$ depletion mouse. We showed distinct cell dynamics of intestinal T_{regs} and $CD4_{IELs}$, which correlate with high or low expression of the transcription factor Thpok, respectively. Upon migration to the epithelium, T_{regs} lose Foxp3 and convert to $CD4_{IELs}$ in a microbiota-dependent fashion, an effect attributed to the loss of Thpok. Finally, we demonstrate that pT_{regs} and $CD4_{IELs}$ perform complementary roles in the regulation of intestinal inflammation. These results reveal intra-tissue specialization of anti-inflammatory T cells shaped by discrete niches of the intestine.



Treg and CD4IEL development

W79. The Activation and Not the Frequency of Neutrophils Correlates with Endoscopic Severity in Adult Crohn's Disease Patients

Amelie Therrien, Laurence Chapuy, Marwa Bsat, Manuel Rubio Trujillo, Gabriel Bernard, Mickael Bouin and Marie Sarfati. Centre Hospitalier de l'Universite de Montreal, Montreal, PQ, Canada

The correlation between the frequency and activation of neutrophils, and endoscopic severity remains unclear in adult Crohn's disease (CD). We evaluated 47 colonic or ileocolonic CD patients, including 12 patients in remission (Simple Endoscopic Score for Crohn's Disease (SES-CD) ≤ 2). Peripheral blood cells were submitted to red blood cells lysis and colonic tissue to mechanic and enzymatic digestion. Blood neutrophils (BoN) and colonic mucosa neutrophils (MuN) were quantified by flow cytometry. We evaluated the frequencies of neutrophils and their activation status (mean fluorescence intensities (MFI) of CD66b and CD64 expression) in relation to SES-CD at the time of tissue sampling (Table 1). We here show that: (1) The frequencies of BoN and MuN were not correlated with SES-CD. (2) CD64 and not CD66b expression on BoN correlated with SES-CD. This correlation was only present in cohorts under treatment (non-biologic and biologic drugs). (3) CD66b and not CD64 expression on MuN correlated with SES-CD, independantly of treatment history. Furthermore, these correlations were also observed in patients with SES-CD>2 (n=35) and more pronounced in patients with exclusive colonic disease (n=26), in blood and mucosa. We conclude that CD64 expression on BoN appears as a good indicator of disease severity in CD patients under treatment, extending recent studies on

pediatric CD patients. Because CD66b expression on MuN and not frequencies of neutrophils correlated with disease severity regardless of treatment history, we propose that activation rather than recruitment of neutrophils in the colon contributes to immunopathogenesis of CD.

Table 1 : Correlation between SES-CD and expression of CD64 on BoN and CD66b on MuN

	MFI CD64 on BoN	MFI CD66b on MuN
All cohort (n=47)*	r = 0.50 p = 0.0003	r = 0.67 p < 0.0001
All active CD patients (n = 35)*	r = 0.49 p = 0.0027	r = 0.51 p = 0.0016
Colonic disease only (n=26)*	r = 0.58 p = 0.0019	r = 0.77 p < 0.0001
No recent treatment (n=9) &	r = -0.26 p = NS	r = 0.89 p = 0.0030
Non biologic treatment (n=16)&	r = 0.69 p = 0.0040	r = 0.79 p = 0.0005
Biologic treatment (n=14)&	r = 0.71 p = 0.0058	r = 0.67 p = 0.0100

* Pearson correlation coefficients
& Spearman rank correlation coefficients

W82. Simultaneous Microbiota-Reactive CD4 T Cell Stimulation and mTORC Inhibition Serve as a Potential Immunotherapy for the Inflammatory Bowel Diseases

Qing Zhao and Charles O. Elson. University of Alabama at Birmingham, Birmingham, AL

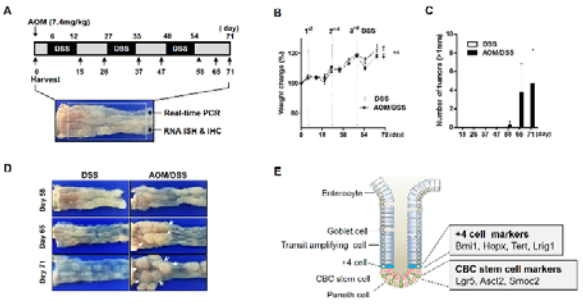
The current paradigm of IBD pathogenesis is that it results from a dysregulated CD4 T cell response to microbiota antigens in genetically-susceptible hosts. CD4 T memory (T_M) cells contribute to the chronicity of the disease. Microbiota-flagellin specific T_M cells are generated during intestinal inflammation and can potentially serve as a reservoir for pathogenic CD4 T effector (T_E) cells during the relapse of disease. T_M cells have a low rate of metabolism until they are re-activated with cognate antigen. The profound metabolic transition from quiescent T_M to expanding T_E requires activation of the mammalian target of rapamycin complex (mTORC). Inhibition of mTORC leads to T_E cell death and anergy, and favors the induction of regulatory T (Treg) cells. We hypothesized that simultaneous T cell receptor (TCR) stimulation and inhibition of mTORC will result in the ablation of microbiota-reactive T_M cells and an altered ratio of T_E/Treg, thus preventing colitis. Using an adoptive T cell transfer model, we found that parenteral immunization induced CD4 T_M response to microbiota CBir1 flagellin in peripheral lymphoid tissues. Simultaneous treatment with mTORC inhibitor rapamycin during immunization significantly dampened CD4 T_M response formation and increased Treg/T_E ratio in an antigen-specific manner. Rapamycin also successfully prevented the development of intestinal inflammation in a CBir1 transgenic CD4 T cell transfer colitis model, decreasing CBir1-specific CD4 T cells and their differentiation into Th1 and Th17 cells in the gut. Thus, we conclude this approach has promise as an immunotherapy for the prevention and/or treatment of IBD.

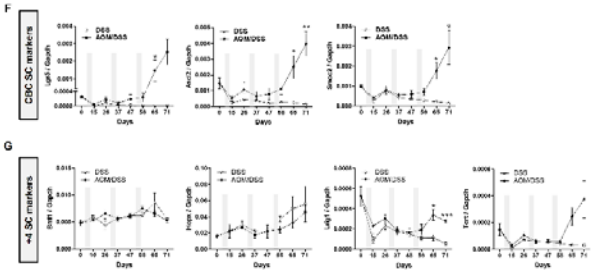
W83. Glucocorticoids Impair Phagocytosis and Inflammatory Response on Macrophages Infected with a Crohn's Disease-Associated Pathogenic Bacteria

Mauricio Javier Olivares[1], Rocio Gutiérrez[1], Isidora Chahuan[1], Alejandro Torres-Riquelme[1], Daniela Parada[1], Karen Dubois[1], Marjorie De la Fuente Lopez[1], Xiaojiang Xu[2], Rodrigo Quera[3], Carolina Figueroa[3], David Diaz-Jimenez[1], John A Cidlowski[2], Roberto Vidal[1] and Marcela A. Hermoso-R[1]. [1]Universidad de Chile, Providencia, Santiago, Region Metropolitana, Chile; [2]National Institute of Environmental Health Sciences, Research Triangle Park, NC; [3]Clinica Las Condes, Santiago, Region Metropolitana, Chili

Glucocorticoids (GC) are a common treatment for inflammatory disorders; however, prolonged use can predispose patients to bacterial infection. Studies addressing GC effects on bactericidal and inflammatory activity of infected macrophages show contradictory results. Here, we examined GC-treated macrophages and their interaction with adherent-invasive *E. coli* (AIEC) strain, CD2-a, a pathogen-associated to Crohn´s disease and isolated from intestinal mucosa, and capable of surviving phagocytosis. Aim: To determine GC effects on bactericidal and inflammatory activity of macrophages infected with CD2-a. THP-1 cells-derived macrophages were infected with CD2-a in the presence or absence of dexamethasone (Dex), and a mRNA microarray was performed. Differentially expressed mRNAs were confirmed by TaqMan-qPCR. Amikacine-protection assay was used to evaluate phagocytic and bactericidal activity of Dex-treated macrophages and infected with *E. coli* strains (CD2-a, HM605, NRG857c, HB101). Cytokine secretion and inflammatory phenotype of macrophages were evaluated by ELISA and flow cytometry, respectively. Results: Microarray analysis showed that CD2-a, Dex and CD2-a+Dex have differential inflammatory genes profiles, many with unique expression pattern in CD2-a+Dex condition. Canonical pathway analysis showed a decreased phagocytosis signaling on Dex-treated macrophages, and an anti-inflammatory polarization on CD2-a + Dex macrophages. Amikacine protection assay showed

reduced phagocytosis ability by Dex. TaqMan-qPCR confirmed Dex inhibition of three phagocytosis-associated genes. All bacteria strains induced TNF-α, IL-6, IL-23, CD40 and CD80 levels that were inhibited by Dex. Conclusions: GC-induced decreased phagocytosis and an anti-inflammatory polarization upon *E. coli* macrophage infection suggesting that AIEC infected patients under this treatment could have an impaired bacterial clearance.

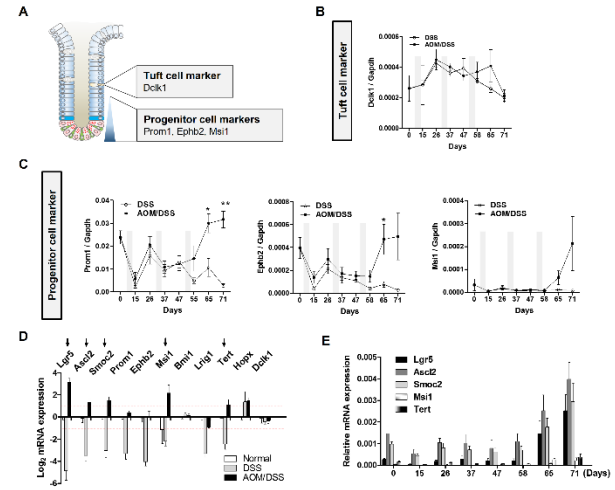


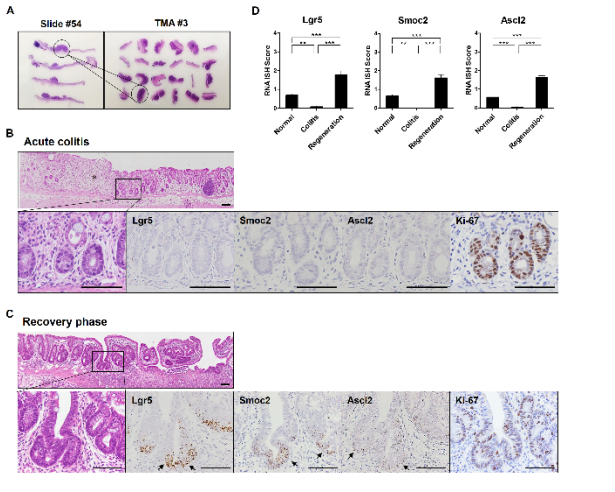


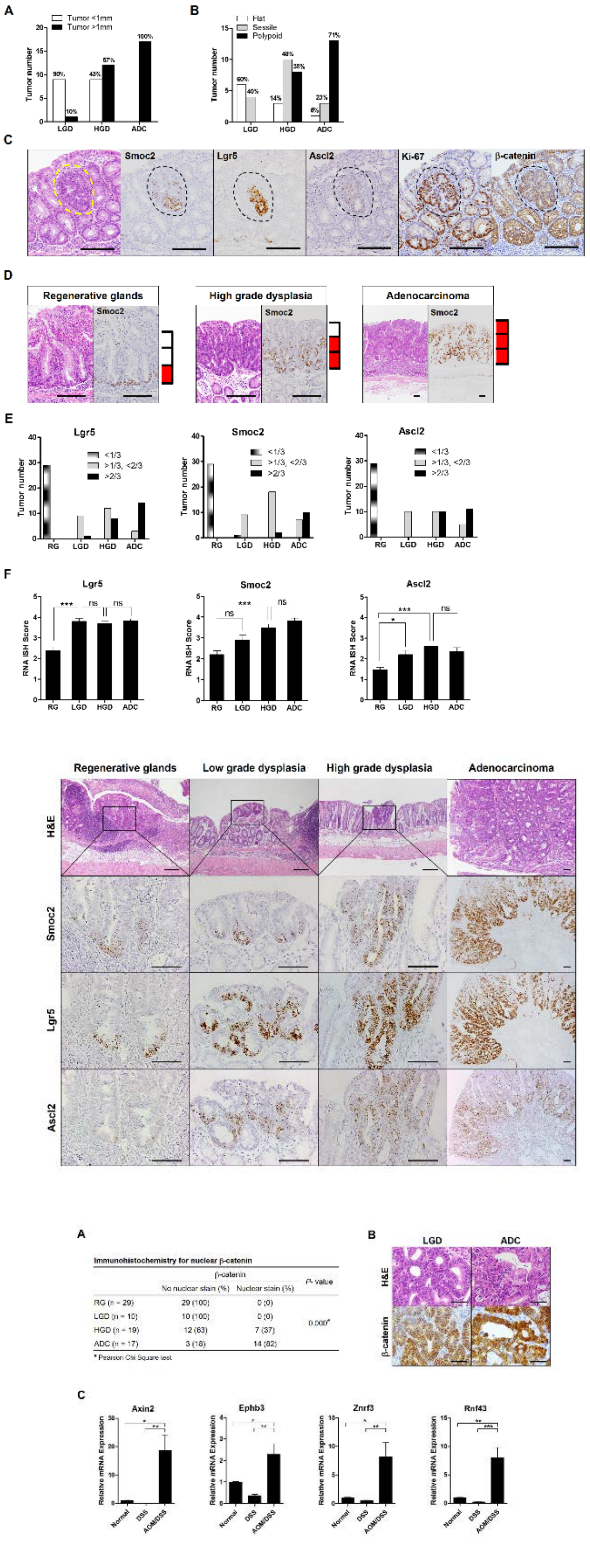
W85. Expression Profile of Intestinal Stem Cell Markers in Colitis-Associated Carcinogenesis

Bogun Jang, Hyesung Kim and Younghee Maeng. Jeju National University, Jejusi, Republic of Korea

The intestinal epithelium has two distinct two stem cell populations, namely, crypt base columnar (CBC) cells and $^{+}$4 cells. Several specific markers have been identified for each stem cell population. In this study, we examined the expression profiles of these markers in colitis-associated carcinogenesis (CAC) to investigate whether they can be used as biomarkers for the early detection of dysplasia. The expression of intestinal stem cell (ISC) markers was measured by real-time polymerase chain reaction during CAC that was induced by azoxymethane and dextran sodium sulfate treatment. CBC stem cell markers increased continuously with tumor development, whereas a $^{+}$4 cell expression profile was not present. CBC stem cell population was suppressed in the acute colitis and then expanded to repopulate the crypts during the regeneration period. Notably, RNA *in situ* hybridization revealed that all dysplasia and cancer samples showed increased expression of CBC stem cell markers in more than one-third of the tumor height, whereas regenerative glands had CBC stem cell markers confined to the lower one-third of the crypt. These results suggest that CBC stem cell markers could be a useful tool for the early detection of colitis-induced tumors.







W86. Persistent *Salmonella enterica serovar typhimurium* Infection Promotes Chronic Intestinal Inflammation in Susceptible Mice

Barbara M. Schultz[1,2], Geraldyne A. Salazar[1,2], Carolina Paduro[1,2], Catalina Pardo-Roa[1,2], Francisco Salazar-Echegarai[1,2], Maria Jose Altamirano[1,2], Javiera Torres[2], Claudia A. Riedel[3], Alexis M. Kalergis[1,2], Manuel M. Álvarez-Lobos[2] and Susan M. Bueno[1,2]. [1]Millennium Institute on Immunology and Immunotherapy, Santiago, Region Metropolitana, Chile; [2]Pontificia Universidad Católica de Chile, Santiago, Region Metropolitana, Chile; [3]Universidad Andrés Bello, Chile, Santiago, Region Metropolitana, Chile

Inflammatory Bowel Diseases (IBD) are chronic intestinal immune disorders that include Crohn's disease and Ulcerative colitis. IBD are the result of an abnormal immune response in susceptible hosts, triggered by a genetic and environmental component. Several reports have described that infection with enteropathogenic bacteria, such as *Salmonella enterica* serovar Typhimurium *(S. typhimurium*), could be a risk factor for these diseases. To evaluate the effect of *S. typhimurium* infection on the onset of IBD, we evaluated two mice model that resemble the characteristics of the disease: the genetic mice model of IL-$10^{-/-}$ mice, which spontaneously develop colitis, and the chemical induction of colitis by Dextran Sulfate Sodium (DSS). Both models were intragastically infected with *S. typhimurium* Wild Type (WT) and treated with antibiotic for 3 weeks. Inflammation of intestine and the persistence of the bacteria in different organs were evaluated at the end of the experiment. We found that mice infected with *S. typhimurium* showed increased levels of inflammation in ileum and colon as compared to non-infected mice. In addition, we detected persistent *Salmonella* infection in different organs of IL-$10^{-/-}$ mice after 42 days of infection, which was not observed in WT mice treated with DSS. Our results suggest that persistent *S. typhimurium* infection promotes chronic inflammation in the intestine that resemble IBD in susceptible hosts, and this chronic inflammation could be related to virulence factors.

W87. Differential T Helper Cell Responses to IL-23 and IL-12 in Colon and Mesenteric Lymph Nodes of Patients with Crohn's Disease and Ulcerative Colitis

Marwa Bsat, Laurence Chapuy, Manuel Rubio Trujillo and Marie Sarfati. University of Montreal, Montreal, Canada, Montreal, PQ, Canada

Crohn's disease (CD) and Ulcerative colitis (UC) are Th17 and Th17/Th1-associated diseases. Hence, IL-23 plays an important role in the pathogenicity of Th17 cells. Ustekinumab, a monoclonal antibody blocking both IL-23 and IL-12 activity, appears to be effective in the treatment of CD patients and is currently in phase III clinical trial for UC patients. We here investigated the $CD4^+$T cell responses to IL-23 and IL-12 in colonic mucosa and mesenteric lymph nodes (mLNs) in CD and UC. Our results indicate that: (1) IL-23 increased the frequencies of IFN-γ^-IL-17^+(Th17) and IFN-γ^+IL-17^+ (Th17/Th1) T cells in the colons of CD, but not UC, patients. (2) Conversely, IL-23 increased the percentage of Th17 and Th17/Th1cells in mLNs of UC, but not CD, patients. (3) IL-12 inhibited the frequencies of Th17 cells in mLNs as well as in mucosa of CD patients only. (4) IL-12 augmented the proportion of single IFN-γ-producing $CD4^+$T cells (Th1 or Th1 ex-Th17) in mLNs and colons of CD and UC patients. Taken together, IL-23 promotes Th17 and Th17/Th1 responses in the colon of CD and in the mLN of UC patients. IL-12 reduced Th17 responses in CD only while it favors Th1 responses in mLNs and colons in both diseases, suggesting a role for IL-12 in the plasticity of Th17 responses. To conclude, these data provide evidence for a differential mode of action at distinct sites in the potential therapeutic efficacy of Ustekinumab in patients with CD and UC.

W131. Inflammatory Bowel Disease Drug Modulation of Autophagy: An Investigation in Pediatric Crohn's Disease

Kirsty M. Hooper[1], Paul Henderson[2], Peter G. Barlow[1], Craig Stevens[1]. [1]Edinburgh Napier University, Edinburgh, United Kingdom, [2]Royal Hospital for Sick Children, Edinburgh, United Kingdom

Crohn's disease (CD), one of the main forms of Inflammatory Bowel Disease (IBD), is a complex disorder characterised by chronic inflammation of the gastrointestinal tract. The pathogenesis of CD is unclear however it involves a combination of genetics and environmental factors that trigger an abnormal immune response to bacteria in the intestines. Genome-wide association studies have strongly linked genes involved in the autophagy pathway to CD. Autophagy is a cellular degradation process that destroys intracellular bacteria and regulates the extent of inflammatory responses. Recent studies suggest that increasing autophagy in CD patients may be therapeutically beneficial. Here we have characterised currently used IBD drugs in the context of autophagy. A better understanding of their mechanism of action is important due to high cost and difficulty associated with development of new drugs. We have characterised the response to IBD drugs by monitoring the autophagy marker LC3 using several complimentary methods including live-cell imaging, flow cytometry and western immunoblotting. Our results show the immunosuppressant azathioprine is a strong inducer of autophagy. We also show that azathioprine induces autophagy in part through inhibition of the mTORC1 pathway. Further, using the Human Autophagy RT² Profiler, we assessed changes in autophagy signalling genes, and among genes up-regulated by azathioprine is PKR-like ER kinase (PERK), which initiates the unfolded protein response. Key results from cell lines are replicated in cells and biopsies isolated from paediatric CD patients and results correlated with genotypic and phenotypic information. This study may contribute to development of personalised treatments for CD.

Inflammatory Bowel Disease – Clinical

OR.69, T72. T Cell Receptor Repertoire Analysis Predicts Post-Operative Recurrence in Crohn's Disease: A Study from the REMIND Group.

Lionel Le Bourhis[1], Claire Auzolle[2], Marjolaine NGollo[1], Hugo Bottois[1], Victor Chardiny[1], Fayçal Djenidi[1], Carmen Stephanescu[3], Stephane Nancey[4], Anthony Buisson[5], Harry Sokol[6], Xavier Treton[3], Nicolas Barnich[5], Philippe Seksik[6] and Matthieu Allez[2].
[1]INSERM UMRS 1160, Paris Diderot, Sorbonne Paris-Cité University, Paris, France; [2]Hôpital Saint-Louis, Paris, France; [3]Hôpital Beaujon, Paris, France; [4]Centre Hospitalier Lyon-Sud, Lyon, France; [5]Centre Hospitalier Clermont-Ferrand, Auvergne, France; [6]Hôpital Saint-Antoine, Paris, France

Persistent T cell clonal expansions are present in the inflamed mucosa of patients with Crohn's Disease (CD) and could impact disease recurrence after surgery. T cells in the mucosa were analyzed by T Cell Receptor (TCR) repertoire sequencing at time of surgery and six months later. The TCR repertoire displayed a high variety of T cell specificities and measures of diversity of the TCR repertoire showed an important range of clonality within the cohort. Importantly, high frequency clones in the surgical specimen were significantly increased in patients presenting 6 months later a post-operative recurrence compared to non-recurring individuals. Furthermore, the clones present at time of surgery were significantly more persistent overtime in patients presenting a recurrence of the disease. High or low clonality could define two subgroups of patients. Transcriptional analysis of high clonality patients presented a CD8 T cell signature compared to a predominant CD4 T cell signature in low clonality patients. Accordingly, expanded clones could be found predominantly in sorted CD8 T cell subsets. In conclusion, high frequency of T cell clonal expansions in the tissue at time of surgery is correlated with increase risk of endoscopic recurrence. Persistent CD8 T cell clones are present in the mucosa of CD patients and are associated with disease progression.

PR.02, T70. Oncostatin M Promotes Intestinal Inflammation by Activating Gut-Resident Stromal Cells and Predicts anti-TNF Refractory Inflammatory Bowel Disease

Nathaniel West, Ahmed Hegazy, Benjamin Owens, Samuel Bullers, Simon Travis and Fiona Powrie.
University of Oxford, England, United Kingdom

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are complex chronic inflammatory conditions of the gastrointestinal tract that are driven by perturbed cytokine pathways. Anti-tumour necrosis factor-α (TNF) antibodies are a mainstay therapeutic approach for IBD. However, up to 40% of patients are non-responsive to anti-TNF agents, and identifying alternative therapeutic targets is a priority. Here we show that expression of the cytokine Oncostatin M (OSM) and its receptor (OSMR) is increased in the inflamed intestine of IBD patients compared to healthy control tissue**,** and correlates closely with histopathological disease severity. OSMR is expressed primarily in non-hematopoietic, non-epithelial intestinal stromal cells, which respond to OSM by producing a range of pro-inflammatory factors including interleukin-6, the leukocyte adhesion factor ICAM-1, and chemokines that attract neutrophils, monocytes, and T cells. In an animal model of anti-TNF refractory intestinal inflammation, genetic deletion, or pharmacological blockade of OSM significantly attenuates colitis. Furthermore, high pre-treatment OSM expression is strongly associated with failure of anti-TNF therapy based on analysis of over 200 IBD patients, including two cohorts from pivotal phase 3 clinical trials. OSM is thus a potential biomarker and therapeutic target for IBD, with particular relevance for anti-TNF refractory patients.

T32. Genetic Architecture of Monogenic Intestinal Disorders in a Large European Cohort of 215 Patients by Targeted Next-Generation Sequencing

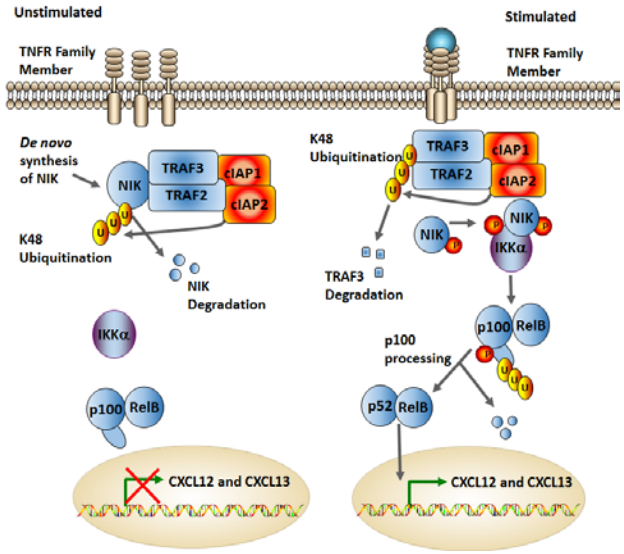
Fabienne Charbit-Henrion[1,2], Marianna Parlato[1], Sylvain Hanein[1], Bernadette Begue[1], Rémi Duclaux-Loras[1], Sabine Rakotobe[1], Jan Nowak[1,3], Julie Bruneau[2], Cécile Fourrage[1,4], Olivier Alibeu[1], Frédéric Rieux-Laucat[1], Eva Levy[1], Marie-Claude Stolzenberg[1], Fabienne Mazerolles[1], Sylvain Latour[1], Christelle Lenoir[1], Alain Fischer[1], Capucine Picard[1,2,5], Genius Group[6], Frank M Ruemmele[1] and Nadine Cerf-Bensussan[1]. [1]Institut Imagine, INSERM UMR 1163, Paris, Ile-de-France, France; [2]Hôpital Necker-Enfants Malades, Paris, Ile-de-France, France; [3]Poznan University of Medical Sciences, Poznan, Poland; [4]Université Paris-Descartes-Paris Sorbonne, Paris, Ile-de-France, France; [5]Investigation Center for Immunodeficiency, Paris, Ile-de-France, France; [6]GENIUS Group, Paris, Ile-de-France, France

Mendelian mutations causing rare but very severe chronic intestinal diseases are identified in an increasing number of genes expressed in hematopoietic immune cells, in epithelial cells or in both. With the goal to facilitate molecular diagnosis, we have set up a custom-made TNGS panel enabling to sequence at once the exons of 66 genes in which mutations causing monogenic intestinal diseases have been reported before June 2015. This tool was used between August 2015 and April 2016 to screen 170 patients without genetic diagnosis in a large cohort of 215 patients recruited between 2009 and 2015 for suspicion of monogenic intestinal disorders. Causative mutations were identified in 56/164 (34%) patients screened by combining functional screening, WES and TNGS, and in 15/51 patients (29.4%) only screened by TNGS. Overall, NGS allows to dissect the spectrum of genes associated to intestinal disorders in our cohort identifying mutations in *CTLA4* (n=2), *EPCAM* (n=1), *FOXP3* (n=4), *IL10R1* (n=1), *LRBA* (n=3), *MYO5B* (n=2), *NCF1* (n=2), *NEUROG3* (n=1), *SKIV2L* (n=3), *TTC37* (n=1), *TTC7A* (n=1), and *XIAP* (n=4). Our results in a large European cohort not only confirm that TNGS is a robust tool for genetic screening of known molecular intestinal disorders but also provides new insight into the phenotypic and genetic diversity of these rare diseases.

T47. Noncanonical NF-κB Signaling is Significantly Up-Regulated in Inflammatory Bowel Disease Patients and May Function as a Biomarker of Therapeutic Response

Irving Coy Allen, Kristin Eden, Dario R. Sorrentino, Vu Q. Nguyen and Kristin M. Knight. Virginia Tech, Blacksburg, VA

Canonical NF-κB signaling is well-studied in inflammatory bowel disease (IBD). However, there is a paucity of data pertaining to noncanonical NF-κB signaling. To better define the contribution of this signaling cascade in human IBD, we collected biopsy specimens from the colon, cecum, or terminal ileum of Crohn's disease, ulcerative colitis, and control patients. For IBD patients, tissue was obtained from both lesion areas and unaffected tissue. These patients were further divided into subgroups based on treatment with infliximab. To assess the activation of pathways associated with inflammation, we utilized RT-PCR based gene expression arrays. Here, we define several genes associated with non-canonical NF-κB signaling that are significantly up-regulated in IBD lesions. Furthermore, we identified a strong positive correlation between increased non-canonical NF-κB signaling and Crohn's disease. We identified 20 genes associated with the noncanoincal NF-κB pathway that are significantly up-regulated in the Crohn's disease patient population. Subsequent analyses revealed that infliximab treatment resulted in significant repression non-canonical NF-κB signaling in Crohn's disease patients, while patients using other therapeutic methods or no treatment continued to have high expression levels. Subsequent bioinformatics analysis revealed that elevation of a panel of chemokines associated with noncanonical NF-κB signaling may be indicators of therapeutic responsiveness, with patients' refractory to infliximab demonstrating significantly increased gene expression. Given the unique expression pattern of key noncanonical NF-κB effector molecules in patients treated with infliximab, it is interesting to speculate that hyperactivation of this pathway may serve as a biomarker of disease activity or predict treatment response.



T73. Bacteroidetes Plays Crucial Role During Combination Therapy of Fecal Microbial Transplantation and Antibiotics for Ulcerative Colitis

Dai Ishikawa[1], Taro Osada[2], Masamichi Takahashi[1], Keiichi Haga[1], Tomoyoshi Shibuya[1] and Sumio Watanabe[1]. [1]Juntendo University Hospital, Tokyo, Japan; [2]Juntendo Urayasu Hospital, Urayasu, Chiba, Japan

The high efficacy of fecal microbiota transplantation (FMT) in therapy-refractory *Clostridium difficile* infection. However, the efficacy of FMT in ulcerative colitis (UC) remains controversial. We previously reported that FMT following the multiple antibiotic therapy (AFM: amoxicillin, fosfomycin and metronidazole) synergistically contributes to the recovery of the phylum *Bacteroidetes* composition, which is associated with high clinical response for UC. Here, we constructed further additional microbial analyses at the species level to confirm whether *Bacteroidetes* species from donors actually colonized and provided therapeutic effects. AFM therapy was administered to patients with UC for 2 weeks until 2 days before FMT. Donor fecal samples were collected on the day of administration and transferred via colonoscopy within 6 h. Microbiome analysis at the species level was performed by a method based on *Bacteroidetes hsp60* sequences using Miseq. We found that dysbiosis in UC was involved the loss of species diversity among *Bacteroidetes* (Simpson's diversity index; UC vs healthy donors, p = .0001). Moreover, in responders, the diversity significantly recovered up to donor's level at 4weeks after FMT via transplantation of *Bacteroidetes* cells as supported by the high similarities of bacterial compositions among patients and their donors. Eradiation of dysbiotic indigenous *Bacteroidetes* species by AFM pretreatment may promote the entry of *Bacteroidetes* species derived from donors, improving the bacterial composition in UC. The strategy presented in our study served as a basis for further investigations of the mechanisms through which the intestinal microbiota is altered following this therapy.

Innate Lymphoid Cells

OR.06, W97. Isolation, *ex vivo* Expansion, Transduction, and Adoptive Transfer of Murine Bone Marrow-Derived Group 2 Innate Lymphoid Cells

Claudia U. Duerr, Connor Carthy, Regina Cencic, Barbara C. Mindt, Jerry Pelletier and Jörg H. Fritz. McGill University, Montreal, PQ, Canada

Group 2 innate lymphoid cells (ILC2) are key players in innate immune responses but if deregulated trigger type 2 immunopathologies such as asthma and asthma exacerbations. However, the scarcity of ILC2 at steady state and inflamed or infected tissues hinders detailed cellular and molecular studies of ILC2. We therefore aimed at developing an easy and efficient method to isolate and expand murine ILC2 *ex vivo*. Murine ILC2 were isolated and cultured and expanded using a specific and novel protocol. The presented protocol is easy to follow, efficient and reliable and yields in a 500-fold expansion. The resulting *ex vivo*-derived ILC2 population shows the same phenotypic and functional characteristic as *in vivo*-derived ILC2 and can be transduced by viral vectors thus allowing selective manipulation of gene expression. Moreover, ILC2 expanded using our novel protocol are functional *in vivo* when adoptively transferred into *Rag2*$^{-/-}$*IL2rg*$^{-/-}$ deficient mice. The method presented herein to isolate, expand transduce and transfer murine bone marrow-derived ILC2 helps to overcome the challenge of working with this rare cell population. Our newly developed tools allow detailed cellular and molecular studies for further deciphering ILC2 biology.

OR.07, W106. Metabolic Control of Innate Lymphoid Cells at Mucosal Sites

Laurel Monticelli, Anne Laure Flamar, Gregory Putzel and David Artis. Weill Cornell Medicine, New York, NY

The family of innate lymphoid cells (ILCs) are enriched at mucosal barrier sites and can serve both pathologic inflammatory and tissue-protective functions in response to cell-extrinsic environmental factors, such as host-derived cytokines and microbial signals. However, the mechanisms by which cell-intrinsic pathways, including metabolic changes, govern ILC function are largely unknown. Recently we discovered that the amino acid enzyme Arginase 1 (Arg1) was a constitutive hallmark of the ILC2 lineage and that deletion of Arg1 limited ILC2 proliferation by inhibiting polyamine synthesis and reducing glycolytic capacity, thereby preventing development of airway inflammation. However, whether this enzyme has roles outside the ILC2 lineage is unknown. Unexpectedly, we found here that Arg1 expression was remarkably heterogeneous across the different ILC subsets. Further, Arg1 expression in each ILC lineage was dynamically regulated at a tissue-specific level in response to intestinal barrier damage, suggesting a potential role for this enzyme in ILC-mediated inflammation or tissue protection at this mucosal site. Consistent with this, selective genetic deletion of ILC Arg1 during murine models of mucosal injury and infection demonstrated that Arg1 is a novel, global instructor of the ILC family that controls the ability of these innate cells to modulate host immunity and inflammation.

OR.08, W105. Involvement of Innate Lymphoid Cells in the Induction of Obesity

Takaharu Sasaki, Kazuyo Moro, Tetsuya Kubota, Naoto Kubota, Tamotsu Kato, Hiroshi Ohno and Shigeo Koyasu. RIKEN IMS, Yokohama, Kanagawa, Japan

Obesity is a worldwide health problem caused by complex interaction between genetic and environmental factors. In this study, we aimed to elucidate the role of lymphocytes in the induction of obesity. We fed high fat diet (HFD) to wild-type (WT) mice, Rag2$^{-/-}$ mice that lack T, B and NKT cells, and γc$^{-/-}$Rag2$^{-/-}$ mice that lack all lymphocytes including innate lymphoid cells (ILCs). While Rag2$^{-/-}$ mice gained weight similar to WT mice, γc$^{-/-}$Rag2$^{-/-}$ mice were resistant to HFD-induced obesity, indicating that ILCs are involved in the induction of obesity. ILCs are classified into three groups based on their cytokine production patterns corresponding to T helper cells: group 1 ILC producing IFNγ similar to Th1 cells, group 2 ILC (ILC2) producing IL-5 and IL-13 similar to Th2 cells, and group 3 ILC (ILC3) producing IL-17 and IL-22 similar to Th17 cells. HFD-fed IL-15$^{-/-}$Rag2$^{-/-}$ mice which lack NK cells, a member of group 1 ILC became obese similar to Rag2$^{-/-}$ mice, but Cre-

ERT2:Gata3flox/flox mice administrated with 4-OHT that are devoid of ILC2, and RorγtGFP/GFP mice which lack ILC3 were resistant to obesity with severer phenotypes of mice lacking ILC2. Adoptive transfer of small intestinal ILC2 (SI-ILC2) but not white adipose tissue ILC2 (WAT-ILC2) into $\gamma c^{-/-}Rag2^{-/-}$ mice restored their body weight gain upon HFD-feeding, indicating the involvement of SI-ILC2 in the induction of obesity. We are now focusing on the mechanisms underlying the induction of obesity by ILC2 based on the functional difference between SI-ILC2 and WAT-ILC2.

OR.09, W103. Intestinal Commensal Bacteria Mediate Lung Mucosal Immunity and Promote Resistance of Newborn Mice to Infection

Hitesh Deshmukh and Jerilyn Gray. Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Immature mucosal defenses contribute to increased susceptibility of newborn infants to pathogens. Sparse knowledge of age-dependent changes in mucosal immunity has hampered improvements in neonatal morbidity due to infections. Here, we report that exposure of neonatal mice to commensal bacteria immediately after birth is required for a robust host defense against bacterial pneumonia, the leading cause of death in newborn infants. This crucial window was characterized by an abrupt influx of interleukin (IL)-22 producing group 3 innate lymphoid cells ($IL22^+ILC3$) into the lungs of newborn mice. This influx was dependent on sensing of commensal bacteria by intestinal mucosal dendritic cells. Disruption of postnatal commensal colonization or selective depletion of dendritic cells interrupted the migratory program of lung $IL\text{-}22^+ILC3$ and made the newborn mice more susceptible to pneumonia, which was reversed by transfer of commensal bacteria after birth. Thus, the resistance of newborn mice to pneumonia relied on commensal bacteria-directed ILC3-influx into the lungs, which mediated IL-22-dependent host resistance to pneumonia during this developmental window. These data establish that postnatal colonization by intestinal commensal bacteria is pivotal in the development of lung defenses in mice.

OR.10, W104. All Subsets of Innate Lymphoid Cells Migrate in Intestinal Lymph

Verena Kaestele[1], Johannes Mayer[1], Alberto Bravo-Blas[1], Hannah Wessel[1], David Withers[2] and Simon Milling[1]. [1]University of Glasgow, Glasgow, Scotland, United Kingdom; [2] University of Birmingham, Birmingham, England, United Kingdom

Innate Lymphoid Cells (ILCs) play an important role in defense against pathogens by producing effector cytokines within hours after infection. ILCs mainly reside at mucosal sites and we recently provided the first evidence that some intestinal ILCs traffic to mesenteric lymph nodes (MLNs). Here we provide a detailed description of those migratory ILCs, identifying for the first time a rare migratory ILC population in the intestinal lymph of mice. Lymph was obtained by thoracic duct cannulation of intact mice or following previous mesenteric lymphadenectomy. We demonstrate that all subsets of ILCs migrate in lymph of mice, with ILC1s ($T\text{-}bet^+$) and ILC2s ($Gata\text{-}3^+$) being the most frequent. We also show that some ILCs, particularly ILC2s and ILC3s ($Ror\gamma t^+$), do not then exit the MLNs. The majority of ILCs exiting the MLNs are ILC1s. Migratory ILCs also comprise $MHCII^+$ ILCs and express high levels of the proliferation marker Ki-67. Interestingly, there is an accumulation of $T\text{-}bet^+Ror\gamma t^+$ co-expressing ILCs in the colon-draining MLN in *Salmonella typhimurium* infected mice. Despite the infection not increasing the total numbers of migratory ILCs in lymph, we observe a corresponding increase in $T\text{-}bet^+Ror\gamma t^+$ co-expressing ILCs in the lymph of infected mice. Our data clearly demonstrate, for the first time, that inflammation can alter migratory ILC populations. This improved understanding of the characteristics of migratory ILCs is important because it helps to understand how they contribute to the control of immune responses.

PR.01, W94. ILC3 Direct Yap1-Mediated Regeneration of Small Intestinal Crypts after Stem Cell Damage

Mónica Romera-Hernández[1], Julien J. Karrich[1], Natalie Papazian[1], Patricia Aparicio-Domingo[2], Janneke Samsom[1] and Tom Cupedo[1]. [1]Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands; [2]Basel University, Laussane, Vaud, Switzerland

Upon injury, the intestinal barrier is rapidly restored by expansion and differentiation of epithelial stem cells enclosed within the intestinal crypts. Recently, we identified a role for group 3 innate lymphoid cells (ILC3) in crypt regeneration after stem cell damage. ILC3-derived IL-22 controls maintenance of intestinal stem cells (ISC), yet the underlying mechanisms inducing ISC renewal and differentiation remain largely unidentified. Here, using IL-22-deficient mice, IL-22 neutralizing antibodies and Stat3 inhibition we show that IL-22 is dispensable for ISC proliferation and intestinal pathology following stem cell injury. Based on these findings we hypothesised that ILC3 modulate damage-driven stem cell-specific signalling pathways controlling expansion and differentiation of stem cells independently of IL-22. To identify ILC3-regulated ISC responses upon injury we generated ILC3-deficient Lgr5-reporter mice, induced stem cell damage and performed RNA-sequencing of ISC. In the absence of ILC3, ISC fail to activate Yap1 signalling, a critical pathway involved in early stem cell responses upon injury. Moreover, in wildtype mice, pharmacological inhibition of Yap1-TEAD interactions aggravates pathology after stem cell damage. Yap1 is known to transiently reprogram Lgr5 ISC and drive differentiation at the expense of stemness. In ILC3-deficient mice, this reprograming is altered and failure to downregulate Wnt signalling prevents the early boost in differentiation. Together this results in diminished crypt output, defective regeneration, and increased pathology. In sum, our findings reveal a role for ILC3 in controlling the evolutionary conserved Yap1 pathway in crypt regeneration, highlighting a previously unappreciated layer of epithelial regulation by cells of the innate immune system.

W100. Innate Lymphoid Cells are Increased in Induced Sputum of Asthma Patients and Affect the Polarization of Macrophages

Jihyun Kim, Boram Bae, Hyun Seung Lee, Kyoung-hee Sohn, Hye-Ryun Kang and Hye Young Kim. Seoul National University, Seoul, Seoul-t'ukpyolsi, Republic of Korea

Asthma is one of chronic respiratory disease that is induced by heterogeneous factors and has various endotypes. It is well known that Th2 cells produce type 2 cytokines, such as IL-5 and IL-13, enhancing allergic inflammation. In addition to T cells, recent studies using mouse model suggest that innate lymphoid cells play an important role in development of asthma. Type 2 ILC produces type 2 cytokines, like Th2 cells, that promote allergic inflammation in lung. Also, type 3 ILC releases IL-17 and IL-22 in non-allergic asthma model. In human, several studies show that ILC2s are increased in blood, bronchoalveolar fluid and sputum of asthma patients. However, it remains unclear that whether and how ILCs are involved in development of asthma in patients. In this study, we analyzed ILCs using induced sputum from asthmatics. Total numbers of ILCs are increased in the induced sputum of asthma patients compared with healthy controls. Because ILCs produce various cytokines that affect other immune cells, we also evaluate subtypes of macrophages, one of major immune cells in induced sputum. Asthma patients have more classically activated macrophages (M1 macrophages) and alternatively activated macrophages (M2 macrophages) than healthy controls. Moreover, the percentage of ILC2 and M2 macrophages in induced sputum show positive correlation, and the percentage of ILC1 or ILC3 is also positively correlated with the percentage of M1 macrophages. Taken together, our data suggest that ILCs contribute to the development of asthma by regulating the polarization of macrophages in human.

W101. Group 2 Innate Lymphoid Cells Recruit and Activate Airway Macrophages to Facilitate Pulmonary Epithelial Regeneration

Alan M. Copenhaver[1], Rania Dagher[1], Aron Berlin[1], Valerie Besnard[2], Marielle Maret[3], Fatima Hamidi[4], Michel Aubier[5], Roland Kolbeck[1], Marina Pretolani[4] and Alison A. Humbles[1]. [1]MedImmune, Gaithersburg, MD; [2]Université Paris, Paris, Ile-de-France, France; [3]IRCAN Inserm UMR1081-CNRS 7284 UNSA, Universite de Nice, Nice, France, Paris, Ile-de-France, France; [4]Inserm UMR1152 - Paris, Ile-de-France, France; [5]Hôpital Bichat-Claude Bernard, Assistance Publique des Hôpitaux de Paris, Paris, Ile-de-France, France

Group 2 innate lymphoid cells (ILC2s) are a subset of ILCs that are resident in many tissues including the lung. These cells respond to epithelial cytokines and in turn potently produce many type 2 associated cytokines, such as IL-4, IL-5, and IL-13. Beyond cytokine production, ILC2s are thought to contribute to aid tissue repair after damage by producing growth factors such as amphiregulin or by expressing the enzyme arginase-1. Alternatively activated macrophages and epithelial progenitor cells also play imporant roles in directing successful repair after damage, but it is not known whether ILC2s work in concert with either of these cells to mediate effective repair. Using a model of sterile epithelial damage, we investigated the role of ILC2s during the course of epithelial repair and regernation. Lung ILC2s were activated 4-6 days after epithelial damage and produced increased levels of GM-CSF, IL-5, and IL-13. Mice deficient in ILCs (*Rag2*$^{-/-}$/*Il2rg*$^{-/-}$) had reduced numbers of macrophages upon injury and subsequently decreased levels of macrophage-derived growth factors, such as IGF-1 and BRP-39. Reconstitution of ILC2s into *Rag2*$^{-/-}$/*Il2rg*$^{-/-}$ mice restored macrophage numbers and growth factor expression within the lung. These data suggest ILC2s are part of a larger, multicellular circuit required for both recruitment and stimulation of alternatively activated macrophages which initiate the epithelial repair process. Thus, ILC2s can mediate both inflammtion and repair at barrier sites by coordinating the activation state of specific effector cells.

W102. Type 2-Biased Immunity in Neonatal Lungs Driven by IL-33 Activated ILC2s

Catherine A. Steer[1], Itziar Martinez-Gonzalez[1], Maryam Ghaedi[2], Patricia Allinger[3], Laura Mathae[1] and Fumio Takei[1]. [1]British Columbia Cancer Agency, Vancouver, BC, Canada; [2]University of British Columbia, Vancouver, BC, Canada; [3] University of Applied Sciences, Vienna, Austria

Early exposure to common allergens has been linked to the development of airway allergic diseases later in life. However, the mechanism is unclear as our understanding of neonatal lung immunity is limited. The neonatal lung generates predominantly T helper (Th)-2 (type 2) biased responses when exposed to air-born antigens soon after birth. Our lab has characterized group 2 innate lymphoid cells (ILC2s) in mouse lungs that respond to IL-33 released upon inhaled allergen exposure and produce IL-5 and IL-13 resulting in type 2 lung inflammation. Consequently, we investigated whether ILC2s drive neonatal type 2 bias. Mouse lung ILC2s rapidly develop after birth and 10-13 day old (D10 – D13) pups have more ILC2s than adults. These ILC2s are activated due to intracellular IL-13 and IL-5 expression and eosinophil lung infiltration, not observed in ILC2-deficient pups. IL-33-deficient D10 pups had fewer activated ILC2s and little eosinophil lung infiltration, suggesting endogenous IL-33 activated ILC2s to produce type 2 cytokines resulting in type 2-biased lung immunity. ILC2s drive Th2-biased cell differentiation, as intranasal (IN) OVA antigen treatment into D10 OTII pups results in more IL-4^{+}IL-13^{+} CD4^{+} T cells in the lung-draining lymph node after 6 days compared to adult OTII mice. Additionally, upon IN protease allergen papain at D10 and again 4 weeks later, higher numbers of lymph node Th2 cells were present compared to in mice without neonatal papain injection. Therefore, ILC2s may play a role in the neonatal sensitization of individuals, causing the development of severe allergic lung inflammation in adults.

W88. IL-1 Regulates Activation and Plasticity of Group 2 Innate Lymphoid Cells

Yoichiro Ohne[1], Jonathan S. Silver[1], Alan M. Copenhaver[1], LuAnn Thompson-Snipes[2], Brandi L. Cantarel[3], Yong-Jun Liu[4] and Alison A. Humbles[1]. [1]MedImmmune, Gaithersburg, MD; [2] Baylor Scott and White Health / Baylor Research Institute, Dallas, TX; [3]University of Texas, Southwestern Medical Center, Dallas, TX; [4]Sanofi, Paris, France

Group 2 innate lymphoid cells (ILC2s) regulate type 2 immune responses by producing the Th2 cytokines IL-5 and IL-13. ILC2s protect against helminth infection, and also regulate lung tissue repair after influenza infection. Since aberrant activation of these cells is linked to pathogenesis in allergic asthma, atopic dermatosis, and pulmonary fibrosis, understanding the biology of human ILC2s is critical for the development of strategies to exploit their capacity to impact the innate immune response. It has been well accepted that epithelial-derived cytokines IL-33, IL-25 and TSLP play important roles in the regulation of these cells. Here, we show that a proinflammatory cytokine IL-1 plays pivotal roles in human ILC2s function. IL-1α and IL-1β robustly induces proliferation and type 2 cytokine production from ILC2s in culture. Importantly IL-1β also upregulates expression of the receptors for the epithelial-derived cytokines, leading to increased sensitivity of ILC2s to these cytokines. IL-1β also governed ILC2 plasticity via induction of low levels of T-bet and IL-12Rβ2, enabling conversion into an ILC1 phenotype in response to IL-12. Notably, the conversion to ILC1-like cells is accompanied by an atypical chromatin landscape characterized by simultaneous transcriptional accessibility of type 1 and type 2 loci. Thus, we have uncovered a novel role for IL-1 in facilitating the maturation and plasticity of ILC2 cells.

W89. Microbiota-Derived Butyrate Suppresses NKp46^{+}CCR6^{-} Group 3 Innate Lymphoid Cells in the Terminal Ileal Peyer's Patch

Sae-Hae Kim and Yong-Suk Jang. Chonbuk National University, Jeonju, Korea, Jeonju-si, Cholla-bukto, Republic of Korea

Anatomical and physiological distinctions in the gut contribute to regional specialization of intestinal immune system by microbiota. Given that the ileal Peyer's patch (PP) belongs anatomically to the small intestine, while it is physiologically exposed to an environment similar to the large intestine with respect to microbes and microbial metabolites, its characteristics may differ from those in PPs in the jejunum. Among the cell types in PPs, group 3 innate lymphoid cells (ILCs) are closely associated with the regulation of commensal bacteria through the suppression of commensal bacteria-specific CD4^{+} T cells, although the regulation of ILCs in ileal PPs is poorly defined. Recent study identifies three subtypes: a CCR6^{+} cells that promotes lymphoid organogenesis and CCR6^{-}NKp46^{+} cells and CCR6^{-} NKp46^{-} cells are related with protective immunity against microbiota. We here found that butyrate plays a role as a regional factor involved in CCR6^{-}NKp46^{+} ILC3s repression in PPs of the terminal ileum. This butyrate-mediated negative regulation of CCR6^{-} NKp46^{+}ILC3s alleviates the tolerogenic mucosal microenvironment by suppressing regulatory T cells in PPs. Collectively, we conclude that the inhibition of CCR6^{-}NKp46^{+} ILC3s by microbiota-derived butyrate confers the functional ability to induce antigen-specific immunity, and that this network contributes to homeostatic regulation of the mucosal immunity. (This study was supported by the Basic Science Research Program, NRF-2016R1A2B2010096 (Y.-S. Jang) through the National Research Foundation (NRF) funded by the Korean Ministry of Science, ICT & Future Planning.)

W90. Intestinal Organoids Co-Cultures as a Novel System to Study Intestinal Lymphocytes

Joana F. Neves, Geraldine Jowett and Graham M. Lord. King's College London, London, England, United Kingdom

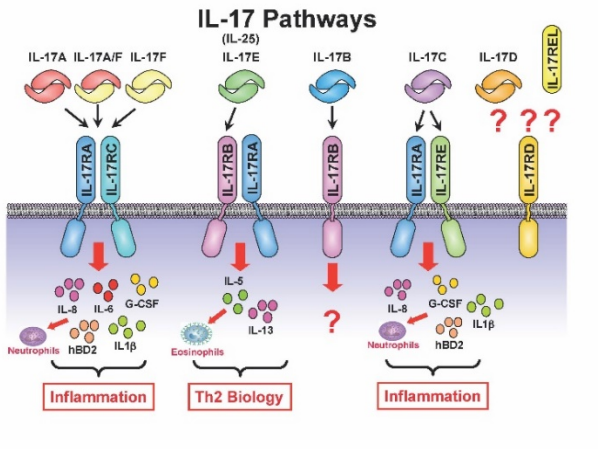
Maintaining intestinal homeostasis depends on interactions between the gut epithelium, the microbiota and the gut-associated immune system; disrupting this delicate balance results in intestinal inflammation, which is a major health problem worldwide, including in Inflammatory Bowel Disease (IBD), patients. Thus, it is important to elucidate the cellular and molecular pathways involved in intestinal homeostasis and inflammation. Innate lymphoid cells ILCs are crucial participants in the defence against infection and in mediating intestinal homeostasis but also inflammation. These tissue-resident cells act rapidly to a threat by producing large amounts of cytokines. The differentiation and functions of ILCs can be modulated by the intestinal environment,

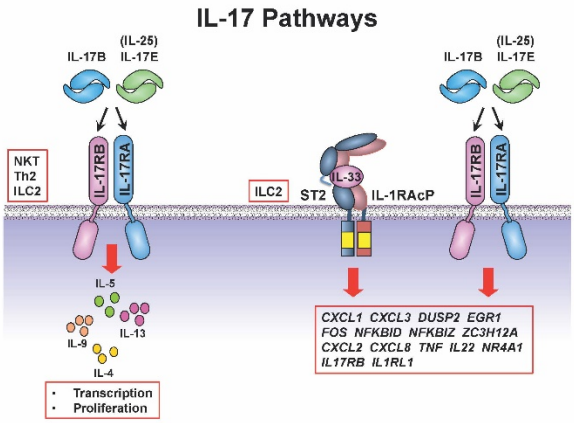
including via interactions with the gut epithelium and the microbiota. Recent data from human studies show the presence of ILC progenitors outside the bone marrow highlighting the importance of studying ILC development and differentiation in different niches. To investigate the cellular and molecular pathways involved in gut homeostasis and inflammation, we have developed a novel *in vitro* system that enables the co-culture of ILCs in intestinal organoids. Intestinal organoids, originally developed by Hans Clevers' group, grow into a three-dimensional structure with defined crypts and villi. Preliminary data show that our model supports the development and differentiation of intestinal ILCs in a reductionist and controlled environment. In addition, the presence of the lymphocytes seems to have beneficial effects for the development and differentiation of the intestinal organoids. Our novel co-culture system is then the first *in vitro* model that allows for the development of ILCs in intestinal conditions.

W91. IL-17B Uses the IL-17RA and IL-17RB to Induce Type-2 Cytokines from Human Immune Cells

Vladimir Ramirez-Carrozzi and Rajita Pappu.
Genentech, South San Francisco, CA

IL-17 family cytokines are critical to leukocyte activation and host defense mechanisms against microbial pathogens. Substantial progress been made in understanding how the IL-17A, IL-17C, IL-17F, and IL-17E (IL-25) members contribute to inflammation, both in terms of biology and receptor specificity. While the IL-17A, IL-17F and IL-17C family members induce overlapping inflammatory cascades that promote neutrophil mediated immunity; IL-25 contributes to Type-2 immune responses and eosinophil activity. We observe analogous properties between IL-17B and IL-25, with both cytokines inducing expression of Type-2 cytokines from human PBMCs. Using blocking reagents to individual IL-17 receptor chains, we demonstrate that like IL-25, IL-17B activity is dependent on the IL-17RB and IL-17RA receptor subunits. These data are consistent with other reports describing IL-17RA as a shared receptor subunit for this cytokine family, and in-vitro data demonstrating a physical interaction between IL-17RB and IL-17B. Receptor profiling revealed that IL-17B targets multiple cell types within the PBMC population including Th2 cells, NKT cells and Group 2 innate lymphoid cells (ILC2s). Here, we demonstrate that IL-17B can directly induce expression of Type-2 cytokines from these cell types. In addition, we show that IL-17B can promote ILC2 proliferation and enhance IL-33 activities in primary human ILC2s. Finally, we characterized the early transcriptional response of ILC2s to IL-17B, IL-25 and IL-33 stimulation. Thus, these data reveal IL-17B is a novel component of the Type-2 immune response poising IL-17B as another IL-17 family member contributing to mucosal immune responses.





W92. The Role of mOASL1 on Innate Lymphoid Cells During Pulmonary Influenza a Virus Infecion

Yuna Chang[1], Ji Sun Kang[2], Young Joon Kim[2] and Hye Young Kim[1]. [1]Seoul National University, Seoul, Seoul-t'ukpyolsi, Republic of Korea; [2]Yonsei University, Seoul, Seoul-t'ukpyolsi, Republic of Korea

Innate lymphoid cells (ILCs) are a group of immune cells that produce cytokines known to be secreted by T cells but stimulated by antigen nonspecific manner. ILCs play important roles in the regulation of tissue homeostasis and immunity especially in mucosal tissues. Oligoadenylate synthetase-like (OASL) is a member of oligoadenylate synthetase (OAS) family, but OASL hasn't catalytic activity because of characteristic changes in the active site. The mouse oasl1 inhibit type I interferon (IFN-I) induction by binding to the 5' UTR of IRF7. Oasl1 deficient mice are resistant to systemic viral infection, but the effects of oasl1 on pulmonary viral infection have not been studied yet. We infected influenza A virus (IAV) to the wild-type (WT) and Oasl1 knockout (KO) mice then analyzed the populations of innate immune cells by flow cytometry. Unexpectedly, Oasl1 KO mice exhibited more intensive lung inflammation following IAV infection and the number of type 2 innate lymphoid cells (ILC2s) was decreased in IAV-infected Oasl1 KO mice. Moreover, type 2 cytokines, such as IL-5 and IL-13, produced by ILC2s were decreased in Oasl1 KO mice in response to IAV infection. On the other hand, dendritic cells (DCs) were increased and displayed enhanced production of interleukin (IL)-1β and tumor necrosis factor (TNF)-α in IAV-infected Oasl1 KO mice. In contrast, there were no differences between WT and Oasl1 KO mice in ovalbumin-induced asthma model. Together, these results demonstrate that Oasl1 deficiency promotes pulmonary inflammation at an early phase of IAV infection and affects the function of ILC2s

W93. Relationship Between ILC3 and Th17 in Nasopharynx-Associated Lymphoid Tissue from Children and Adults and their Responses to Staphylococcal Stimulation

Lualuaa Zaki, Shamsher Ahmed, Neil French, and Qibo Zhang. University of Liverpool, Liverpool, England, United Kingdom

Innate lymphoid cells (ILCs) are increasingly appreciated as being critical in immune homeostasis and inflammation. It has been suggested there is a reciprocal interaction between ILC and T cell response. Current data on the relationship between ILC3 and Th17 in humans are limited. We have studied the frequencies of ILC3 and Th17 in the nasopharynx-associated lymphoid tissue (NALT, adenotonsillar tissue) from children and adults, and their relationships with age. We have studied the relationship between IL-17-producing ILC3 and Th17 induction in NALT following *Staphylococcal aureus* stimulation, and also studied the effect of temporary RORgt inhibition on the ILC3 and Th17 response. ILC3 and Th17 frequencies were analyzed with flow-cytometry following staining for linage markers, CD127, NKP44, c-kit, IL17A and IL-22. Although no significant difference in ILC3 frequency was shown between children and adults, there was an age associated increase in Th17 frequency in NALT. A dose-dependent increase in Th17 induction/response was shown following Staphylococcal stimulation, but that was associated with a dose-dependent decrease in the ILC3 frequency. Temporary blocking of RORgt by the RORgt inhibitor (GSK805) inhibited the Th17 response but not the ILC3 frequency in NALT following *Staphylococcal aureus* stimulation. Our results suggest an important role of ILC3 in regulating Th17-mediated inflammatory response induced by nasopharyngeal pathogens in the human nasopharynx. Further studies are ongoing to interrogate the cellular interactions between ILC3 and Th17 in the context of nasopharyngeal bacterial infection.

W95. Role of ILC2-Derived Interleukin-4 in Immune Responses

Yasutaka Motomura, Shigeo Koyasu and Kazuyo Moro. RIKEN IMS, Yokohama City, Kanagawa, Japan

Interleukin (IL)-4 plays a crucial role in type 2 immune responses such as immunity against helminth infection and allergic responses, through the induction of IgE production from B cells and Th2 differentiation, leading to the initiation of acquired immunity. Group 2 innate lymphoid cells (ILC2s), a new type of innate lymphocyte that we originally reported as natural helper cells, are known to regulate immunity against helminth infection and allergic responses. ILC2s rapidly produce large amounts of IL-5 and IL-13, which are hallmark cytokines of Th2 cells, prior to the acquired immune response, suggesting that ILC2s regulate the initiation of allergic disorders. Although Th2 cells produce IL-4 as well as IL-5 and IL-13 after

TCR stimulation by antigen, ILC2s fail to produce IL-4 even after stimulation with IL-33 or IL-25, which are known to induce production of IL-5 and IL-13 in ILC2s. For this reason, ILC2s are not thought to contribute to IL-4-mediated immune responses. However, we found that the IL-4 gene locus is already open, even in naïve ILC2s, strongly suggesting that ILC2s have the ability to produce IL-4. Further, we identified the physiological condition that induces IL-4 production in ILC2s even in the absence of antigen, which is distinct from that in Th2 cells. Here we propose a perspective on the molecular mechanisms of IL-4 production in ILC2s and the role of ILC2-derived IL-4 during the pathogenesis of allergic disorders.

W96. Dysfunction in Expansion and Effector Function of Group 2 Innate Lymphoid Cells (ILC2s) Activated by Microbiota-Induced IL-33 cContributes to the Pathogenesis in Crohn's-Like Ileitis

Kristine-Ann G. Buela and Theresa T. Pizarro. Case Western Reserve University, Cleveland, OH

Expression of IL-33 and its receptor, ST2, is increased in several autoimmune and inflammatory disorders, including inflammatory bowel disease (IBD). The contribution of IL-33 in acute, primarily chemically-induced models of colitis is contentious, indicating both pathogenic and protective functions. We recently described that IL-33 signaling blockade ameliorates chronic inflammation in ileitis-prone SAMP1/YitFc (SAMP) mice, and that the gut microbiome is essential for IL-33 expression in these mice. To this point, this study aims to determine the role of IL-33-responsive group 2 innate lymphoid cells (ILC2s) to chronic intestinal inflammation in SAMP mice. Our results showed remarkable increases in frequency and absolute numbers of ILC2s within draining mesenteric lymph nodes (MLNs) and ileal lamina propria of SAMP vs. AKR (control) mice that augmented with age as disease worsened in 20- vs. 4-week-old SAMP, and demonstrated increased levels of IL-5. Additionally, $ST2^+$ ILC2s potently expanded after exogenous IL-33 administration. We also observed that at peak levels of ileitis severity, T cell absence in SAMPxRAG2$^{-/-}$ mice did not affect total ileal inflammatory scores or Th2 cytokine expression compared to wildtype (WT)-SAMP, suggesting that ILC2 Th2 cytokine expression is sufficient to induce ileitis. Interestingly, SAMP lacking the bacterial sensor for muramyl dipeptide (SAMPxNOD2$^{-/-}$) and germ-free (GF)-SAMP showed decrease in MLN-derived ILC2s vs. WT-SAMP, suggesting that gut microbiome components may be necessary for ILC development and expansion via IL-33 during chronic gut inflammation. Collectively, these indicate that the IL-33/ST2 axis may mechanistically contribute to chronic intestinal inflammation, such as in IBD, through pathogenic ILC2 development.

W98. Analysis of ILC2 Deficiency During Pulmonary Cryptococcosis

Markus Kindermann, David Vöhringer, Markus F. Neurath and Stefan Wirtz. University Hospital Erlangen, Erlangen, Bayern, Germany

Pulmonary infections with the opportunistic pathogen *Cryptococcus neoformans* are one of the major causes of mortality of immunocompromised HIV patients. The main infection route is via inhalation of desiccated fungi or spores deeply into the lungs. Once incorporated, the pathogen gets in touch with phagocytosing macrophages and dendritic cells, which modulate the immune response by direct killing, cytokine secretion and antigen presentation. While type 1/3 immunity is associated with fungal control and clearance during Cryptococcosis, type 2 immunity is linked with a detrimental course of disease. However, little is known about regulatory pathways modulating and balancing immune responses during the onset of *Cryptococci* infections. Given that pulmonary numbers of ILC2 are largely increased in a mouse model of Cryptococcosis, we took advantage of conditional RORalpha deficient mice lacking ILC2 to assess their functional role during different phases of *Cryptococci* infection. Here, we provide evidence that RORalpha deficient mice preferentially evolve a more prominent type 1/3 immunity indicated by decreased numbers of eosinophils at sites of infection accompanied by increased levels of type 1/3 signature cytokines. Hence, this shift of the immune response leads to a more efficient fungal control concomitant with less severe lung pathology and prolonged survival. In conclusion, we show evidence that RORalpha functions as a regulatory checkpoint of immune responses during pulmonary Cryptococcosis by favoring detrimental type 2 immunity. This regulatory switch may have potential relevance for anti-mycotic treatment in human patients.

W99. Microbe-Associated Molecular Patterns Inhibit ILC2 Mediated Allergic Lung Inflammation

Maryam Ghaedi[1], Grace Poon[1], Itziar Martinez-Gonzalez[2] and Fumio Takei[2]. [1]University of British Columbia, Vancouver, BC, Canada; [2]Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, Canada, Vancouver, BC, Canada

Allergic lung inflammation is driven by overproduction of type 2 cytokines in response to inhaled allergens. Upon allergic encounter, a damaged lung epithelium releases alarmin IL-33 and activates group 2 innate lymphoid cells (ILC2s). Activated ILC2s produce type 2 cytokines and induce type 2 inflammation. Chronic type 2 inflammation can cause allergy and asthma. According to hygiene hypothesis, microbial infections that induce a type 1 immune response prevent the development of type 2 immune responses such as allergy and asthma. Recent studies have indicated that interferons (IFN), prototypic type 1 cytokines, are potent inhibitors of ILC2s. We found that a single intranasal injection of bacteria-derived lipopolysaccharide (LPS) strongly inhibited IL-33-induced ILC2 activation and eosinophilic lung inflammation in mice. The effect of LPS on ILC2s was dependent on Toll-like receptor (TLR) 4 and IFNγ but independent of T and B cells. In LPS-injected mice, fractions of NK, NKT cells and ILC1s were IFNγ$^+$. Intranasal injections of IL-12, which is known to activate NK, NKT and ILC1s, also inhibited lung ILC2s. As ILC2s do not express TLR4 but express the IFNγ receptor, these results suggested that LPS stimulates TLR4$^+$ myeloid cells to produce IL-12, which activates IFNγ production by NK, NKT cells and ILC1s. We also found that LPS treatment induced upregulation of the pro-type 1 transcription factor T-bet, which might be responsible for the inhibition of ILC2s. In addition to LPS, intranasal Poly I:C (double stranded RNA and TLR3 ligand) also inhibited IL-33-induced ILC2 proliferation and eosinophilic lung inflammation. These results showed that microbe associated molecular patterns inhibit ILC2-mediated allergic lung inflammation and provided experimental evidence for the hygiene hypothesis.

Monocytes and Macrophages

OR.59, T78. Distinct Niche-Dependent but Commensal-Independent Control of Resident Macrophage Ontogeny in the Gastrointestinal Tract

John Grainger, Tovah Shaw, Stephanie Houston, Hayley Bridgeman, Thomas Barbera, Patrick Strangward, Andrew MacDonald and Joanne E. Konkel. University of Manchester, Manchester, England, United Kingdom

A defining feature of resident gut macrophages is their high turnover from blood monocytes, which is attributed to tonic commensal-driven inflammation and is considered a unique feature of this tissue. However, this tenet runs contrary to the established importance of long-term micro-environmental training in conferring tissue-specialisation of macrophage function. Here we show unappreciated heterogeneity in the gut macrophage compartment, identifying three distinct populations that segregate based on phenotype and ontogeny. Unexpectedly, one population identified by the apoptotic cell uptake receptor Tim-4 was found to be maintained largely independently of blood monocytes and localised in the *lamina proporia* and intraepithelial layer. Contrasting this, muscularis macrophages were rapidly replaced from blood monocytes. Furthermore, ontogeny of gut macrophages was found to change with physiologic ageing of the tissue but was crucially not dependent on the presence of a live commensal microbiota. These findings challenge the current paradigm of a commensal-dependent pathway of gut macrophage replenishment from blood monocytes. Instead, supplanting it with a model in which macrophage-monocyte turnover is highly-specialised to niche occupation.

OR.60, T81. Long-Lived Yolk-Sac Derived Intestinal Macrophages Interact with Submucosal and Myenteric Neurons

Sebastiaan De Schepper[1], Simon Verheijden[1] and Guy E. Boeckxstaens[2]. [1]KU Leuven, Leuven, Vlaams-Brabant, Belgium

Macrophages located in the lamina propria (LpMϕ) are continuously exposed to foreign antigens, demanding constant replenishment by monocytes to sample the lumen. In contrast, much less is known about the origin and longevity of macrophages in other layers of the intestine, such as the submucosal and muscularis externa macrophages (MMϕ). Here, we used a fate-mapping model by crossing *Cx3cr1-CreER* mice with Rosa26^{YFP} reporter animals to irreversibly target *Cx3Cr1*-expressing macrophages upon tamoxifen treatment. This approach induced YFP-expression in 76.9 ± 10.4% of CD64^{+} LpMϕ and 80.4 ± 4.9% of CD64^{+} MMϕ 1 week post-tamoxifen injection. Of interest, a fraction of YFP^{+} LpMϕ (7.4 ± 0.3%) and MMϕ (22.4 ± 1.3%) persisted into adulthood for at least 8 months. Immunostaining confirmed this flow cytometry data and showed YFP^{+} expression in the villi (92 ± 3.4 % of F4/80 Mϕ), submucosa (78.8 ± 8.8 %) and muscularis layer (78.4 ± 11.8 %) at 1 week post-injection. With time, YFP labeling progressively dropped in the villi, submucosal and muscularis. However, at the level of the submucosal and myenteric plexus a population of long-lived YFP^{+} macrophages persisted in contact to enteric neurons. Moreover, pregnant *Cx3cr1-CreER*. Rosa26^{YFP} animals were injected at E8.5 with tamoxifen. Using this approach, 40% of F4/80^{+}CD11b^{low} cells were YFP^{+} on the day of birth, indicating a prenatal origin. In conclusion, our results demonstrate a long-lived macrophage population originating from the yolk sac within the submucosa and muscularis layer of the intestine, emphasizing the strong heterogeneity in intestinal macrophage ontogeny.

OR.61, T16. Interaction Between Enteric Glia and Myeloid Cells as Critical Players in iIntestinal Immune Homeostasis

Gera Goverse[1], Merle van Winge[1], Hevy Nawzad[1], Michelle Stakenborg[1], Evelien Labeeuw[1], Marlene Hao[1], Pedro J. Gomez-Pinilla[1], Pieter Vandenberghe[1], Guy E. Boeckxstaens[2] and Gianluca Matteoli[2]. [1]University of Leuven, Leuven, Belgium; [2]KU Leuven, Translational Research Center for Gastrointestinal Disorders, Leuven, Vlaams-Brabant, Belgium

In the gastrointestinal tract, a balance between immune activation and tolerance is essential to preserve intestinal homoeostasis. Recently we have demonstrated that stimulation of the enteric neurons has potent anti-inflammatory effects, mainly via modulation of macrophages (MFs) and dendritic cells (DCs). In the current study, we investigate if also enteric glial cells (EGCs), the major cellular constituent of the enteric nervous system, may have immunomodulatory properties. Immunohistochemical analysis revealed that EGCs are in close contact with MFs within all layers of the intestine. Moreover, we found that glial-secreted molecules were able to decrease expression of pro-inflammatory cytokines, such as IL-12 and IL-6, while anti-inflammatory cytokine IL-10 was further increased in MFs in response to LPS stimulation. In addition, typical anti-inflammatory MF markers, such as MRC-1 and MSR-1, were increased in MFs stimulated with supernatant of EGCs. Analysis of the EGC phenotype both *in vivo* and *in vitro* during inflammation revealed expression of several monocyte-chemoattractant proteins among which CX3CR1L, as well as genes involved in the proliferation and generation of a tolerogenic phenotype in monocytes. In line, we were able to demonstrate that EGCs are able to specifically attract $CX3CR1^+$ monocytes. Overall, we provide evidence suggesting that EGCs exert immunomodulatory effects on intestinal myeloid cells. Taken together, our data indicate that interactions between enteric glia and the intestinal immune system might be crucial to maintain intestinal immune homeostasis and prevent immune-mediated diseases such as inflammatory bowel disease.

OR.63, T79. TNFα Production by Monocytic Precursors of Human Intestinal Macrophages is Poorly Controlled by IL-10 in Patients with IBD

Inva Hoti[1], Neil E. McCarthy[1], Edward M. Giles[2], Ibrahim Ayada[1], Hannah Gordon[2], James O. Lindsay[2] and Andrew J. Stagg[1,3]. [1]Blizard Institute, London, England, United Kingdom; [2] Royal London Hospital, London, England, United Kingdom; [3]Queen Mary University of London, London, England, United Kingdom

Intestinal macrophages, derived from monocytic precursors, are critical targets of the IL-10 which prevents development of colitis in mice. In humans, loss-of-function IL-10R mutations cause early-onset IBD. We aimed to test the hypothesis that IL-10 responsiveness in key myeloid cells is suboptimal in adult IBD patients even in the presence of functional IL-10 signaling. LPS-induced TNFα production, STAT3 phosphorylation, and IL-10Rα expression, were measured by flow cytometry using cells from blood or extracted from colonic biopsies. LPS-induced TNFα production by recently recruited $CD14^{hi}$ myeloid cells from the healthy intestine was inhibited by IL-10. LPS-induced TNFα production by circulating $CD14^+CD16^-$ classical monocytes (78±4.46% $TNFα^+$), the presumptive precursors of these intestinal myeloid cells, was also significantly ($p < 0.001$) inhibited by IL-10 in healthy controls. A similar frequency (89±2.39%) of intermediate monocytes ($CD14^+CD16^+$) produced TNFα but this response was significantly ($p=0.009$) less well controlled by IL-10 despite higher IL-10Rα expression and similar IL-10-induced STAT3 phosphorylation. Fewer LPS-stimulated non-classical monocytes ($CD14^-CD16^+$) produced TNFα (33±6.24%; $p < 0.001$), but this response was not significantly inhibited by IL-10 due to poor IL-10-induced STAT3 phosphorylation as a consequence of low STAT3 availability. IL-10 was significantly less effective at inhibiting TNFα production by classical monocytes in IBD patients than controls ($p=0.026$), despite increased expression of IL-10Rα and IL-10-induced STAT3 phosphorylation. Thus, the ability of IL-10 to control inflammatory activity in monocytes is subpopulation dependent; intermediate and non-classical cells may increasingly contribute in IL-10-rich environments. Furthermore, reduced responsiveness to IL-10 in classical monocytic precursors of intestinal macrophages may contribute to inflammation in IBD.

T74. Muscularis Macrophages Regulate Tissue Adaptation to Enteric Infection

Christina L. Graves, Ilana Gabyani, Diego Borges, Paul Muller and Daniel Mucida. Rockefeller University, New York, NY

The extensive enteric immune and nervous systems, and interactions between them, play a critical role in gastrointestinal (GI) physiology and in response to pathogens. While immune responses at the intestinal interface are critical for resistant to pathogens, exaggerated inflammation or deficient tissue repair mechanisms often result in chronic immunopathology. We have recently demonstrated that intestinal muscularis macrophages (MMs) residing within the myenteric plexus are skewed towards a tissue-protective gene program, which is reinforced upon enteric infection as a result of extrinsic noradrenergic neuronal activation and signaling through adrenergic receptor beta 2 (b_2AR) on MMs. We investigated the role of MM during enteric infections and how activation of b_2ARs impacts infection-induced tissue damage using a newly established mouse model of post-infectious IBS. We observed that upon oral infection with Salmonella typhimurium mutant *spiB*, which is cleared one week-post infection, wild-type mice develop persistent low-grade inflammation and show signs of long-lasting enteric neuropathy including increased total transit time and altered intestinal muscle-ring contraction. To assess the role of b_2AR signaling in MMs we crossed the myeloid Lyz2^{cre} strain with Adrb2$^{flox/flox}$ mice (*LysMΔAdrb2*). We observed that *LysMΔAdrb2* mice show enhanced neuropathy and tissue inflammation post *spiB* infection when compared to Cre^{-} littermate controls. Transcriptomic analyses at early and late time-points post infection revealed reduced tissue-protective markers and increased levels pro-inflammatory molecules in sorted MMs isolated from *LysMΔAdrb2* mice. Our results suggest that b2AR-mediated signaling on MMs is part of a neuro-protective response during infection and may facilitate functional recovery of the tissue.

T75. Monocyte-Derived Cells are Important Players in the Tissue Destruction Undermining Gingival Tissue Integrity in Periodontitis

Sofia Björnfot Holmström[1], Reuben Clark[1], Stephanie Zwicker[1], Daniela Bureik[1], Egle Kvedaraite[1], Eric Bernasconi[2], Gunnar Johannsen[1], Benjamin Marsland[2], Elisabeth Almer boström[1] and Mattias Svensson[1]. [1]Karolinska Institutet, Stockholm, Stockholms Lan, Sweden; [2]University of Lausanne, Lausanne, Vaud, Switzerland

Periodontitis (PD) is a chronic inflammatory disease of tooth supportive tissues. The mucosa in PD is characterized by an increased infiltrate of immune cells; among these the monocytes. We hypothesize that monocytes, followed by their differentiation and subsequent production of inflammatory mediators, contribute to the tissue degradation in PD. Gingival biopsies were collected from PD and control patients. In parallel, we set up a human organotypic oral mucosa model, composed of gingival fibroblasts, blood monocytes and the TERT-immortalized human oral keratinocyte line OKF6-TERT2, to study monocyte-derived cells in tissue inflammation. The biopsies and models were analyzed with Multiplex, Western blot, immunofluorescence, and multicolor flow cytometry. We demonstrated that PD gingiva is associated with increased expression of matrix metalloproteinase (MMP)12. In addition, we found that monocyte-derived cells were the predominant source of MMP12 in PD. The organotypic model facilitated differentiation of monocytes into macrophage like monocyte-derived cells, shown to be responsible for the induced production of MMP12. We demonstrated that the PGE2/COX-2 pathway is not involved in the regulation of MMP12, while CSF-2 was a potent inducer. MMP12 added to the model resulted in loss of Tropoelastin, which was confirmed in PD gingival tissue, suggesting a role of MMP12 in undermining tissue integrity in PD. To the best of our knowledge this is the first study demonstrating an association of PD and increased MMP12 production by monocyte-derived cells. Also, we have successfully established a model system, which can be utilized to target and modulate pathways of tissue destruction in PD.

T76. Alpha Toxin Activation of the Inflammasome Distracts Mitochondria from their Role in the Killing of *S. Aureus*

Taylor Cohen, Omari Jones-Nelson, Christine Tkaczyk, C. Kendall Stover and Bret Sellman. Medimmune, Gaithersburg, MD

Alpha toxin (AT) expression correlates with the severity of clinical *Staphylococcus aureus* (SA) respiratory infection. At the cellular level, AT pore formation in the host cell membrane leads to activation of the NLRP3 inflammasome. Prior studies have suggested that caspase-1 activation, a downstream effector of the NLRP3 inflammasome, is required for killing of SA. However, others have shown that *Nlrp3*$^{-/-}$ mice are protected from SApneumonia. To study the role of NLRP3 complex formation and inflammation associated with inflammasome dependent cytokines (IL-1β/IL-18) in host defense against SA, we administered neutralizing antibodies (24h prior to infection) against either AT or the downstream cytokines and monitored survival (lethal intranasal infection, 2e8 CFU/mouse) or bacterial clearance (sublethal intranasal infection, 5e7 CFU/mouse). We found that neither IL-1β nor IL-18 neutralization protected against lethal infection (30-50% survival anti-IL-1β or anti-IL-18) to the same degree as AT neutralization (100% survival). Cytokine neutralization did not promote bacterial clearance during sublethal infection while AT neutralization significantly ($P = 0.0006$) reduced bacterial numbers recovered from the lung 24h post infection. *In vitro*, wild type (WT) and AT null (D*hla*) SA induced caspase-1 activation, although levels were lower in D*hla*-SA infected human monocytes. Inhibition of caspase-1 reduced WT and D*hla*-SAkilling ($P=0.0251$ and < 0.0001 respectively) by human monocytes, while the NLRP3 inhibitor MCC950 increased killing ($P < 0.0001$) of WT SA. We used confocal microscopy to visualize where bacteria were being trafficked in relation to active caspase-1. When monocytes were incubated with WT-SA, we found that activated caspase-1 (YVAD-FLICA) and phagocytosed SAdid not co-localize within the cell. When incubated with D*hla*-SA, active caspase-1 co-localized with internalized bacteria. Mitochondrial reactive oxygen species (mitoROS) have been implicated in caspase-1 activation, therefore we tested the ability of a mitoROS inhibitor, mitoTEMPO, to prevent killing of WT or D*hla*-SA. MitoTEMPO inhibited killing of D*hla* ($P < 0.0001$), not WT SA suggesting that AT uncouples mitochondria from their involvement in killing internalized bacteria, presumably by recruiting mitochondria to the inflammasome complex. When incubated with WT as compared with D*hla* SA, mitochondria co-localization with internalized bacteria was significantly reduced ($P < 0.0001$). Therefore, despite increased caspase-1 activation in response to AT activation of the NLRP3 inflammasome, WT-SA killing is reduced as both mitochondria and caspase-1 do not traffic to the site of internalized bacteria. We conclude that AT induction of the inflammasome protects SAfrom immune clearance by improperly activating key antimicrobial complexes within phagocytes impairing bacterial killing.

T77. Deficiency of Axl Receptor Increases the Percentage of Phagocytes in the Lung Mucosae during Homeostasis

Kamila Guimarães-Pinto[1], Estefania Claudio[2], Ulrich Siebenlist[2], Brian L. Kelsall[3] and Alessandra A. Filardy[1]. [1]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil; [2]National Institutes of Health, Bethesda, MD; [3]National Institute of Allergy and Infectious Diseases, Bethesda, MD

Alveolar macrophages (AMs) and lung conventional dendritic cells (cDCs) are key cells for the establishment of pulmonary homeostasis as they efficiently kill microbes yet produces regulatory cytokines to maintain tolerance to microbiota and inhaled harmless antigens. Efferocytosis is one of the major regulatory mechanisms to maintain tolerance and hemostasis in sites with high apoptosis rate due to continuous regeneration of the epithelial layer. The TAM family of receptor tyrosine kinases (Tyro3, Axl and MerTK) mediates non-inflammatory efferocytosis by phagocytes through the bridging phosphatidylserine-binding molecules growth arrest-specific 6 (Gas6) or Protein S. We found that under homeostatic conditions, partial deficiency of efferocytosis in Axl deficient mice (Axl$^{-/-}$) increases the *in vivo* percentage of AMs (SiglecF^{+}CD11c^{+}) and parenchymal CD11b$^{+/-}$CD103^{+} cDCs, key cells for mediating efferocytosis, compared to wild-type (WT) mice. In contrast, the frequencies of CD11b^{+}CD24^{+}CD103^{-}CD64^{-} cDCs and CD11b^{+}CD24^{+}CD103^{-}CD64^{+} mono-DCs in the lung parenchyma of Axl$^{-/-}$ mice are similar to WT mice. Functionally, isolated lung mucosal CD11c^{+} phagocytes from Axl$^{-/-}$ mice produce more pro-inflammatory

cytokines (TNF-α and IL-6) and less IL-10 in response to LPS *in vitro*, than their counterparts from WT mice. These data support the hypothesis that in the setting of Axl-deficiency factors released from poorly cleared apoptotic cells undergoing secondary necrosis promote lung inflammation by driving an accumulation of AMs and inflammatory $CD11b^{+/-}$ $CD103^{+}$ cDCs within the pulmonary mucosae.

T80. Characterization and Proliferation of Human Intestinal Macrophages in Inflammatory Bowel Disease

Elizabeth McDonald[1], Pamela Wright[1], Calum Bain[2], Allan Mowat[1], Elaine Robertson[3], Daniel Gaya[3] and Simon Milling[1]. [1]University of Glasgow, Glasgow, Scotland, United Kingdom; [2]University of Edinburgh, Edinburgh, Scotland, United Kingdom; [3]Glasgow Royal Infirmary, Glasgow, Scotland, United Kingdom

Inflammatory bowel disease (IBD) is dependent on macrophages. However, the specific roles played by different subpopulations in intestinal inflammation of Crohn's disease (CD) and ulcerative colitis (UC) are not yet understood. This cross-sectional study developed 12-parameter flow cytometry protocols to analyse colonic biopsies from patients with active CD, UC and non-inflamed controls. *We aimed to accurately identify specific macrophage subpopulations and to ascertain potentially pathogenic functions of macrophages in IBD patients.* Within the intestinal lamina propria, we unambiguously differentiate macrophages from dendritic cells (DCs: $CD45^{+}CD64^{-}$ $HLA\text{-}DR^{+}CD11c^{+}$; macrophages: $CD45^{+}CD64^{+}HLA\text{-}DR^{+}$). Colonic macrophages express CD33, a known marker of human macrophages. Additional characterisation of the macrophage population showed two distinct subsets, further differentiated by heterogeneous expression of mannose receptor, CD206. RNAseq analysis comparing $CD206^{+}$ and $CD206^{-}$ cells revealed 835 genes with significantly altered expression. $CD206^{+}$ macrophages express markers consistent with a mature macrophage phenotype: high expression of CD68 and CD163, and lower of Trem1. We therefore identify $CD64^{+}$ $HLA\text{-}DR^{-}$ $CD206^{-}$ cells as intermediate-stage macrophages, similar to the well-characterised monocyte-derived cells that generate intestinal macrophages in mice. To address the contribution of $CD206^{+}$ and $CD206^{-}$ populations to inflammation we assessed their frequency and functions. Surprisingly, in human tissues, both populations are highly proliferative, independent of inflammatory status. We have identified and thoroughly characterised macrophage populations from intestinal lamina propria. This reveals that human colonic macrophages appear to be derived from a monocytic precursor and, unlike murine intestinal macrophages, are highly proliferative.

T82. Context-Dependent Differentiation of Monocyte to Macrophage in the Steady State and Inflamed Intestine

Girmay Desalegn and Oliver Pabst. RWTH Aachen, Aachen, Nordrhein-Westfalen, Germany

In the steady state, these intestinal macrophages are constantly replenished by circulating blood monocytes and functionally contribute to establish oral tolerance and intestinal homeostasis. In contrast to steady state, during inflammation, there is increased recruitment of monocytes and a change in their differentiation pattern towards pro-inflammatory macrophages state that exacerbates pathology. Here we characterized monocyte to macrophage differentiation during steady state and inflammation. To study monocyte differentiation *in vivo,* bone marrow $Ly6C^{hi}$ monocytes were purified and intravenously transferred into either unmanipulated recipients or recipients undergoing mechanical manipulation of small intestine to induce inflammation before adoptive transfer. Transferred cells were isolated from recipient's intestinal lamina-propria 1,2,5, and 9 days after transfer. Flow-cytometry revealed that in healthy recipients, monocyte progressed over several days from monocytes to $CX3CR1^{high}$ anti-inflammatory macrophages. As judged by expression of Ly6C, MHCII, CD64, CD11c and CX3CR1 this process seemed reminiscent in inflamed intestine except of low CX3CR1 expression at later stages in inflamed compared to healthy recipients. However, Nanostring transcriptome analysis revealed a more profound difference between healthy and inflamed intestines. Already one day after adoptive transfer, transferred monocyte adopted a distinct gene expression profile that was clearly indicative of the healthy or inflamed intestine. Moreover, we identified factors that could potentially control context-dependent differentiation of monocyte into two distinct monocyte/macrophages population with divergent function. Our results indicate that anti-versus pro-inflammatory macrophage development might not result from

gradual adaptation to the local environment but rather stem from a fundamental transcriptomic programming already apparent at early stages of monocyte to macrophage development.

T83. Chemokines Over-Expressed in Colorectal Cancer with a Potential Role in the Tumor Associated Macrophages (TAMs) Profile

Marjorie De la Fuente Lopez[1,2], Daniela Parada[2], Daniela Simian[1], Maripaz Martinez[1], Francisco Lopez-K[1], Udo Kronberg[1], Sebastian López[3], Antonella Sanguinetti[3], Mario Abedrapo[3], Rodrigo Quera[1] and Marcela A. Hermoso-R[2]. [1]Clinica Las Condes, Santiago, Region Metropolitana, Chile; [2]Universidad de Chile, Santiago, Region Metropolitana, Chile; [3]Hospital Clinico Universidad de Chile, Santiago, Region Metropolitana, Chile

The recruitment and activation of hematopoietic cells, including macrophages, to the supporting tumor stroma is fundamental in the carcinogenesis and metastasis. According to the important role of chemokines in chemotaxis, cell trafficking and inflammation, a complex network of these molecules can influence cancer progression and tumor-associated macrophages (TAMs) infiltration. Aim: To investigate tissue and plasma levels of chemokines involved in macrophages recruitment and their association with progression of cancer and their role in TAMs activation. Plasma and tumor/healthy mucosa were obtained from Chilean patients with colon cancer (n= 25) under surgery. Protein extract from tissue were used to evaluate concentration of chemokines involved in macrophage recruitment (CCL2, CCL3, CCL4, CCL5 and CX3CL1), through Luminex assays. Statistical analysis was performed using non-parametric Wilcoxon matched-pair test, considering $p<0,05$. CCL3 plasma levels were evaluated by ELISA. THP-1-differentiated macrophages were stimulated with human CCL3 in combination with IFN-gamma and LPS, and expression of inflammatory makers was evaluated by flow cytometry. Tumor levels of CCL2 (Median= 216.90 pg/mg), CCL3 (Median= 67.63 pg/mg) and CCL4 (Median= 46.30 pg/mg) were significantly higher than those in healthy tissue (Median= 154.40, 23.00 and 24.11 pg/mg, respectively). CCL3 plasma levels do not correlate with chemokine tissue content. CCL3 in combination with proinflammatory cytokines can enhance the expression of CD80, a marker of pro-inflammatory macrophages. Conclusions: High expression of CCL2, CCL3 and CCL4 in colon cancer could induce the infiltration of TAMs, and specifically CCL3 promote an inflammatory macrophage profile representing a potential therapeutic target.

Mucosal B Cells

F100. Breast Milk Lymphocytes Response to Prenatal Vaccination with Pneumococcal Conjugate Vaccine-13

Mustapha Jaiteh, Sophie Roetynck and Ed Clarke. Gambia Medical Research Council, Banjul, Western, Gambia

Breast milk (BM) is a complex, dynamic, bio-fluid containing nutrients and immunological components that enhance neonatal growth and protection. Leukocytes can represent 70% of the cells in colostrum and 2% in matured BM, and increase up to 94% in the case of infection. In healthy mothers, lymphocytes make up 5 to 9% of BM leukocytes and as compared to blood, they comprise a larger population of activated and memory cells, believed to extravasate into neonatal circulation and enhance immune maturation. Nothing is known on the effects of maternal vaccination on the BM lymphocyte in humans. Our main objective is thus to characterize the lymphocyte response, with specific focus to B cells, to Pneumococcal Conjugate Vaccine (PCV-13) in BM of mothers vaccinated between 28-32 weeks gestation. This exploratory study is part of a phase III clinical trial. BM lymphocyte population from 60 mothers, randomized to receive PCV-13, will be characterized using flowcytometry and pneumococcal specific memory B cells will be analyzed by ELIspot, both in BM and peripheral blood from mothers and infants at delivery. Both B and T cells were observed in colostrum, IgG+ memory B cells were detected and could comprise about 1% of pneumococcal specific cells. This preliminary data suggests the presence of pneumococcal specific memory B cells in colostrum, which could be attributed to maternal vaccination and/or colonization. Data will be analyzed according to vaccination and colonization status. This study could reveal one of the potential mechanisms underlying neonatal protection via maternal immunization through breastfeeding.

F104. Anatomically Remote Education of B Cells is Required for Colonic Health

Neil Surana[1] and Dennis Kasper[2]. [1]Boston Children's Hospital, Boston, MA; [2] Harvard Medical School, Boston, MA

Mucosa-associated lymphoid tissues contain roughly 80% of all immune cells and produce virtually all of the body's IgA. Although the majority of IgA-secreting cells educated within a mucosal site home back to the same anatomic region, some cells are also found in distant mucosal tissues. These observations underlie the notion of a common mucosal immune system, which holds that anatomically unrelated mucosal sites are functionally connected by a shared immune system. However, the ontological basis of this separation between site of immune education and functionality has remained elusive. Here we show that mice lacking Peyer's patches (PPs)—small-intestinal lymphoid tissue covered by antigen-sampling M cells—have no immunologic defect in the small-intestinal lamina propria. Surprisingly, the primary immunological abnormality in PP-deficient mice was a reduction in colonic B cells, including plasmablasts but not plasma cells. Adoptive transfer experiments conclusively demonstrated that PP-derived cells preferentially give rise to colonic—but not small-intestinal—B cells and plasmablasts. Finally, these PP-derived colonic B cells were critical for restraining colonic inflammation. Thus, PPs bridge the small-intestinal and colonic immune systems and provide a clear example of immune education being required in an anatomic compartment distinct from the effector site. Our findings, which highlight that the majority of fecal IgA is produced by colonic plasmablasts that originate from PPs, will help inform design of mucosal vaccines.

F106. Ontogeny of B Cell Activation and IgA Class Switching in the First Year of Life in HIV-Exposed Uninfected Infants in Uganda

Sara R. Saporta-Keating[1], Elizabeth J. McFarland[1], Tina M. Powell[1], Carolyne Onyango-Makumbi[2], Samuel Okurut[3], Michael A. Eller[4], James T. Gaensbauer[1], Mary Glenn Fowler[5] and Edward N. Janoff[6]. [1]University of Colorado - Anschutz Medical Campus, Aurora, CO; [2]Makerere University–Johns Hopkins University Research Collaboration, Kampala, Uganda; [3]Makerere University Walter Reed Project, Kampala, Uganda; [4]US Military HIV Research Program, Walter Reed Army Institute of Research, Silver Springs, MD; [5]Johns Hopkins University School of Medicine, Baltimore, MD; [6]Mucosal and Vaccine Research Program Colorado (MAVRC), Aurora, CO

Development of IgA^+ plasma cells in the gut lamina propria is delayed until after 1mo of age as is IgA in blood. We propose that the introduction and activation of IgA^+ B cells in blood over time after birth are derived from their mucosal origins. Among 78 healthy HIV-exposed, uninfected infants in Uganda, we characterized the memory phenotype of B cells (naive [IgD^+A^-], IgA^+ [IgD^-IgA^+] and other class-switched (CS) cells [IgD^-IgA^-] by flow cytometry at 0, 2, 6, 12, 18, 24, and 48 weeks of life and expression of activation markers ($CD95^+$, $CD86^+$ and $CD40^-$) in each subset. Gut homing was predicted by co-expression of α4β7 integrin (β7 stain). IgA^+ B cells were not detected until 6 weeks of life, and remained a minority population that increased in frequency from birth to 48 weeks (0 to 0.53±0.2%). The majority of IgA^+ B cells expressed CD95 (≈75%), fewer CD86 (≈20%), the latter comparable to $CD40^-$ cells. IgA^+ cells showed greater activation than the majority naïve cells as did the other CS cells. Expression of high levels of β7 ($\beta7^{hi}$) most clearly distinguished IgA^+ B cells from other CS and naïve cells. Almost all $IgA^+\beta7^{hi}$ B cells expressed CD95 and one-third of $IgA^+\beta7^{hi}$cells expressed CD86. Conclusion: IgA^+ B cells increase in frequency over time, most prominently expressing the β7 homing marker. β7 expression is associated with activation of IgA^+ B cells. These findings suggest that mucosal sites likely serve as the origin and stimulus for development of this discrete B cell population.

F97. Oral Vitamin A Supplementation of Porcine Epidemic Diarrhea Virus (PEDV)-Infected Gilts Enhances the Gut-Mammary-Secretory IgA (sIgA) Axis and Passive Protection in Nursing Piglets

Stephanie Neal Langel[1], Anastasia N. Vlasova[1], Francine C. Paim[1], Moyasar A. Alhamo[1], Kelly Lager[2] and Linda J. Saif[1]. [1]Ohio State University, Wooster, OH; [2]National Animal Disease Center, United States Department of Agriculture, Agricultural Research Service, Ames, IA

Stimulation of lactogenic immunity in gilts via the gut-mammary gland-sIgA axis is critical for passive protection of nursing piglets against PEDV. Vitamin A (VitA) imprints gut homing of B and T cells and enhances mediators for differentiation of IgA antibody-secreting cells (ASC). We hypothesized that VitA supplementation of gilts would enhance both mucosal immune responses and the gut-mammary-sIgA axis to boost lactogenic immunity and passive protection of nursing piglets against PEDV challenge. Gilts received oral retinol palmitate daily (30,000 IU) from gestation day 76 throughout lactation. At 3-4 wks pre-partum, both VitA-supplemented (PEDV+VitA, n=3) and non-supplemented (PEDV, n=4) gilts were orally inoculated with PEDV; non-supplemented gilts received MEM (Mock, n=4). All piglets were PEDV-challenged at 3-5 days post-partum. The mortality rate of PEDV-challenged piglets of PEDV+VitA gilts was 6.25% compared with 33.3% and 94.3% for PEDV and Mock litters, respectively. Piglets born to PEDV+VitA gilts had lower diarrhea scores at multiple post-challenge days (PCD). PEDV-specific IgA ASC appeared earlier in peripheral blood [post-inoculation day (PID) 6-8] of PEDV+VitA gilts compared with PEDV gilts (PID 12-17) and PEDV+VitA gilts had higher frequencies of IgA^+ and $IgA^+\beta7^+$ mononuclear cells in blood at PID 6-8. In colostrum, milk and serum, PEDV+VitA gilts had higher mean PEDV neutralizing antibody titers and milk PEDV-specific IgA ASC compared with PEDV gilts at various piglet PCDs. This innovative approach and our findings suggest that oral VitA supplementation stimulates mucosal immunity and the gut-mammary-sIgA axis in gilts resulting in increased lactogenic immunity and protection in neonatal piglets.

F98. Antigen-Specific PP B Cells Migrate from Germinal Centers to M Cells in the Subepithelial Dome when They Respond to Antigen after Oral Immunization

Mats Bemark[1], Rathan Komban[1], Anneli Strömberg[1], Adi Biram[2], Ziv Shulman[2] and Nils Lycke[1]. [1]University of Gothenburg, Gothenburg, Vastra Gotaland, Sweden; [2] Weizmann Institute of Science, Rehovot, Tel Aviv, Israel

Here we study movements of GFP-expressing antigen-specific B cells after oral immunization with the hapten NP conjugated to CT as an antigen. When B cells respond to this antigens in the small intestine, they invade pre-existing Peyer´s patch (PP) germinal centres rather than form new ones. We find two populations of responding B cells, one that expresses the germinal centre marker GL7 and one that does not. A majority of both cell types are found within germinal centres, but RNA seq analysis demonstrate that the populations differ in their expression of homing receptors. $GL7^-$ antigen-specific B cells that express high levels of CCR6 are indeed found in the subepthelial dome, where they make close contact with M cells. Antigen-specific B cells are also found in the thoracic duct during the response. We have previously demonstrated that B cell responses are synchronized between PP during immune responses to select high affinity variants. How antigen recognition by B cells in the SED can shape this selection process will be discussed.

OR.104, F99. Specificity of IgA Responses

Jeffrey Bunker[1], Steven Erickson[1], Theodore Flynn[2], Marlies Meisel[1], Carole Henry Dunand[1], Bana Jabri[1], Patrick Wilson[1], Dionysios Antonopoulos[2] and Albert Bendelac[1]. [1]University of Chicago, Chicago, IL; [2]Argonne National Laboratory, Lemont, IL

Immunoglobulin A (IgA) is prominently secreted at mucosal surfaces and coats a fraction of the intestinal microbiota. However, the commensal bacteria bound by IgA are poorly characterized and the type of humoral immunity they elicit remains elusive. We used bacterial flow cytometry coupled with 16S rRNA gene sequencing (IgA-Seq) in murine models of immunodeficiency to identify IgA-bound bacteria and elucidate mechanisms of commensal IgA targeting. We found that residence in the small intestine, rather than bacterial identity, dictated induction of specific IgA. Most commensals elicited strong T-independent (TI) responses that originated from the orphan B1b lineage and from B2 cells, although atypical commensals including segmented filamentous bacteria and *Mucispirillum* evaded TI responses but elicited T-dependent IgA. We further probed the clonal reactivities of the IgA repertoire by cloning and characterizing hundreds of monoclonal antibodies from murine IgA plasma cells, precursors, and naive B cell subsets. Though polyreactivity is strongly counterselected during B cell development, IgA plasma cells typically produced polyreactive and microbiota-reactive antibodies. However, naturally microbiota-reactive and polyreactive specificities resembling IgAs were also observed among naive B cells. Natural antibodies were selected into the IgA repertoire at a pre-IgA stage in Peyer's patches, prior to somatic hypermutation. IgA selection itself occurred naturally and was preserved in the absence of microbiota or dietary antigens. Homeostatic IgAs are therefore natural antibodies selected for polyreactivity to microbiota and numerous potential antigens.

OR.105, F103. Antigen Presenting Group 3 ILCs Regulate Mucosal T Cell-Dependent IgA Responses and Coating of Colonic Commensal Bacteria

Felipe Melo-Gonzalez, Markella Papadopoulou and Matthew Ross Hepworth. University of Manchester, Manchester, England, United Kingdom

The generation of an appropriate humoral response in the intestine is essential for healthy host-commensal microbiota interactions. In particular, IgA secreted into the intestinal lumen by tissue-resident B cells acts to segregate and neutralize bacteria with the potential to induce inflammation, while further ensuring a healthy balance of commensal species within the complex microbiota. IgA class switching can occur via both T cell-independent and T cell-dependent mechanisms, with commensal bacteria species inhabiting atypical niches or exhibiting pathobiont potential preferentially stimulating T cell-dependent antigen-specific IgA responses. Group 3 innate lymphoid cells (ILC3) are constitutively present in the intestinal tract and associated lymph nodes. A subset of ILC3 migrate to, and reside within, the interfollicular regions of the mesenteric lymph node (mLN) where they are ideally placed to interact with the adaptive immune system. In particular, mLN-resident ILC3 express MHCII and have antigen-

presenting capacity. Here we demonstrate that mice lacking ILC3-intrinsic MHCII expression develop elevated T follicular helper cell (TfH) responses, germinal center formation and IgA class switching in the mLN and have increased numbers of IgA-secreting plasma cells in the colon at both steady state and following inflammatory insult. Utilizing IgA-seq we are defining the commensal bacterial species that are targeted by T cell responses elicited in the absence of regulation by antigen-presenting ILC3. In addition, we are dissecting the molecular requirements for ILC3 regulation of TfH responses and B cell class switching and exploring the consequences of this dysregulation in the context of chronic inflammatory disease.

OR.106, F102. IgA Production Requires B Cell Interaction with Subepithelial Dendritic Cells in Peyer's Patches

Andrea Reboldi[1], Tal Arnon[2], Lauren Rodda[3], Amha Atakilit[3], Dean Sheppard[3] and Jason Cyster[3]. [1]University of Massachusetts Medical School, Worcester, MA; [2]University of Oxford, Oxford, United Kingdom; [3]University of California San Francisco, San Francisco, CA

Immunoglobulin A (IgA) represents the main antibody class secreted by B lymphocytes at the mucosal surfaces, and is critical in maintaining intestinal homeostasis Secretory Immunoglobulin A (IgA) has roles both in protection from enteric pathogens and in maintaining homeostasis of intestinal commensals. IgA production occurs mainly in Peyer's patches (PPs). However, the cellular interactions necessary for IgA class switching are poorly defined. Here we show that in mice, the chemokine receptor CCR6 is used by activated B cells to access the subepithelial dome (SED) of PPs. CCR6 upregulation in PP B cells occurs in a $CD40^-$, and thus most likely T cell–dependent, manner. Intravital microscopy revealed that B cells undergo prolonged interactions with SED dendritic cells (DCs). SED DC are maintained in a lymphotoxin-β receptor (LTβR)–dependent by innate lymphoid cells, and PP IgA class switching requires both population. PP DCs augment IgA production by integrin αvβ8-mediated activation of transforming growth factor–β (TGFβ). In mice where B cells are unable to access the SED, IgA responses against oral antigen and gut commensals are impaired. Our study establishes the PP SED as a niche supporting DC–B cell interactions needed for TGFβ activation and induction of mucosal IgA responses. By defining a network of interactions required for IgA switching, this study also identifies approaches that could be used to augment IgA responses in a context of mucosal vaccination.

OR.107, F101. *In Utero* Lymphotoxin-Beta Receptor Signalling Shapes Gut Stromal Cell Populations and Orchestrates Mucosal B Cell Response in Adulthood

Conglei Li, Evelyn Lam, Lesley Ward, Tian Sun, Alberto Martin and Jennifer Gommerman. University of Toronto, Toronto, ON, Canada

Lymphotoxin-beta Receptor (LTβR) signalling is required for the development of secondary lymphoid organs, including Peyer's patches, and for maintaining the immune system homoestasis during adulthood. Intestinal IgA levels are also severely reduced in LT-deficient mice; however, the underlying mechanisms are unclear. Using genetic and pharmacological approaches, we found that LTβR signalling is dispensable for mucosal IgA responses in mice where the LT pathway is blocked during adult life. In contrast, we discovered that LTβR signalling is critically required during fetal life for the generation of IgA-producing cells specific for a T-dependent antigen (Rotavirus). Specifically, in WT→$LT\beta^{-/-}$ chimeras, plasma cell differentiation and generation of rotavirus-specific antibody-secreting cells (ASC) in the mesenteric lymph nodes were modestly reduced concomitant with a severe defect of ASC accumulation in the lamina propria, suggesting ASC migration or/and ASC survival in the lamina propria may be dependent on *in utero* LTβR signaling. We also found that while an altered microbiome contributed to defective homeostatic fecal IgA in LT-deficient mice, fecal anti-rotavirus IgA responses in LT-deficient mice were reduced independent of animal husbandry practices that affect the microbiome. Lastly, mice treated *in utero* with LTβR-Ig, a blocking agent, recapitulated the phenotype of WT→$LT\beta^{-/-}$ chimeras, and this treatment led to the alterations in gene expression of lamina propria stromal cells in adulthood. Collectively, our data demonstrate that *in utero* LTβR signalling shapes gut stromal cell populations and has an impact on mucosal B cell response during adulthood.

OR.108, F105. Microbiota-Dependent Inhibition of IgA B-Cell Responses and Paradoxical Enhancement of Intestinal Th17 Cells in IL-21 Receptor Deficiency

Hyeseon Cho[1], Henrique Jaime[2], Byunghyun Kang[1], Rafael Pires Oliveira[3], Rosanne Spolski[4], Warren Leonard[5] and Brian L. Kelsall[1]. [1]National Institute of Allergy and Infectious Diseases, Bethesda, MD; [2]Thomas Jefferson University, Philadelphia, PA; [3]Federal Institute of Parana, Palmas, Parana, Brazil; [4]National Heart, Lung, and Blood Institute, Bethesda, MD; [5] National Institutes of Health, Bethesda, MD

Despite studies indicating a role for IL-21 in intestinal inflammation, how it precisely affects intestinal homeostasis and immunity to infection is not clear. Here, we report a potent effect of commensal microbiota on the phenotypic manifestations of IL-21 receptor deficiency. IL-21 is expressed by $CD4^+$ T cells of Peyer's patches (PPs) and small intestine lamina propria (SILP) and strongly induced by co-housing with SFB-positive mice. IL-21R-deficient mice exhibit reduced numbers of germinal center B cells, AID expression, and IgA^+ B cells in PPs, consistent with the known role for IL-21 in B cell class switching. Furthermore, IL-21R KO mice have reduced IgA^+ plasmablasts and plasma cells in the SILP, but not colon LP. Higher levels of SFB and expression of SAA1, Reg3β, and Reg3γ were found in the terminal ilium of KO mice consistent with the hypothesis that a defective IgA response to SFB results in enhanced bacterial accumulation and epithelial cell contact. Consistent with this possibility, and contrary to prior studies showing a direct role for IL-21 in enhancing Th17 differentiation, increased Th17 and $CD4^+Foxp3^+$Treg cells were found in the SILP of IL-21R KO compared to WT mice, while neither Th1 nor ILC3 populations were altered. Finally, during *Citrobacter rodentium* infection, IL-21R deficiency lead to strikingly reduced tissue pathology without affecting bacterial clearance, associated with dampened levels of pathologic IFNγ, IL-12, and IL-1β. These data demonstrate microbiota-dependent regional and pleotropic effects of IL-21 signaling that affect intestinal immune homeostasis and play a critical role in infection-induced immunopathology.

Mucosal Immunology in the Neonate

OR.32, W117. Sterile-Activated cDC1 Cells in Neonatal Lung Induce Lymph Node Stroma Maturation While Limiting CD4-T Cell Responses

Aaron Silva Sanchez, Selene Meza-Perez, Sara L. Stone, Fran Lund and Troy Randall. University of Alabama at Birmingham, Birmingham, AL

Neonates are more susceptible to respiratory infections and make poor responses to vaccination, possibly due to immature populations of dendritic cells (DCs) in the lung. Here we examined the development and function of lung DCs in neonatal mice. In addition to the CD11b^{hi} and CD103^{hi} DCs that populate the adult lung, we found a population of CD103^{int} DCs that transiently appeared in the lungs between birth and 2 weeks of age. These CD103^{int} DCs were the dominant population of DCs in the lungs and mediastinal LNs (MedLN) of neonates, whereas CD11b^{hi} DCs were the dominant population in adults. CD103^{int} DCs expressed XCR1 and CD205 and were dependent on the transcription factor BATF3, indicating that they are a subset of cDC1 cells. CD103^{int} DCs appeared activated and constitutively expressed high levels of MHC-II, CD40, CCR7 and PDL-1, independently of microbial exposure or MyD88-dependent signaling. Consistent with the idea of sterile activation, CD103^{int} DCs have a transcriptome that is distinct from either resting or activated CD103^{hi} DCs. CD103^{int} DCs poorly phagocytose, process and present antigens and have a limited capacity to prime CD4^{+} T cells. However, they spontaneously migrate to the draining LN, where they promote the maturation of high endothelial venules (HEVs) and fibroblastic reticular cells (FRCs) and increase the overall size and cellularity of the LN. Our results suggest that CD103^{int} DCs are developmentally programmed to promote LN maturation in the absence of inflammation or microbial colonization, at the expense of limiting CD4^{+} T cell responses.

OR.33, W107. Active Suppression of Intestinal CD4^{+}TCRαβ^{+} T Lymphocyte Maturation During the Postnatal Period

Natalia Torow[1], Kai Yu[2], Marijana Basic[2], Andre Bleich[2], Sigfried Weiss[3], Vuk Cerovic[1], Oliver Pabst[1] and Mathias Hornef[1]. [1]RWTH University Hospital, Aachen, Nordrhein-Westfalen, Germany; [2]Hannover Medical School, Hannover, Niedersachsen, Germany; [3]Helmholtz Centre for Infection Research, Braunschweig, Niedersachsen, Germany

Priming of the mucosal immune system during the early postnatal period substantially influences host-microbial interactions and susceptibility to immune-mediated diseases in adult life. The underlying mechanisms, however, are ill defined. Whereas in the adult T cells occupy both secondary lymphoid tissues as well as effector compartments of the small intestine, in the neonate T cells emerge around birth and locate exclusively to the Peyer's patches during the postnatal period. Surprisingly, neonatal CD4 T cells remain naive throughout the postnatal period, despite exposure to the rapidly evolving enteric microbiota and in stark contrast to the adult situation. Maternal SIgA and mucosal regulatory T cells act in concert to prevent immune stimulation and maintain the immature phenotype of CD4 T cells in the postnatal intestine during homeostasis. Yet, neonatal CD4 T cells are able to readily undergo maturation and gain effector function upon barrier disruption by invasive infection with rotavirus or *Salmonella enterica* as well as upon adoptive transfer of antigen-specific T cells. Our work aims to better understand the mechanisms that maintain the naiveté of the adaptive mucosal immune system at steady state during postnatal development by characterizing the availability of routes for luminal antigen in the neonate. Our results identify mechanisms that actively suppress CD4 T cell maturation during the postnatal period that might contribute to prevent autoreactivity, sustain a broad TCR repertoire and establish life-long immune homeostasis.

OR.35, W113. Microbiota and Plasmacytoid Dendritic Cells Influence Thymic Development in Neonates

Nitya Jain[1,2], Maria Ennamorati[2], Kara Clerkin[2], Stefan Halvorsen[2], Kaitlin Berry[2], Caryn Porter[2], Huajun Wang[2], Vladimir Yeliseyev[3], Meredith Weglarz[2], Lynn Bry[3], Slim Sassi[2] and Brian Seed[2]. [1]Harvard Medical School, Charlestown, MA; [2]Massachusetts General Hospital, Boston, MA; [3]Brigham and Women's Hospital, Boston, MA

Mammalian intestinal microbial communities harbor both an enormous source of commensals as well as potential pathogens. In the postnatal period, the immune system must rapidly develop a discrimination of microbial friend from foe akin to that of self-non-self recognition. Lymphocytes of the T cell lineage that participate in this mucosal process develop in the thymus. We show here that thymic development is influenced by the intestinal microbiota, and that plasmacytoid dendritic cells (PDCs) are one class of antigen presenting cell that transit from the intestine to the thymus during the postnatal period. Experimental perturbation of bacteria and PDCs produces changes in the thymic distribution and residency of PLZF$^+$ innate-like cells. Thus, PDCs may act as sensors of intestinal microbial status that convey compartment information to the thymus to promote appropriate immune responses to the microbiota.

PR.05, W116. Induction of RORγt$^+$ Regulatory T Cells for the Prevention of Allergic Diseases is Controlled by Microbial and Maternal Factors During Early Life

Kathryn Knoop[1], Keely G. McDonald[1], Jenny Gustafsson[1], Simon Hogan[2], Charles Elson[3], Phillip Tarr[1] and Rodney Newberry[1]. [1]Washington University School of Medicine, Saint Louis, MO; [2]Cincinnati Childrens Hospital Medical Center, Cincinnati, OH; [3]University of Alabama at Birmingham, Birmingham, AL

Allergic disorders are rapidly increasing in children in Western societies. Decades of observational studies and clinical trials have arrived at breastfeeding along with complimentary introduction of food allergens and avoidance of oral antibiotics in the first year of life as practices to reduce allergic outcomes in at risk children. However, the biologic basis for these recommendations and the reason the benefit of these practices is restricted to a specific time in early life are not known. We identified a pre-weaning interval during which gut luminal antigens are shunted to the colonic immune system to stimulate development of RORγt$^+$ inducible regulatory T cells (iTregs) thereby enhancing immune tolerance. Luminal antigen delivery in early life was mediated by the formation of goblet cell-associated antigen passages (GAPs), which were regulated by goblet cell intrinsic sensing of breast milk derived epidermal growth factor and the blooming gut microbiota. Disruption of the gut microbiota or GAP antigen delivery during early life resulted in abrogation of tolerance to dietary antigens encountered during this time, a long-lived decrease in RORγt$^+$ iTregs, and skewing of the immune system toward systemic allergic Th2 responses. We propose the microbiota, in combination with maternal factors, control antigen delivery as well as the induction of RORγt$^+$ iTregs during early life, facilitating enhanced tolerance to dietary antigens encountered during this time and allowing maturation of a balanced and durable immune system necessary to suppress inappropriate Th2 responses.

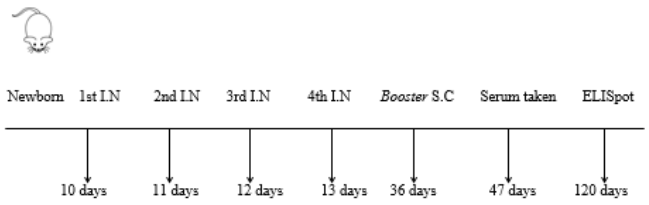
W02. Evaluation Memory B-Cells in Neonatal Mice Immunized of *Bordetella Pertussis* Complexed with Dioctadecyldimethylammonium Bromide (DODAB-BF) as Adjuvant by ELISpot Assay

Fernanda Ayane de Oliveira Santos and Elizabeth De Gaspari. Adolfo Lutz Institute, São Paulo, São Paulo, Brazil

Despite over 50 years of population-wide vaccination, whooping cough incidence is on the rise. At the start of 1980, more than 40,000 cases of pertussis (incidence rate, >30/100,000 habitants-year) were reported annually in Brazil (http://www.cve.saude.sp.gov.br). The age groups at highest risk are infants and, to a lower extent, newborns who can get infected before receiving the first dose of vaccine and develop a severe course of the disease. Possible strategies to control these negative trends are to develop novel more effective vaccines using new adjuvants. The Enzyme-Linked Immunosorbant Spot (ELISpot) assay was first developed in 1983 as a highly sensitive method for the identification of antibody secretion at the single-cell level. This is the first study to show with *Bordetella pertussis* (Bp) acellular antigens complexed with (DODAB-BF) as adjuvant using prime booster system

of immunization in male and female outbred mice. Mice immunized intranasally (I.N) received the booster for subcutaneous route (S.C) with cationic complexes of Bp 25 µg/ml in 0.1 mM DODAB-BF were colloidally stable, exhibiting a mean diameter of 211,0 ± 3,7 optimal for antigen presentation. Magnitudes of the total B cell response were analyzed IgA, IgM and IgG by ELISpot four months after the last immunization in male and female outbred mice immunized from I.N/S.C. Bp+DODAB-BF were elicited significant levels of isotypes IgM> IgA> IgG and difference between the gender was not detectable. Our findings give supportive evidence that DODAB-BF showed to be a good adjuvant with potential use for the future development of vaccines.

A



A: Prime booster immunization in neonatal mice. Magnitudes of the total B cell response were analyzed IgA, IgM and IgG by ELIspot four months after the last immunization in male and female (♂♀) outbred mice immunized using the prime-boost system I.N/S.C.

B

IgA ♀	IgA ♂
Bp = 89 spots	Bp = 170 spots
Bp+DODAB-BF = 137 spots	Bp+DODAB-BF = 207 spots

IgM ♀	IgM ♂
Bp = 272 spots	Bp = 74 spots
Bp+DODAB-BF = 479 spots	Bp+DODAB-BF = > 500 spots

IgG ♀	IgG ♂
Bp = 44 spots	Bp = 81 spots
Bp+DODAB-BF = 360 spots	Bp+DODAB-BF = 286 spots

B: Splenocytes were adjusted to a density (1 x 107 cells/mL) and plated into 96-well ELIspot plates (Mabtech) coated with an anti-mouse IgM, IgA and IgG antibodies at 180 µL/well. The spots represent the antibodies produced post-stimulation with 20 µg/mL of Bp. The ELIspot plates were developed according to the manufacture's manual and read with AID ELISpot (Autoimmun Diagnostika GMBH, Germany).

W03. Evaluation of Difference Immune Response in Neonatal Mice using Outer Membrane Vesicles of *Neisseria meningitidis* B Complexed with Two Different Adjuvants

Fernanda Ayane de Oliveira Santos[1] and Elizabeth De Gaspari[2]. [1]São Paulo University, São Paulo, Sao Paulo, Brazil; [2]Adolfo Lutz Institute, São Paulo, Sao Paulo, Brazil

Aluminum hydroxide (HA) is the most widely used commercially to improve the immunogenicity of the vaccine. The cationic lipid dioctadecyldimethylammonium bromide (DODAB-BF) is an effective adjuvant and has been studied as a model for vaccines via mucosal and parenteral. The research process of a new adjuvant with lower cytotoxicity with good efficiency in modulation of immune response, biocompatibility and easy formulation using new antigenic preparations, is important of investigation against *Neisseria meningitidis.* The aim of this study was to evaluate the immunogenicity of antigenic from OMVs of *N. meningitidis* B complexed with two different adjuvants: DODAB-BF and HA comparing the evaluation of subcutaneous and intranasal immunization for the first time using the prime-boost system in outbred neonatal mice. As universal methods of antibody detection were used: Immunoblot, DOT-ELISA, ELISA and ELISpot aiming for the humoral and cellular immune response and of male and female mice. By Immunoblot analysis the specificity of antibodies with the homologous strain *N. meningitidis* B:4:P1.19.15. By DOT-ELISA was verified the cross-reactivity with different sorogroups (B, C, W and Y) that was not observed with HA. By ELISA the antibodies titers were quantified and compared in the sera of mice immunized with DODAB-BF+OMVs and HA+OMVs for IgG, IgG1 and IgG2a. The immunization routes used exhibited IgG titers, and both adjuvants promoted the production of IgG1 and IgG2a varying according to the route of immunization used. By

ELISpot was analyze IFN-γ- and IL-4 and the results showed the response directly to Th1 and Th2 profile.

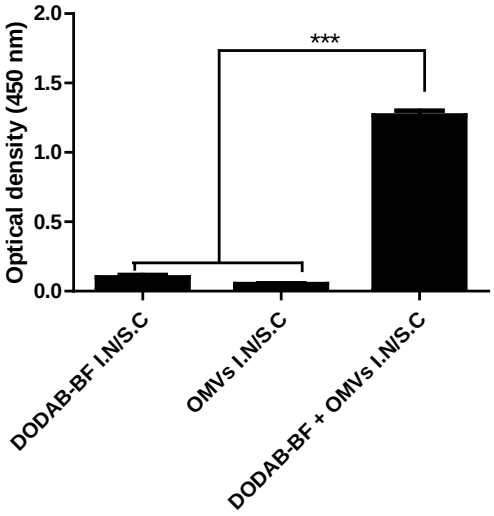


Figure 1: ELISA for the evaluation of IgG antibodies produced of male mice serum immunized with DODAB-BF, OMVs and DODAB-BF+OMVs by the immunization using the prime-boost system I.N/S.C at 47 age in outbred mice. The results represent sera 1:200 diluted. DODAB-BF, OMVs and DODAB-BF+OMVs. DODAB-BF control sera from mice immunized with DODAB-BF only. OMVs control sera from mice immunized with OMVs of *N. meningitidis* B:4 P:1.15,19. DODAB-BF+OMVs sera from mice immunized with DODAB-BF+OMVs from *N. meningitidis* B:4 P:1.15,19. Results are represented as mean ± SEM. *** P <0.0001.

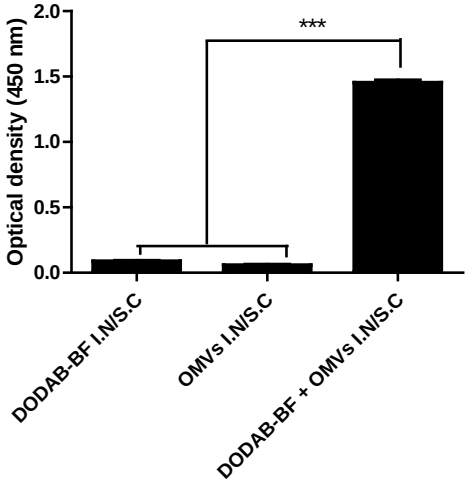


Figure 2: ELISA for the evaluation of IgG antibodies produced of female mice serum immunized with DODAB-BF, OMVs and DODAB-BF+OMVs by the immunization using the prime-boost system I.N/S.C at 47 days of age in outbred mice. The results represent sera 1:200 diluted. DODAB-BF, OMVs and DODAB-BF+OMVs. DODAB-BF control sera from mice immunized with DODAB-BF only. OMVs control sera from mice immunized with OMVs of *N. meningitidis* B:4 P:1.15,19. DODAB-BF+OMVs sera from mice immunized with DODAB-BF+OMVs from *N. meningitidis* B:4 P:1.15,19. Results are represented as mean ± SEM. *** P <0.0001

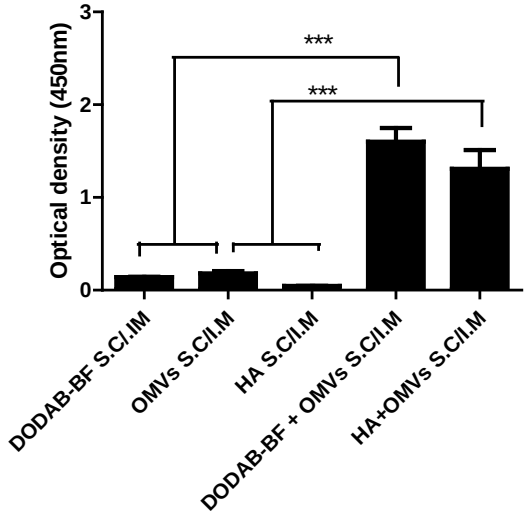


Figure 3: ELISA for the evaluation of IgG antibodies produced of male mice serum immunized using the prime-boost system S.C/I.M at 47 days of age in outbred mice. The results represent sera 1:500 diluted. DODAB-BF, OMVs, HA, DODAB-BF+OMVs and HA+OMVs. DODAB-BF control sera from mice immunized with DODAB-BF only. OMVs control sera from mice immunized with OMVs of *N. meningitidis* B: 4P: 1.15,19. HA control sera from mice immunized with HA only. DODAB-BF+OMVs sera from mice immunized with DODAB-BF+OMVs from *N. meningitidis* B:4 P:1.15,19. HA+OMVs sera from mice immunized with HA+OMVs of *N. meningitidis* B:4 P:1.15,19. Results are represented as mean ± SEM. *** P <0.0001

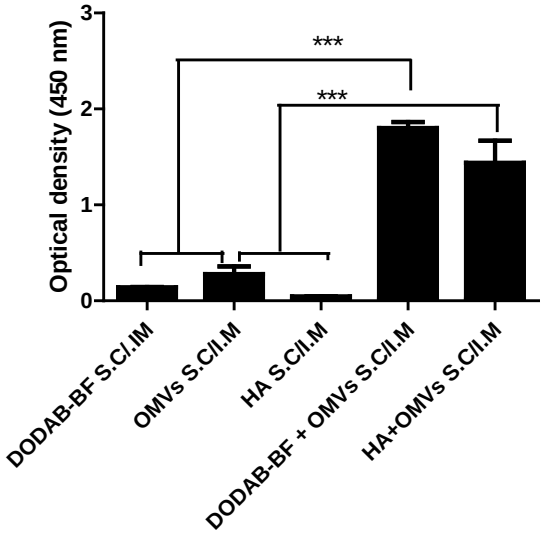


Figure 4: ELISA for the evaluation of IgG antibodies produced of female mice serum immunized using the prime-boost system S.C/I.M at 47 days of age in outbred mice. The results represent sera 1:500 diluted. DODAB-BF, OMVs, HA, DODAB-BF+OMVs and HA+OMVs. **DODAB-BF** control sera from mice immunized with DODAB-BF only. **OMVs** control sera from mice immunized with OMVs of *N. meningitidis* B:4 P:1.15,19.

HA control sera from mice immunized with HA only. **DODAB-BF+OMVs** sera from mice immunized with DODAB-BF+OMVs from *N. meningitidis* B:4 P:1.15,19. **HA+OMVs** sera from mice immunized with HA+OMVs of *N. meningitidis* B:4 P:1.15,19. Results are represented as mean ± SEM. *** *P* <0.0001.

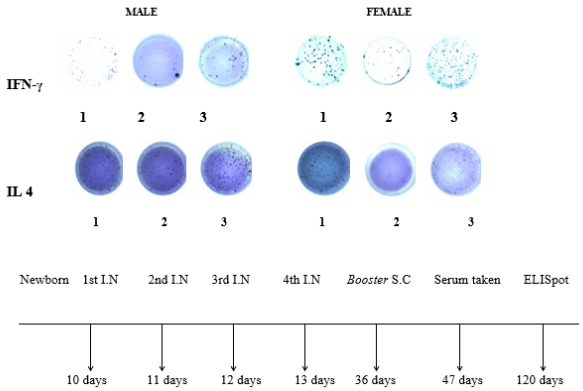


Figure 5: Magnitudes of the total T cell response were analyzed by IFN-γ and IL4 ELIspot four months after the last immunization in male and female outbred mice immunized using the prime-boost system I.N/S.C. **1** OMVs I.N/S.C; **2** DODAB-BF I.N/S.C; **3** DODAB-BF+OMVs I.N/S.C. Splenocytes were adjusted to a density (1 x 10^6 cells/mL) and plated into 96-well ELIspot plates (Mabtech) coated with an anti-mouse INF-γ and IL4 antibodies at 180 µL/well The spots represent interleukin produced post-stimulation with 20 µg/mL of OMVs of *N. meningitidis* B. ELIspot plates were developed according to the manufacture's manual and read with AID ELISpot (Autoimmun Diagnostika GMBH,Germany).

W108. Immunomodulatory Factors Present in Human Milk Induce T Cell-Independent IgA Class Switch Recombination in Naïve B Cells

John J. Varrone, Sade T. Fridy, Antti E. Seppo and Kirsi M. Jarvinen. University of Rochester Medical Center, Rochester, NY

Human milk provides passive immunity during the first weeks of life, when infant mucosal IgA production is deficient yet the developing gut is challenged with numerous immune stimuli. However, it remains unknown whether breast milk actively promotes infant IgA production in situ. Two IgA class switch recombination (CSR) factors, IL-10 and TGF-β, have been previously discovered in breast milk. Here we report abundant levels of APRIL, a key cytokine in the induction of T cell-independent IgA CSR, in milk samples from a diverse population of mothers. To address the role of these cytokines on IgA CSR, we developed an *in vitro* assay where naïve B-cells were isolated from cord blood by IgD+ selection, and then stimulated with IL-10, TGF-β, and APRIL or sterile milk serum for seven days before analysis of B-cell IgA production by flow cytometry. To elicit TGF-β biological activity, we also included acid-activated milk serum in our assays. We observed an increase in IgA_1 expression in B-cells treated with the cytokines or acid-activated milk serum (2- and 5-fold, resp.; n.s. and p=0.0028) as compared to unstimulated B-cells, but no change in B-cells treated with milk serum alone. To our knowledge, this is the first study to describe the presence of APRIL in human milk, and to demonstrate T cell-independent IgA CSR induced by breast milk that is dependent on acid activation, suggestive of the role of TGF-β. Further experiments are underway to dissect the relative individual contribution of each cytokine in breast milk-induced IgA CSR.

W109. Maternal Microbiota Educates Neonatal IgA Response

Qinghui Mu[1], Husen Zhang[2], Jay Kirby[1] and Xin Luo[1]. [1]Virginia Tech, Blacksburg, VA; [2] National Institute of Health, Bethesda, MD

Maternal microbiota plays a key role in governing the establishment of neonatal gut microbiota. However, whether and how maternal microbiota from breast milk would affect the development of neonatal immune response remains unclear. Here we report that *de novo* synthesis of neonatal IgA in pre-weaning Rag1-sufficient mouse pups could be super-induced by the milk of $Rag1^{-/-}$ nursing moms. 16S rRNA sequencing analysis showed that the gut microbiota from the upper intestine of Rag1-sufficient pups were distinct depending on the genotypes of their nursing moms, with those nursed by $Rag1^{-/-}$ moms exhibiting a higher bacterial diversity than those nursed by Rag1-sufficient moms. Interestingly, when $Rag1^{-/-}$ moms were co-housed (littermates) with Rag1-sufficient moms, or treated with antibiotics, the pups that they nursed no longer exhibited the heightened IgA synthesis. Further studies revealed that the number of type 3 innate lymphoid cells (ILC3s) in the pups' intestine was significantly increased when the pups were nursed by $Rag1^{-/-}$ moms. In addition, the super-

induction of neonatal IgA was abrogated when pups were treated with an aryl hydrocarbon receptor antagonist that is known to reduce the number and function of ILC3s. These results suggest that the maternal microbiota may educate neonatal IgA response through an ILC3-dependent mechanism.

W110. A 3-D Mouse Brain Microphysiological System (mBMPS) to Study Necrotizing Enterocolitis (NEC) Induced Brain Injury

Qinjie Zhou[1], Diego Nino[1], David Pamies[1], Helena Hogberg[1], Thomas Prindle[1,2], Chhinder Sodhi[1,2] and David Hackam[1,2]. [1]Johns Hopkins University, Baltimore, MD; [2]Johns Hopkins Hospital, Baltimore, Baltimore, MD

Necrotizing enterocolitis (NEC) is a devastating gastrointestinal disease that occurs to premature infants with high morbidity and mortality. For those who survived, neonates with NEC have significantly higher tendency for neurodevelopmental impairment compared to neonates without NEC. However, the underlying link between gut and brain remains to be elucidated. To enable unbiased large-scale screening for these factors from peripheral system, a physiologically relevant *in vitro* brain organoid system is therefore in demand. Here we have developed a 3-D mouse brain microphysiological system (mBMPS) to resemble *in vivo* mouse brain. mBMPS was comprised of differentiated and mature neurons and glial cells after 4-5 weeks' *in vitro* incubation, based on time course characterization of markers by real time PCR and immunostaining analysis. mBMPS demonstrated active neuronal activities characterized by calcium influx upon glutamic acid stimuli and harbored spontaneous electrical activity at basal level as measured by Maestro multielectrode array (MEA). Upon acut treatment of Lipopolysaccharide (LPS), proinflammatory cytokines such as IL-1β, TNFα, iNOS and Lipocalin 2 were induced which were largely dependent on functional TLR4 receptor as mBMPS derived from TLR4 knockout mouse had compromised reaction. Next, we will adopt large scale screening with factors derived from the peripheral system in NEC animals to identify potential factors to target to reverse NEC induced brain injuries, and further develop translational therapies for neonates who suffer from cognitive impairment due to NEC.

W111. Protein Malnutrition Alters Tryptophan and Angiotensin Converting Enzyme 2 Homeostasis and Impairs Adaptive Immune Responses in Gnotobiotic Pigs Transplanted with Human Infant Fecal Microbiota and Infected with Human Rotavirus

David D. Fischer[1], Sukumar Kandasamy[1], Ayako Miyazaki[1], Francine C. Paim[1], Stephanie N. Langel[1], Lulu Shao[2], Moyasar A. Alhamo[1], Juliet Chepngeno[1], Huang-Chi Huang[1], Anand Kumar[3], Gireesh Rajashekara[1], Linda J. Saif[1] and Anastasia N. Vlasova[1]. [1]Ohio State University, Wooster, OH; [2]University of Pittsburgh, Pittsburgh, PA; [3]Los Alamos National Laboratory, Los Alamos, NM

Malnutrition leads to stunted growth and increased morbidity contributing to nearly half of all deaths in children under 5. Mortality due to rotavirus (RV) diarrhea is common in areas where malnutrition is prevalent; however, the relationship between malnutrition and RV infection remains unclear. In this study, gnotobiotic pigs were fed protein-sufficient or -deficient diets with or without tryptophan supplementation, transplanted with an infant's fecal microbiota and infected with virulent human RV (HRV). Protein deficiency decreased HRV antibody and total IgA concentrations, systemic T helper and cytotoxic T cell frequencies, and serum tryptophan and angiotensin I converting enzyme 2 (ACE2) post-infection. Deficient diet pigs had impaired tryptophan catabolism post-infection compared with sufficient diet pigs; tryptophan supplementation increased the frequencies of regulatory T cells in deficient and sufficient diet pigs. These results indicate that a protein-deficient diet impairs activation of the adaptive immune response following HRV infection and alters tryptophan homeostasis.

W112. Maternal-Derived IgG/IgE Immune Complexes Prime Cord Blood Basophils for Anti-IgE and Anti-IgG Activation

Sara Paveglio[1], Karim Rezaul[1] and Adam Matson[1,2]. [1]University of Connecticut Health, Farmington, CT; [2]Connecticut Children's Medical Center, Farmington, CT

The placenta expresses high levels of the neonatal Fc receptor, FcRn, which facilitates the transplacental passage of maternal IgG. Prior work performed in our laboratory has provided compelling evidence that

maternal IgE traverses the placenta as IgG/IgE immune complexes (ICs). We determined that essentially all IgE in cord blood (CB) serum is bound by IgG, strongly suggesting that this is the predominant mechanism for fetal IgE acquisition. We hypothesize that following entry into the fetal circulation, maternal IgG/IgE ICs bind to FcεRI-expressing cells such as basophils, leading to sensitization and subsequent histamine release. Preliminary data to support this hypothesis demonstrated that cross-linking maternal ICs on CB basophils using either anti-IgE or anti-IgG, resulted in increased surface expression of the basophil activation markers CD63 and CD203c. Preincubation of maternal serum with omalizumab, a humanized anti-IgE that prevents IgE from binding to FcεRI, diminished this activation. Similarly, maternal ICs purified from serum via protein A chromatography, spontaneously induced β-hexosaminidase release in RBL-SX38 cells, a rat basophil leukemia cell line that expresses human FcεRI. The spontaneous degranulation was limited by preincubation of the ICs with omalizumab. These data are significant as we have previously shown that histamine impairs the ability of CB myeloid dendritic cells (mDCs) to properly respond to innate immune ligands such as lipopolysaccharide (LPS). Thus, an overall goal of our research is to determine the ability of maternal ICs to mediate fetal basophil histamine release and alter mDC responsiveness to microbial ligands that are typically associated with anti-allergenic Th1 responses.

W114. Neonatal Mice Possess Two Phenotypically and Functionally Distinct Lung-Migratory CD103$^+$ Dendritic Cell Populations Following Respiratory Infection

Tracy Ruckwardt, Kaitlyn Morabito, Erez Bar-Haim, Deepika Nair and Barney Graham. National Institutes of Health/National Institute of Allergy and Infectious Diseases, Bethesda, MD

The CD103$^+$ subset of lung migratory dendritic cells (DCs) plays an important role in the generation of CD8$^+$ T cell responses following respiratory infection. Here, we demonstrate that the dependence on CD103$^+$ DCs for stimulation of RSV-specific T cells is both epitope and age-dependent. CD103$^+$ DCs in neonatal mice develop two phenotypically and functionally distinct populations following respiratory infection. Neonatal CD103$^+$ DCs expressing low levels of CD103 (CD103lo DCs) and other lineage and maturation markers including costimulatory molecules are phenotypically immature and functionally limited. Expression of GM-CSF is lower in the lungs of naïve and infected neonates than in their adult counterparts, which may limit the development of CD103$^+$ DC in the lung. CD103lo DCs sorted from infected neonates were unable to stimulate cells of the $K^dM2_{82\text{-}90}$ specificity, which are potently stimulated by CD103hi DCs sorted from the same animals. These data suggest that the delayed maturation of CD103+ DCs in the neonate limits the $K^dM2_{82\text{-}90}$-specific response and explain the distinct CD8$^+$ T cell response hierarchy displayed in neonatal mice that differs from the hierarchy seen in adult mice. These findings have implications for the development of early-life vaccines, where the promotion of responses with less age bias may prove advantageous. Alternately, specific approaches may be used to enhance the maturation and function of the CD103lo DC population in neonates to promote more adult-like T cell responses.

W115. Relationship Between Antigen Presenting Cells and iNKT Cells During Early-Life and how this Could Influence the Development of Later Life Susceptibility to Colitis

Thomas Gensollen. Brigham and Women's Hospital, Boston, MA

Substantial evidence indicates that the interactions between the commensal microbiota and the immune system of the intestinal tissues of the host during childhood may set the stage for later development of the immune system and susceptibility to inflammatory bowel diseases (IBD) in a genetically susceptible host. However, the mechanisms by which exposure to microbes during early life is influencing susceptibility to IBD remains poorly understood. Among the numerous immune cells that are involved in the pathogenesis of IBD, invariant natural killer T (iNKT) cells are increasingly recognized to play an important role. The absence of microbiota during early life but not thereafter leads to iNKT cell accumulation in the colon and later life susceptibility to colitis initiated by an environmental trigger. The mechanisms underlying the accumulation of iNKT cells and how they are regulated by the microbiota exclusively during the "neonatal window of opportunity" remains elusive. Using specific models of transgenic mice that allow for inducible cell depletion, we show that signal(s)

regulating iNKT cell accumulation during early life that are influenced by the microbiota are specifically provided by colonic hematopoietic cells. We characterized the relation between these cells and iNKT cells during early-life and how this could influence the development of later life susceptibility to colitis.

W118. Necrotizing Enterocolitis Associated Lung Injury, Pathogenesis and Therapeutic Strategy

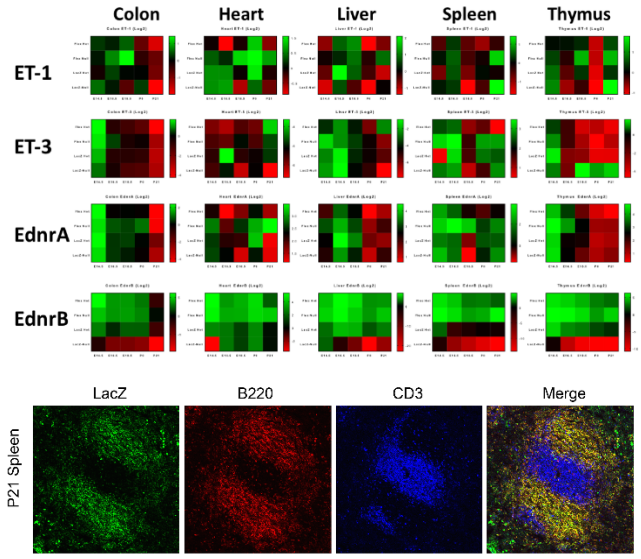
Hongpeng Jia[1,2], Chhinder Sodhi[1,2], Yukihiro Yamaguchi[1,2], Peng Lu[1,2], Laura Martin[2], Misty Good[3], Qinjie Zhou[2], Jungeun Sung[2], William B. Fulton[1], Diego F. Nino[2], Thomas Prindle[1,2], John Ozolek[4] and David Hackam[1,2]. [1]Johns Hopkins Hospital, Baltimore, Baltimore, MD; [2]Johns Hopkins University, Baltimore, MD; [3]Washington University in St. Louis, St. Louis, MO; [4]Children's Hospital of Pittsburgh, Pittsburgh, PA;

Necrotizing enterocolitis (NEC) is the leading life-threatening gastrointestinal disease in premature infants with extremely high mortality and morbidity. In those patients who survive NEC, one of the most important long-term consequences is the development of severe lung disease, which is more severe than the lung disease that develops in premature infants that do not develop NEC, suggesting that the lung disease is linked to the gut inflammation. However, the underlying mechanism remains incompletely understood. The overall goal of this study is to understand the mechanisms leading to the development of NEC-associated lung injury, and to identify novel preventative or therapeutic approaches for this devastating complication of prematurity. We found in our study that TLR4 expression in the lung gradually increases during postnatal development, and that mice and humans with NEC-associated lung inflammation express higher levels of pulmonary TLR4 than age-matched controls. NEC in wild-type newborn mice resulted in significant pulmonary injury that was prevented by deletion of TLR4 from the pulmonary epithelium, indicating a role for pulmonary TLR4 in lung injury development. Mechanistically, intestinal epithelial TLR4 activation induced high mobility group box-1 (HMGB1) release from the intestine which activated pulmonary epithelial TLR4, leading to the induction of the neutrophil recruiting C-X-C motif chemokine-5 (CXCL5) and the influx of pro-inflammatory neutrophils to the lung. Strikingly, the aerosolized administration of a novel carbohydrate TLR4 inhibitor prevented CXCL5 upregulation and blocked NEC-induced lung injury in mice

W119. Expression Patterns of the Endothelin Axis in Sites of Extramedullary Hematopoiesis in Hirschsprung Disease

Giuliana Medrano[1], Peihong Guan[1], Amanda Barlow-Anacker[2] and Ankush Gosain[1,3]. [1]University of Tennessee Health Science Center, Memphis, TN; [2]University of Wisconsin, Madison, WI; [3]Le Bonheur Children's Hospital, Memphis, TN

Extramedullary Hematopoiesis (EH) is a component of development and can also be triggered during pathological conditions. Endothelins (Et1, Et3) and their receptors (EdnrA, EdnrB) modulate lymphocyte function. Gene defects in EdnrB/Et3 result in Hirschsprung disease (HSCR). Hirschsprung-Associated Enterocolitis (HAEC), the most serious complication of HSCR, is associated with altered lymphocyte populations in bowel and spleen. We sought to determine the temporal and spatial expression pattern of the endothelin axis and the cellular sources of expression in the developing hematopoietic system and in HSCR. Using an embryonic and early post-natal time course in WT and two murine models of HSCR/HAEC, we found that Et1 was stably expressed in heart, spleen and thymus, with increased post-natal expression in liver and colon. Et3 was highly expressed in the early embryonic colon and spleen. EdnrA was highly expressed in the embryonic colon, spleen and thymus, as well as post-natal heart and liver. EdnrB was highly expressed in the embryonic colon, spleen, and heart, as well as post-natal liver. Within the post-natal spleen, B lymphocytes were identified as expressing EdnrB. Variations in expression of all four genes were seen in HSCR/HAEC. We conclude that the components of the endothelin axis are widely expressed in sites of EH and that murine HSCR/HAEC includes significant alterations in this pattern of expression. The precise identity of endothelin axis-expressing cells and their function in HAEC remain to be elucidated.



W43. Prevention of Allergy Development by Early Administration of Probiotic Strain *E. Coli* O83:K24:H31. Possible Effect of this Probiotic Strain on Immune System of Newborns

Jiri Hrdy, Lenka Sukenikova, Olga Novotna, Petra Petraskova, Kristyna Borakova and Ludmila Prokesova. Charles University and General University Hospital in Prague, Prague, Stredocesky kraj, Czech Republic

Allergy belongs to one of the most common diseases with steadily increasing incidence. Early administration of selected probiotic strains could prevent allergy development. We have shown the decreased allergy incidence in children after postnatal application of the probiotic strain *Escherichia coli* O83:K24:H31 (EcO83). The effect of EcO83 on newborn immune system and the capacity of EcO83 to promote dendritic cell (DC) maturation and polarisation of immune responses were followed. Increased presence of activation marker CD83 was observed on DC stimulated *in vitro* by EcO83, DC of newborns of allergic mothers having significantly higher increase of CD83 surface expression than DC of children of healthy mothers. Increased gene expression and secretion of IL-10 was detected in DC stimulated with EcO83, the increase being higher in DC of newborns of healthy mothers in comparison with that of allergic ones. Coculture of EcO83 stimulated DC with $CD4^+$T cells generally increased the presence of intracellular cytokines tested. Intracellular presence of Th1 and Th2 cytokine was more pronounced in T cells of newborns of allergic mothers whereas IL-10 was increased CD4+T cells of newborns of healthy mothers. We can conclude that newborns of allergic mothers have generally increased reactivity of both DC and $CD4^+$T cells which together with decreased capacity of DC of newborns of allergic mothers to produce IL-10 could support inappropriate immune responses development after allergen encounter. EcO83 induces dendritic cell maturation and increases their production of IL-10. This modulation could help to suppress allergy development. This work was supported by AZVCR15-26877A.

Mucosal Infections

L.02, F107. Determination of IgA1 and IgA2 in Human Colostrum and its Association with the Number of Infections in the Newborn

Erick Saul Sanchez[1], Leopoldo Santos[1], Kaleb Mondragon[2], Victor Manuel Contreras[3], Ma del Rocío Thomson Bonilla[3], Arturo Cérbulo Vázquez[4], Julio Cesar Alcántara Montiel[5] and Hector Romero Ramírez[1]. [1]Cinvestav-IPN, City of Mexico, Distrito Federal, Mexico; [2]IPN, Cuautla, Morelos, Mexico; [3]Hospital Regional 1º de octubre ISSSTE, City of Mexico, Distrito Federal, Mexico; [4] Hospital de la Mujer. Secretaria de Salud División de Enseñanza e Investigación, City of Mexico, Distrito Federal, Mexico; [5]Hospital de Alta Especialidad Ixtapaluca, City of Mexico, Distrito Federal, Mexico

At birth, newborns interact with many environmental antigens with an immature immune system. Thus, their defense depends on various factors they receive from their mothers before and after birth. Colostrum and breast milk provide not only sources of food but also contain cells and proteins for their defense. One of the most important factors is the SIgA which is produced by the plasma cells in the mammary acini and transported into the colostrum and milk. SIgA in the newborn will play an important role in protection by immune exclusion and in the homeostasis between the mucosa and normal microbiota. A decrease in systemic IgA levels is related to increased susceptibility to infectious diseases at different stages of life of the individual, but this is especially important during the lactation of the newborn. In Mexico, there are no studies quantifying different isotypes of immunoglobulin in colostrum. Thus, we quantified their presence in colostrum by ELISA with emphasis in levels of IgA1 and IgA2. The results were correlated to the number of infections presented by the mothers during the last six months of pregnancy and with the newborns, during the first three months of life. The results, so far, have indicated a correlation of low levels of IgA with increase in the number of respiratory, gastrointestinal, urogenital, cutaneous, autoimmune, and allergic diseases by the mothers. The analysis of newborns has not started yet. The results will allow a better understanding of the role of IgA in the health of newborn children.

OR.12, W121. Rectal LCMV Infection Results in Systemic Viral Dissemination Mediated by Inflammation Driven Influx of Immune Cells

Martin Trapecar[1], Ben Cohn[1], Shahzada Khan[1], Eric Woodruff[1], Julie Luong[1], Frank Wu[1] and Shomyseh Sanjabi[1,2]. [1]Gladstone Institute of Virology and Immunology, San Francisco, CA; [2]University of California, San Francisco, San Francisco, CA

Understanding the immune response to vaginal and rectal viral exposure is required to combat sexual transmission. We vaginally infected wild-type mice with lymphocytic choriomeningitis virus (LCMV) or Zika virus (ZIKV) and showed that both replicate in the vaginal mucosa with minimal induction of type I/III interferons (IFN), causing dampened innate-mediated control and failure to mature local antigen-presenting cells (APCs). Lack of APC maturation in the mucosa delayed CD8 T cell activation in the draining lymph node and hindered timely appearance of effector CD8 T cells in vaginal mucosa, postponing viral control. However, LCMV replication remained localized without systemic dissemination (Khan et al. 2016). In contrast, we found that rectal inoculation of LCMV triggers early viral RNA sensing, leading to induction of IFNs and upregulation of RIG-I/MDA5. This response is followed by induction of chemokines that lead to recruitment of various immune cells to the colon. Rectal infection of IFNAR-/- mice reduced IFN and inflammatory signals and curbed leukocyte influx into the colon, which resulted in decreased viral spread to the spleen. A similar delay in early viral spread was obtained infecting $CCR2^{-/-}$ mice and at later time points in $RAG1^{-/-}$ mice. Viral induced inflammation in the rectum promotes immune cell trafficking and systemic dissemination prior to adaptive immune activation. Furthermore, viral persistence is prolonged in the colon compared to other organs. Our findings demonstrate the risk of rapid viral dissemination associated with rectal viral infections and provide evidence for dampened immunity that leads to viral persistence in the colon.

OR.13, W122. IgA Antibodies Targeting Phosphoprotein Inhibit Measles Virus Replication by Blocking Formation of RdRp Complex and Securing Type I Interferon Pathway of Epithelial Cells

Yi Yang[1], Dihan Zhou[1,2] and Huimin Yan[1,2]. [1]State Key Laboratory of Virology, Wuhan, Hubei, China; [2]Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China

Most viruses enter our body through mucosa, and the mucosal epithelial cells (ECs) are usually the very initial target cells for viral early replication. Secretory IgA antibody mediates primary defense functions against viral infection related to mucosal ECs. Besides the immune exclusion activity to block viral attachment from apical surface of ECs, IgA is usually located within ECs by transcytosis and able to interact with viral targets inside ECs if virus gets entry into ECs. This provides the cellular basis for IgA to inhibit early viral replication by intracellular neutralization. More importantly, the intracellular neutralization activity endows IgA with more potential anti-viral activities specific not only to viral surface components but also to viral non-surface and non-structure proteins. We have previously demonstrated that measles virus (MV) matrix protein specific IgA can interact with newly synthesized matrix protein during IgA transport through the ECs, and indeed neutralize MV replication intracellularly. In the present study, we further screened and obtained two monoclonal IgA antibodies specific to phosphoprotein of MV. We observed that both of the IgA MAbs can suppress MV replication efficiently in Caco-2 cells, but one of them cannot inhibit viral replication in Vero-IgR cells. Further investigations showed that one IgA inhibits MV replication by blocking formation of viral RdRp complex, and another one inhibits MV replication by securing type I interferon pathway of ECs. These data imply that IgA antibodies against viral non-structural components have unique anti-viral functions, which may help to design novel anti-viral drugs and vaccines.

OR.30, W120. A Mechanism for the Induction of Type 2 Immune Responses by a Protease Allergen in the Female Genital Mucosa

Ji Eun Oh, Dong Sun Oh, Hi Eun Jung and Heung Kyu Lee. Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea

The genital mucosa is a barrier that is constantly exposed to a variety of pathogens, allergens, and external stimuli. Although both allergen exposure and parasite infections frequently occur in the genital area, the mechanism by which immune responses – particularly type 2 immunity – are induced has rarely been studied in the genital mucosa. Here, we demonstrate the induction of T helper type 2 immunity in the genital mucosa in response to a model allergen, the protease papain. Intravaginal papain immunization induced type 2 immunity in a manner that was dependent on protease activity and the estrous phase of the mice. In addition, IL-33 was released from the vaginal epithelia after intravaginal papain immunization, leading to the activation of type 2 innate lymphoid cells. Moreover, the IL-33-MyD88 signalling pathway was critical for the induction of type 2 immunity. We also found that Th2 differentiation in response to intravaginal papain treatment requires a specific DC subset that is controlled by IRF4. These findings suggest that type 2 immunity is induced by a unique mechanism in the genital tract, which is an important, but often overlooked, barrier surface.

OR.64, T87. T Cells, Not Autoantibodies, Drive Susceptibility to Mucosal Candidiasis in Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy (APECED)

Timothy Break and Michail S. Lionakis. National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, MD

APECED, caused by mutations in autoimmune regulator (*AIRE*), is characterized by multisystem autoimmunity and susceptibility to chronic mucocutaneous candidiasis (CMC). AIRE is necessary for the negative selection of self-reactive *T cell*s in the thymus, which explains why AIRE-deficiency leads to autoimmunity. How AIRE-deficiency causes CMC susceptibility, however, remains elusive. It has been postulated that autoantibodies against IL-17/IL-22 drive CMC susceptibility, but correlation between

CMC and these autoantibodies is only seen in ~70% of patients, suggesting that other factors may also play a role. Here, we have established the first mouse model of mucosal candidiasis in Aire-deficient mice, which have significantly elevated fungal load and mucosal injury post-oral infection, despite the absence of IL-17/IL-22 autoantibodies; in agreement, transfer of *Aire*$^{-/-}$ serum into *Aire*$^{+/+}$ mice does not promote *Candida* susceptibility. Strikingly, induction of IL-17A/F, IL-22 and IL-17-dependent antimicrobial peptides, was not impaired in the oral mucosa of *Aire*$^{-/-}$ mice and APECED patients. Furthermore, neutralization of IL-17A/IL-17F or IL-22 in *Aire*$^{-/-}$ mice further increased mucosal fungal burden. Remarkably, *Aire*$^{-/-}$*Tcr-alpha*$^{-/-}$ double-knockout mice control the infection, indicating that *T cell*s drive infection susceptibility. Indeed, significantly greater numbers of activated CD4 and CD8 *T cell*s accumulate in the *Aire*$^{-/-}$ oral mucosa, and adoptive transfer of *T cell*s obtained from *Aire*$^{-/-}$ but not *Aire*$^{+/+}$ mice into Rag knockout recipient mice was sufficient to promote increased oral mucosal fungal load post-infection. Ongoing work is aimed at characterizing the phenotype, polarization, repertoire, and function of *T cell*s in the oral mucosa of *Aire*$^{-/-}$ mice and the mechanisms by which they mediate fungal infection susceptibility.

OR.65, T99. Pulmonary Innate Type 2 Inflammatory Responses Protect Against *Staphylococcus Aureus*-Induced Sepsis

Paulette Krishack, Tyler Louviere, Anne Sperling, and Philip Verhoef. University of Chicago, Chicago, IL

Sepsis is defined as life-threatening organ dysfunction caused by the body's response to infection. Sepsis is associated with overwhelming type 1 or type 17 immunologic inflammation, but the role of type 2 immune responses in sepsis remains unknown. To test the role of type 2 immune responses in sepsis, we treated mice with intratracheal IL-33 to induce an innate pulmonary type 2 response, followed by intravenous infection with *S. aureus* to induce sepsis. Surprisingly, type 2 responses were beneficial during acute infection, as IL-33 treatment protected mice from *S. aureus*-induced death. An increase in the ratio of lung eosinophils to neutrophils was revealed in IL-33 treated, *S. aureus* infected mice, suggesting that type 2 responses suppress lung neutrophilia. Further, little difference was found in splenic granulocytes or lymphocytes, suggesting that activation of pulmonary immune responses alone was sufficient for protection. Infection of PLZF null mice, which have defective NKT and ILC2 cells normally required for type 2 innate responses after IL-33 treatment, resulted in accelerated mortality compared with wild type littermate controls. Moreover, IL-33 did not rescue PLZF null mice from death. However, infection of CD1d null mice, which lack only NKT cells but have intact ILC2s, demonstrated no difference in mortality. Thus, pulmonary type 2 innate immunity provided by ILC2s is critical for protection against *S. aureus*-mediated death.

OR.66, T91. Retinoic Acid Induces CD11b^{+} Leukocyte Production of IL-17 and T Cell Responses that Protect the Vitamin A Deficient (A^{-}) Host from Gastrointestinal Infection

Lindsay Snyder, Veronika Weaver, A. Catharine Ross and Margherita Cantorna. Pennsylvania State University, University Park, PA

Vitamin A deficiency affects approximately 250 million preschool age children worldwide and is associated with increased susceptibility to enteric disease. Similarly, vitamin A deficient (A^{-}) mice are extremely susceptible to *Citrobacter rodentium* infection. Treating A^{-} mice with retinoic acid (RA) twice (2x) at d7 and d9 post-infection effectively cleared the infection. To determine the role of retinoid signaling in T cells during infection, mice with *T cell* specific disruption of retinoid signaling (T-dnRAR) were infected with *C. rodentium*. T-dnRAR mice became chronically infected with C. *rodentium* and had higher systemic bacterial burdens at d10 compared to WT littermates. Similar to A^{-} mice, T-dnRAR mice were unable to clear the infection. RA treatment of chronically infected T-dnRAR mice at d37 was ineffective for eliminating the *C. rodentium*, suggesting that retinoid signaling in T cells is required for clearance. To elucidate the immunological changes being induced by RA, infected A^{-} and 2x RA treated A^{-} mice were sacrificed at d10 post-infection. In the colon, 2x RA treatment induced *il17a* mRNA and increased the number of IL17a producing cells. The majority of IL17a producing cells were CD11b+ cells (~80%), including neutrophils and macrophages. CD4 *T cell*s accounted for only a small fraction of the IL17a+ cells. These data demonstrate novel retinoid targets including innate cell-mediated production of IL17a at the peak of infection and clearance-inducing T cell responses late in infection. RA regulation of innate and adaptive immune

responses is associated with protection from gastrointestinal infection in A- hosts.

OR.67, T110. Contribution of Inflammatory Monocytes to the Pathogenesis of *Salmonella*-Induced Colitis

Patrick A. McLaughlin[1], Julie A. Bettke[1], Jason W. Tam[2], Joanna D. Luke[1], Jesse Leeds[1], James B. Bliska[1], Brian P. Butler[3] and Adrianus WM van der Velden[1]. [1]Stony Brook University, Stony Brook, NY; [2]University of North Carolina, Chapel Hill, Chapel Hill, NC; [3]St. George's University, True Blue, Grenada

Salmonella are pathogenic bacteria that invade intestinal epithelial cells and induce a secretory response that initiates the recruitment of innate immune cells. While this inflammatory response helps limit *Salmonella* invasion in the long term, recent studies have shown that the inflammatory response also provides nitrate, a respiratory electron acceptor used by *Salmonella* for growth in the inflamed intestine. The generation of inflammation-derived nitrate is dependent on *Nos2*, which encodes inducible nitric oxide synthase (iNOS), an enzyme that catalyzes the production of nitric oxide (NO). However, the cellular source(s) of iNOS and, therefore, NO-derived nitrate used by *Salmonella* for growth in the inflamed intestine have yet to be identified. We previously demonstrated that inflammatory monocytes recruited into tissues during *Salmonella* infection are major producers of NO and that their ability to produce NO is dependent on iNOS. Here, we report that inflammatory monocytes infiltrate ceca of mice infected with *Salmonella*. We show that *Ccr2*, which encodes CC-chemokine receptor 2 (CCR2), is required for inflammatory monocytes to infiltrate ceca of mice infected with *Salmonella* and that the severity of the pathology induced during *Salmonella* infection is reduced in mice that lack *Ccr2*. We also show that nitrate levels and *Nos2* transcript levels are reduced in ceca of *Salmonella*-infected mice that lack *Ccr2*, and that the ability of *Salmonella* to use nitrate respiration for growth in the inflamed intestine is reduced in mice that lack *Ccr2*. Collectively, these results suggest that inflammatory monocytes play a key role in the pathogenesis of *Salmonella*-induced colitis.

OR.68, T97. Goblet Cell Associated Antigen Passages are Inhibited by and Used as a Portal of Entry During Enteric Infection

Devesha Kulkarni, Keely G. McDonald, Mark Miller and Rodney Newberry. Washington Univesity School of Medicine, St. Louis, MO

Appropriate exposure of the immune system to dietary antigens is crucial to induce tolerance and maintain intestinal homeostasis. However, exposure to innocuous luminal substances during an enteric infection can lead to inappropriate inflammatory responses, suggesting the existence of mechanisms to limit steady state luminal sampling during infection. We recently demonstrated a role for small intestinal (SI) goblet cells (GCs) in delivering luminal antigens across the epithelium by forming goblet cell associated antigen passages (GAPs) in the steady state. How pathogenic bacteria affect the formation of GAPs and the ability of pathogens to use GAPs as a portal of entry are unexplored. We observed that GAPs, antigen delivery to the LP-DCs, and immune responses to luminal antigen in the MLN were acutely inhibited by wildtype, but not mutant non-invasive *Salmonella typhimurium (St)* infection. GAP inhibition was mediated by IL-1b activating Myd88 and EGFR in GCs, suppressing GAP formation. *St* preferentially localized around and within the GCs forming GAPs, and the translocation *St* to the draining MLN required GCs and correlated with GAP density. Overriding GAP inhibition during *St* infection by deletion of Myd88 or EGFR in GCs resulted in the return of immune responses to dietary antigen in the MLN, increased pathogen dissemination, increased inflammation, and shortened survival. These observations indicate that steady state sampling pathways are closely regulated during infection to prevent inappropriate responses to innocuous antigens, to limit pathogen entry, and to shift immune responses to promote pathogen clearance.

T100. The Key Role of TPL2 in Regulating *C. Difficile* Infection-Mediated Inflammation

Xingmin Sun. University of South Florida, Tampa, FL

Tumor progression locus 2 (TPL-2) plays a critical role in the response to inflammatory signals as it functions as a serine/threonine kinase in the MAPK signal transduction cascade known to regulate both innate and adaptive immunity. The pro-inflammatory actions of TPL-2 are mediated by the activation of MAPKs,

including ERK, c-Jun NH_2-termianl kinase (JNK) and p38 MAPK. Both *Clostridium difficile* TcdA and TcdB are capable of inducing pro-inflammatory cytokines including TNF-α, IL-1β and IL-6, which are implicated in the development and progression of *C. difficile* infection (CDI). Previously, we showed that both TcdA and TcdB could activate p38 MAPK and ERK in both Raw264.7 macrophages and mouse bone marrow-derived dendritic cells (BMDCs). We also found that TcdA-mediated TNF-α production in RAW264.7 cells was mediated through p38 MAP kinase and MEK/ERK signaling pathways. In this study, we investigated whether TPL2 play a central role in CDI severity by mediating the production of pro-inflammatory cytokines including TNF-α, IL-1β and IL-6. We report here that in BMDCs or bone marrow derived macrophages (BMDMs), a TPL2 specific inhibitor abolished or significantly reduced TcdB-induced production of TNF-α, IL-1β and IL-6. We further demonstrated that TPL2 inhibitor dramatically blocked TcdB-induced activation of ERK and p38 MAP kinase, but not of JNK in BMDCs. We confirmed these results using BMDCs or BMDMs extracted from TPL2-knock out (TPL2-KO) mice. Finally, we demonstrated TPL2-KO mice were significantly more resistant than wild-type mice to *C. difficile* infection in mice. Taken together, our data suggest TPL2 represents a potential therapeutic target for CDI treatment.

T101. Immune Regulation in the Gut During Persistent Virus Infection

Bethany Macleod[1], Heidi Elsaesser[2], Cynthia Guidos[3] and David Brooks[2]. [1]University Health Network, Toronto, ON, Canada; [2]Princess Margaret Cancer Centre, Toronto, ON, Canada; [3] Sickkids, Toronto, ON, Canada

During chronic virus infection the host immune response is unable to clear the pathogen and the virus persists long term. The sustained antigen load creates an immunosuppressive environment that results in the functional alteration of multiple immune subsets, including CD4 and CD8 T cells, B cells, dendritic cells and macrophages. Persistent infection of mice with LCMV Clone-13 results in profound T cell exhaustion in both lymphoid organs and certain peripheral tissues such as the CNS and kidneys. However, the extent to which virally exhausted cells are able to persist and function in the mucosa is poorly understood. We utilized mass cytometric analysis of 33 immune cell markers to build a global "immunological footprint" of innate and adaptive immune cell exhaustion in lymphoid organs, the circulation, and, peripheral mucosal tissues (the GI tract) during LCMV Cl-13 infection. Furthermore, we compared the immunological footprints of mice following immunotherapy. These studies provide novel insight into the unique mucosal environment in the context of persistent virus infection, and, during immunotherapy-mediated viral control.

T102. Reduced ILC3, Th17, and FOXP3/RORt$^+$ T Reg Cells and Severe C. Rodentium Infection in Vitamin D Deficient Mice

Yang-Ding Lin and Margherita Cantorna. Pennsylvania State University, University Park, PA

Vitamin D deficient (D^-) mice are extremely susceptible to *Citrobacter rodentium* infection. Th17 cells are required for clearance of *C. rodentium*. Innate lymphoid cells (ILC) are early sources of IL-22 (ILC3) that regulate T cell responses. Vitamin D sufficient (D^+) and D^- mice had equal numbers of $CD3^+$, $CD4^+$ and $TCRab^+$ T cells in the colon. The ILC3 and Th17 frequencies were lower in the D^- colon. D^+ colonic ILC3s and $CD4^+$ T cells produced significantly more IL-22 and IL-17 than D^- both before and after infection. In addition, $FOXP3^+$ and FOXP3/$RORgt^+$ T regs were significantly higher in D^+ than D^- mice. Conversely, frequencies of the FOXP3/GATA3 T cells were lower in D^+ than D^- mice; demonstrating a shift in the $FOXP3^+$ phenotype in the D^- host. Treatment of the D^- mice with the active form of vitamin D ($1,25(OH)_2D$, 1,25D) resulted in the recovery of Th17 and ILC3 subsets but not the FOXP3/RORgt population. Vitamin D is required for ILC3, Th17 and RORgt/FoxP3 T cells in the colon. The data suggests a developmental role for vitamin D in RORgt T reg cells that cannot be recovered by 1,25D treatments. Increased susceptibility of D^- mice to *C. rodentium* infection was associated with reduced ILC3, Th17 cells that produce IL-22 and IL-17 early and late during infection.

T103. LINGO3 Regulates Inflammation and Host Clearance during Enteropathogenic Bacterial Infection

Kelly Zullo, Li-Yin Hung and De'Broski Herbert. University of Pennsylvania, Philadelphia, PA

Immunoregulatory mechanisms operating at the mucosal interface are incompletely understood. Here in, we focused on Leucine rich repeat Ig-like containing domain 3 (LINGO3), a type I membrane protein expressed in the stomach, small intestine, and colon, as a potential regulator of innate and adaptive immunity against mucosal pathogens. To this end, LINGO3 deficient mice were generated and studied during infection with *Citrobacter rodentium (C. rodentium),* an enteropathogenic bacterium. It is known that *C. rodentium* infection expands IL-22 secreting ILC3 as an early response (day 0-8) whereas T_H1, T_H17, and T_H22 $CD4^+$ T cells are induced at later stages (day 15-21). Wild-type (WT) and LINGO3 deficient mice were inoculated with 10^8 colony forming units (CFU) and evaluated between (0-26 days post infection) to assess *C. rodentium* bacterial burden. Results show that compared to WT mice, LINGO3 deficiency significantly reduced fecal CFU during the early response (0-13 dpi), but paradoxically increased fecal CFUs during later stages (day 15-21). Evaluation of mesenteric lymph node cytokine secretion at day 13 demonstrated increased levels and frequency of IFNg, IL-22, and IL-17A $CD4^+$ *T cell*s in LINGO3 deficient mice as compared to WT. Surprisingly, we found no differences between strains regarding the frequency or number of $IL\text{-}22^+$ ILC3 cells or anti-microbial peptide induction at 13 dpi. Combined, we propose that LINGO3 functions as negative regulator of early inflammatory responses, but a positive regulator of host-protective immunity during latter stages of infection. Further investigation of this dichotomy may yield important insights to the regulation of gastrointestinal immunity.

T104. Airway Epithelial Cells Orchestrate Innate Lymphocyte-Mediated Antifungal Immunity

Nydiaris Hernandez-Santos, Jeffrey S. Fites, Darin Wiesner and Bruce S. Klein. University of Wisconsin, Madison, WI

Airway epithelial cells are the first point of contact with microorganisms entering the lung, and they are key elements of the immune response against such microorganisms. Although the epithelial cell response against viruses and bacteria is well documented, little is known about epithelial cell-mediated antifungal immunity. Here we show that NFκB signaling in lung epithelial cells is required to restrain the growth of *Blastomyces dermatitidis,* a causative agent of fungal pneumonia. Epithelial cell-mediated immunity to *B. dermatitidis* is dependent on two important factors: (1) production of IL-17A and GM-CSF by TCRβ+CD4+ and TCRγδ+ innate lymphocytes, as demonstrated by the decreased frequency of cytokine-producing cells in mice where NFκB signaling in airway epithelial cells is ablated, and (2) Interleukin 1 receptor (IL-1R) signaling in epithelial cells. The latter is evident in chimeric animals with IL-1R-deficient epithelial cells and WT hematopoietic cells. These animas exhibit a significantly higher fungal burden than mice sufficient for IL-1R in both epithelial and hematopoietic cells. Our data strongly suggest that CCL20, the ligand for CCR6, provides an important link between epithelial cell function and cytokine production by innate lymphocytes. Here we show that CCL20 is induced as early as 24 hours post-infection with *B. dermatitidis* and its production is contingent upon IL-1R signaling, and expression of NFkB in epithelial cells. Therefore, airway epithelial cells mount a robust response against *B. dermatitidis* in an IL-1R-dependent manner via the actions of IL-17A- and GM-CSF-producing innate lymphocytes, whose functions may be orchestrated by epithelial cell-derived CCL20.

T105. The Role of Antibodies Against Mouse Enolase-Induced by *Treponema Denticola* in the Experimental Periodontitis of Mice

Ahreum Lee, Keumjin Baek, Dong Il Kwon, Jehan Alam and Youngnim Choi. Seoul National University, Seoul, Seoul-t'ukpyolsi, Republic of Korea

Antibodies against alpha-enolase (ENO1) have been detected in various autoimmune and infectious diseases. The enolase of *Treponema denticola* (tENO), a periodontal pathogen, has 53% identities and 70% homology with human ENO1. In our previous study, antibodies against tENO purified from the sera of severe periodontitis patients cross-reacted with ENO1, and the levels of anti-ENO1 antibodies had positive correlations with the periodontal clinical parameters of patients. We hypothesized that anti-ENO1 antibodies are produced during immune response to tENO and that the anti-ENO1 antibodies may contribute to the progression of periodontitis. Repeated immunization with tENO increased the levels of antibodies to mouse ENO1 as well as those to tENO, the levels of which were highly correlated to each other. Although the Pg+ tENO group slightly increased the levels of alveolar bone loss and TNFa expressed in the gingival tissues compared to the Pg group, differences between the two groups were not

significant. The tENO group expressed significantly increased levels of TNFa in the gingival tissues compared to the sham group. A significant positive correlation between the levels of alveolar bone loss and those of TNFa was confirmed. Although the levels of anti-mENO antibodies had a weak positive correlation with those of TNFa, their effect on alveolar bone loss was not observed. Collectively, exposure to tENO can induce the production of anti-ENO1 antibodies, however, the role of the anti-ENO1 antibodies in the progression of periodontitis is minimal at least in the experimental periodontitis of mice.

T106. Characterization of the Immune Response in the Lungs of Mice Vaccinated with a Pneumococcal Protein Chimera

Tanila dos Santos[1], Thiago Rojas Converso[2], Dunia Rodriguez[2], Cibelly Goulart[2], Luciana Leite[2], Henrique Rofatto[2], Lucio Ferraz[3] and Michelle Darrieux[3]. [1]São Paulo University, Bragança Paulista, Brazil; [2]Butantan Institute, São Paulo, Sao Paulo, Brazil; [3]São Francisco University, Bragança Paulista, Brazil

Streptococcus pneumoniae is responsible for over one million annual deaths. The current polysaccharide vaccines are too expensive to be implemented in lower income countries, reinforcing the need for lower cost vaccines with high coverage. PspA and Pneumolysin are two virulence factors able to elicit protection in different models of pneumococcal infection. The combination of PspA and the pneumolysin derivative PlD1 induced the production of high antibody levels against each protein, and protected mice against invasive challenge. The aim of the present study was to investigate the cellular response induced by such vaccine, and to evaluate protection in a model of focal pneumococcal pneumonia. A model of focal pneumococcal pneumonia was developed in BALB/c mice by nasal instillation of a high dose of a serotype 14 strain with low virulence. Airway inflammation was confirmed by total and differential cell counts in BAL and by histological analysis of the lungs, and bacterial loads were measured 7 days after challenge. Cytokine levels were determined in the BALF and lungs of mice immunized with PspA-PlD1 fusion after challenge, by flow cytometry and RT-PCR, respectively. After challenge, the mice developed lung inflammation with no invasion of other sites, as demonstrated by histological analysis of lung tissue. We detected a significant production of TNF-α and IL-6 in the BALF, which correlated with protection against pneumonia in the group immunized with PspA-PlD1. Conclusion: PspA-PlD1fusion is protective against invasive infections and pneumonia in mice. Supported by FAPESP.

T107. Lipopolysaccharide Induces Immunoglobulin A Production and Transcytosis in Airways

Anderson Archelus[1], Séverine Létuvé[2], Claire Carrion[1], Sandrine Lecardeur[1], Christelle Oblet[1], Jean-Claude Aldigier[1], Michel Cogné[1], Anne Druilhe[1]. [1]Université de Limoges, Limoges, France [2]Université Paris Diderot, PRES Sorbonne Paris Cité, Paris, France

Immunoglobulin (Ig)A play a major role as a microbial homeostatic agent in mucosa. Regulation of IgA production and transport into mucosal secretion is studied mainly in the gut. In this study, we evaluated the effect of lipopolysaccharide (LPS) on IgA in airways. We show that intratracheal injection of LPS induces a huge increase of IgA level in bronchoalveolar lavage fluids (BALF) at days two and five without altering the levels of other Ig isotypes in BALF or of IgA in plasma. Albumin level in BALF was not affected. LPS promotes the expression of mRNA coding for the secreted form of IgA but fails to recruit B, B1a, B1b, plasma cells and to augment the proportion of germinal center or of IgA-positive B cells in lungs. An augmentation of J chain and polymeric Ig receptor (pIgR) expression in lungs and of secretory IgA in BALF was also observed. Five days after LPS treatment, human monoclonal IgAs containing a low (LpIgA) or a high (HpIgA) proportion of the polymeric IgJ-associated form were injected in blood and two hours later BALF was collected. HpIgA was found in BALF in contrast to LpIgA that was undetectable. Neutrophils, but not T cells or monocytes/macrophages, as well as pro-IgA cytokines, including IL17 and APRIL, were increased in BALF and in lungs. Depletion of neutrophils in blood, lung and BALF and intratracheal injection of APRIL failed to alter IgA level in BALF and secreted IgA and pIgR mRNA in lungs. IL17 provokes the increased expression of pIgR in mouse lungs and in differentiated primary human bronchial epithelial cells. Altogether these results suggest that LPS increases IgA level in BALF by promoting both IgA-

positive plasma cell activation and IL17-mediated IgA transcytosis in lungs.

T108. CD4+ T Cells Play a Pathogenic Role During *Clostridium difficile* Infection in Hosts with a History of Colitis

Mahmoud Saleh, Alyse Frisbee, Erica Buonomo, Carrie Cowardin and William A. Petri. University of Virginia, Charlottesville, VA

Clostridium difficile infections (CDIs) are the number one cause of hospital-acquired diarrhea in the United States. Several clinical studies have reported higher incidence and severity of CDI in patients with one of the two major forms of inflammatory bowel disease (IBD): ulcerative colitis and Crohn's disease. In order to understand the factors underlying increased severity of CDI in IBD patients, we utilized a Dextran Sulfate Sodium (DSS) murine model of inflammatory colitis. In support of clinical observations, our data showed that mice treated with DSS and then infected with *C. difficile* developed a more severe *C. difficile* disease compared to untreated mice. Increased severity of disease was measured by increased mortality, weight loss and clinical scores. Importantly, comparison of *C. difficile* burden between mice with prior DSS colitis and untreated controls showed no differences in *C. difficile* colonization between the two groups, suggesting that increased severity of disease might be due to the host immune response to infection. Immunophenotyping of immune cells recruited to the colon at the peak of CDI revealed increased levels of CD4+ T cells at the site of infection in mice with prior DSS colitis. Furthermore, depletion of CD4+ T cells using a monoclonal antibody protected mice with prior DSS colitis from severe *C. difficile* disease. These data have identified a novel role for CD4+ T cells in contributing to increased severity of CDI. Our current studies are aimed at understanding the downstream mechanisms by which these T cells are acting to drive severe disease.

T109. The *Candida albicans* Peptide Toxin, Candidalysin, Activates Epithelial Cell Signalling and Immune Responses via Epidermal Growth Factor Receptor (EGFR)

Jemima Ho, Spyridoula Nikou, Jonathan Richardson, David Moyes and Julian Naglik. King's College London, London, England, United Kingdom

Candida infections are of great clinical importance, giving rise to severe morbidity and mortality in millions worldwide. Immunosuppressed individuals are particularly susceptible and a dramatic surge in the prevalence of *Candida* infections has recently been observed. Candidalysin is a newly identified *Candida albicans*-derived peptide toxin that causes cell damage and epithelial immune activation during *C. albicans* mucosal infection (Moyes et al. Nature 2016, 532, 64-68). Preliminary data indicate that Candidalysin mediates its immune effects via the epidermal growth factor receptor (EGFR). TR146 cells were treated with and without EGFR kinase inhibitors (Gefitinib or PD153035) prior to Candidalysin exposure. Phosphorylation of EGFR and intracellular signalling proteins was determined by Western blotting. EGFR ligand and cytokine release was measured by ELISA/Luminex. Candidalysin-treated epithelial cells secreted elevated levels of EGFR ligands (e.g. epiregulin, amphiregulin) and inflammatory cytokines (IL-1β, GM-CSF, G-CSF). EGFR phosphorylation and expression of downstream signalling molecules, c-Fos and pMKP1, were also upregulated. Inhibition of EGFR kinase activity resulted in significant suppression of pEGFR, signalling molecules and cytokine release. Conclusion: Activation of EGFR in epithelial cells is critical for intracellular signalling and cytokine release in response to Candidalysin. Further investigations are required to determine the potential therapeutic value of targeting EGFR or associated signalling components during *C. albicans* infections.

T111. A Protein Chimera Including PspA in Fusion with PotD is Protective Against Invasive Pneumococcal Infection and Reduces Nasopharyngeal Colonization in Mice

Thiago Rojas Converso[1], Cibelly Goulart[1], Michelle Darrieux[2] and Luciana Leite[1]. [1]Butantan Institute, São Paulo, Sao Paulo, Brazil; [2]Universidade São Francisco, São Paulo, Sao Paulo, Brazil

Despite the success of the available polysaccharide-based vaccines against *Streptococcus pneumoniae* in preventing invasive diseases, this bacterium remains a major cause of death in many parts of the world. Since carriage is being largely studied in order to prevent transmission, new vaccine strategies are needed to prevent new colonization cases. Thus, the utilization of fusion proteins is being investigated as alternative to current formulations. The *pspA-potD* hybrid was

generated by ligation of *potD* gene into pET-28a-pspA vector. The proteins were expressed in *E. coli* and purified by chromatography. BALB/c mice were immunized with 3 doses of the proteins biweekly. Antibodies titers were evaluated by ELISA; the binding and opsonophagocytic function were assayed. The splenocytes were cultured for cytokine production. Immunized mice were challenged for survival and colonization evaluation. In the present work, we demonstrate that a chimeric protein, composed of PspA and PotD in fusion is able to maintain the protective characteristics of both parental proteins, providing protection against systemic infection while reducing nasal colonization. The mechanisms underlying the protective efficacy of the rPspA-PotD hybrid protein were investigated, revealing the production of specific antibodies with an increased binding capacity to pneumococcal strains of diverse serotypes and genetic backgrounds, enhanced opsonophagocytosis, and secretion of IL-17 – an important cytokine related with colonization protection, by splenocytes. These findings reinforce the use of chimeric proteins based on surface antigens as an effective strategy to protect against pneumococcal diseases, and to reduce colonization, an important step for transmission of the bacterium. Supported by FAPESP.

T112. Hypo-Inflammatory Innate Immune Response Against Viral and Bacterial Infection in Mouse Pulmonary Fibrosis Model

Yunyun Zou[1], Sang-Uk Seo[1] and Mi-Na Kweon[2]. [1]Seoul National University, Hongcheon-gun, Kangwon-do, Republic of Korea; [2]University of Ulsan Collge of Medicine, Seoul, Seoul-t'ukpyolsi, Republic of Korea

Cystic fibrosis and idiopathic pulmonary fibrosis are ultimately fatal diseases with no known cure. Pulmonary fibrosis can be characterized by dyspnoea and frequent lung infections. In most cases, bacterial and some viral infections are associated with the exacerbation of lung disease. Thickened secretions gradually block and remodel the airway and lung, which lead to infections even more difficult to eradicate. Infection-induced innate immune responses are crucial host defense mechanism against invading microbes. To study the role of the innate immune response in pathogenesis after infection, we used bleomycin (BLM) to induce fibrosis in C57BL/6 mice. BLM-treated mice were then subsequently infected with *Streptococcus pneumoniae* or influenza virus. *S. pneumoniae* infected BLM-treated mice exhibited increased weight loss and higher mortality compared to S. penumoniae infected control mice. Conversely, BLM-treated mice were less susceptible to influenza virus infection compared to control mice. We further performed experiments to assess inflammatory responses in BLM-treated mice and found that innate immune cell recruitment and inflammatory cytokine productions were decreased in both virus and bacteria-infected mice. From our data, we suggest that hypo-inflammatory innate immune response in the lung may lead to opposing disease outcomes in different types of infections.

T84. Epithelial Cell-Derived Phospholipase A2 Group 1B (PLA2g1B) is an Endogenous Anthelmintic

Lewis J. Entwistle[1], Victoria S. Pelly[1], Stephanie M. Coomes[1], Yashaswini Kannan[1], Stephanie Czieso[1], Abdul Sesay[1], Nikolay Nikolov[1], Amina Metidji[1], Helena Helmby[2], David Y. Hui[3] and Mark S. Wilson[1]. [1]Francis Crick Institute, London, England, United Kingdom; [2]London School of Hygiene & Tropical Medicine, London, England, United Kingdom; [3]University of Cincinnati, Cincinnati, OH

Immunity to intestinal helminth infections has been well studied; however, the endogenous mechanism of helminth killing at mucosal surfaces remains unknown. To identify novel mechanisms of intestinal immunity and parasite killing we compared the small intestinal transcriptome of mice that were susceptible (primary infected, *H. p.* 1°) or resistant (secondary challenge infected, *H. p.* 2°) to the murine intestinal helminth *Heligmosomoides polygyrus*. Transcriptional analysis identified elevated expression of the lipid catabolising enzyme, phospholipase A_2 group 1B (*Pla2g1b*), in resistant, but not susceptible mice. Elevated *Pla2g1b* was dependent upon drug-mediated killing of *H. polygyrus* and its expression was restricted to epithelial cells of the small intestine. Elevated expression of *Pla2g1b* was critical for immunity to *H. polygyrus*, as *Pla2g1b*$^{-/-}$mice failed to expel a challenge infection with *H. polygyrus*. The failure to expel *H. polygyrus* in *Pla2g1b*$^{-/-}$ mice was not due to an ineffectual or aberrant immune response, with previously described essential mediators of anti-helminth immunity fully intact in *Pla2g1b*$^{-/-}$mice. Instead, we identified that PLA_2g1B had a direct effect on *H. polygyrus* larvae, with *in vitro* treatment of L3 larvae compromising their ability to establish *in vivo*.

Importantly, treatment of *H. polygyrus* larvae could restore immunity and expulsion of *H. polygyrus* in *Pla2g1b*$^{-/-}$ mice. Paradoxically, IL-4Rα signalling negatively regulated *Pla2g1b* expression in intestinal organoid cultures, uncoupling PLA_2g1B-mediated helminth killing from type 2 immune mediated expulsion. Together, these data indicate that endogenous epithelial cell-associated PLA_2g1B is required for direct killing of invading larvae; revealing a previously unrecognised PLA_2g1B-dependent mechanism of anti-helminth immunity.

T85. Intestinal Immune Responses to Chicken Coccidiosis in the Context of Th1 and Th17 Responses

Woohyun Kim and Hyun Lillehoj. United States Department of Agriculture, Agricultural Research Service, Beltsville, MD

Coccidiosis is one of the most economically important diseases of the chickens caused by *Eimeria* spp. as it destroys intestinal epithelium resulting in nutrient malabsorption, body weight loss, and in severe cases, death. In the context of adaptive T cell immunity, it has known that IFN-γ-mediated Th1 response is dominant in *Eimeria* infection. However, since the discovery of Th17 lineage which is distinct from the Th1 and Th2 lineages in the early 2000s, it has become clear that Th17 cells play an important role in host defense in various infection and in inflammations, especially at mucosal surface. In order to determine if Th17 response is induced by *Eimeria* infection in chickens and to understand its role in the course of infection, we investigated expression levels of Th17 cells-related cytokines at the intestinal site of *E. tenella*-infected chickens in together with Th1 cells-related cytokines. We found an increase of the proportions of both $CD4^+IFN\text{-}\gamma^+$ cells and $CD4^+IL\text{-}17A^+$ cells in *E. tenella*-infected cecum with increasing number of parasites in the intestine. We also found that the mRNA levels of IL-17A and IFN-γ increased in *Eimeria* antigen-stimulated splenic $CD4^+$ cells and that the Th1- and Th17-related cytokines increased in infected tissues. Collectively, our study demonstrates that the both Th1 and Th17 immune responses are implicated in *E. tenella* infection in chickens.

T86. Innate Immunity-Mediated Restriction of *Citrobacter rodentium* Disease Induction to an Early Window of Opportunity

Stefanie Buschor, Miguelangel Cuenca, Stephanie Uster, Christian Schürch and Siegfried Hapfelmeier. University of Bern, Bern, Switzerland

The pathogenesis of the intestinal mouse pathogen *Citrobacter rodentium*, similar to the important human diarrheal pathogen EPEC, depends on its unique ability to form characteristic intestinal epithelium-associated microcolonies, also known as “attachment/effacement” (A/E) lesions. These are microcolonies of bacteria that intimately attach to the apical surface of epithelial cells and induce the local destruction (“effacement”) of the epithelial brush border. Disease severity and mucosal inflammation correlate with the extent of A/E lesion formation. To compensate continuous shedding of infected cells driven by the rapid intestinal epithelial turnover, A/E microcolonies must be dynamic and able to renew by continuous epithelial re-infection. The route and dynamics of this re-infection required for sustained epithelial A/E pathogenesis are ill-defined. Re-infection by planktonic luminal bacteria and local cell-to-cell spread of sessile bacteria without planktonic phase may both contribute. We show that colonic epithelial A/E microcolonies consist exclusively of clonal bacterial populations that depend on local cell-to-cell spread to persist through the course of infection. Their establishment by luminal bacteria is limited to merely the first *C. rodentium* in an A/E virulence gene expression-dependent manner. Our data demonstrate that the establishment of *C. rodentium* pathogenesisis normally restricted to a short window of opportunity that determines disease outcome. Thus, the therapeutic targeting of this vulnerability may also have merit in the treatment or prevention of human A/E infection.

T88. The Oral Bacteriome/Mycobiome in Patients with Loss of Function STAT3 Mutations

Loreto Abusleme[1], Patricia I. Diaz[2], Alexandra F. Freeman[3], Teresa Greenwell-Wild[1], Jay H. Jo[4], Jigarkumar V. Desai[3], Michail S. Lionakis[3], Steven M. Holland[3], Julia A. Segre[5], Heidi Kong[4] and Niki M. Moutsopoulos[1]. [1]Natonal Institutes of Health/National Institute of Dental and Craniofacial Research, Chevy Chase, MD; [2]University of Connecticut Health Center, Farmington, CT; [3] National Institutes of Health/National Institutes of Allergy and Infectious Disease, Bethesda, MD; [4]National Institutes of Health/National Cancer Institute, Bethesda, MD; [5]National Institutes of Health/National Human Genome Research Institute, Bethesda, MD

Th17 cells are key regulators involved in barrier surveillance and anti-fungal immunity. Patients with autosomal dominant hyper-IgE syndrome (AD-HIES) present with Loss of Function *STAT3*-mutations, which impair cytokine signaling and Th17 differentiation, rendering these individuals susceptible to mucocutaneous candidiasis. We investigated the prevalence and severity of oral fungal disease in AD-HIES patients and evaluated their bacterial and fungal mucosal oral communities with high-throughput sequencing of 16SrRNA and ITS1 libraries, respectively. Detailed oral disease phenotyping in a cohort (n=35) of adult AD-HIES patients revealed significant susceptibility to oral candidiasis with 83.3% of AD-HIES patients reporting recurrent oral candidiasis and 50% of them presented with active lesions at the time of sampling, despite the administration of antifungal prophylaxis. Analysis of the oral bacteriome/mycobiome from a subset of patients (n=18) revealed that AD-HIES patients harbor unique bacterial and fungal communities that separate clearly from those of age and gender-matched healthy individuals (n=18). AD-HIES communities exhibit a marked decrease in fungal and bacterial diversity compared to healthy donors with a clear predominance of *Candida* species (mostly *C. albicans*), particularly in subjects with active fungal lesions. The AD-HIES microbiome also displayed significant depletion of health-associated taxa, with overrepresentation of select oral commensal bacteria. In summary, our data show that defective oral mucosal Th17 immunity, in the context of AD-HIES, predisposes to oral fungal infections and significant microbial community shifts. Our current work aims to further define how the interplay of defective Th17 immunity, bacterial commensal colonization and fungal overgrowth collectively contribute to fungal disease susceptibility.

T89. Identification of a Role For Casz1 in Th Differentiation and Anti Fungal Host Adaptive Immunity in Oral Mucosa

Pushpa Pandiyan, Natarajan Bhaskaran and Aaron Weinberg. Case Western Reserve University, Cleveland, OH

Th17 cells are instrumental in mucosal host defense against extracellular bacteria and fungi. Here we show that Casz1, whose function is previously unknown in $CD4^+$ T cells, determines the balance between various Th subsets. Conditional deletion of Casz1 in $CD4^+$ T cells significantly inhibits Th17 cell differentiation, but promotes Th1 differentiation. Casz1 also modulates Foxp3 expression depending on the cytokine milieu. Loss of Casz1 in the context of oral mucosal *Candida* infection severely impairs Th17 responses, causes alterations in the frequency of $Foxp3^+$ T cells, and lowers the ability of the mice to clear the secondary infection. These results underscore the critical role of Casz1 in determining Th17 lineage differentiation *in vivo*. Transcriptome analyses of Casz1 deficient $CD4^+$ T cells show a signature consistent with defective Th17 differentiation but enhanced Th1 differentiation. Taken together, these data identify Casz1 as a new Th plasticity regulator having important clinical implications for mucosal immunity.

T90. The Intersection of Immune Responses, Microbiota and Pathogenesis in Giardiasis

Marc Y. Fink, Jenny G. Maloney, Aleksander M. Keselman, Erqiu Li and Steven M. Singer. Georgetown University, Washington, DC

Infection with the protozoan parasite, *Giardia*, can produce a range of symptoms including diarrhea, severe cramps, and nutrient malabsorption. Our lab and others have previously indicated that immune responses can contribute to pathogenesis. Additionally, it has also been reported that intestinal microbiota can affect the ability of the parasite to colonize laboratory mice. Our goal was to determine if intestinal immune cells are activated following infection and how the microbiota interacts

with intestinal immune responses. Wild-type or CCR2 deficient mice were treated with a cocktail of antibiotics and infected with the GS strain. Control mice were not treated with antibiotics and/or were not infected. Changes in the intestinal microbiome and immune responses were determined using 16S sequencing and flow cytometry of isolated lamina propria and intraepithelial lymphocyte populations, respectively. Sucrase activity was also measured in mucosal lysates. Mice treated with antibiotics or infected with *Giardia* exhibited shifts in their intestinal microbiomes. With or without antibiotics, we observed an increase in the frequency of $CD4^+$ T cells in the lamina propria at day 7 post-infection. We also observed an increase in macrophage populations and activated $CD8^+$ T cells, but only in mice not treated with antibiotics. Macrophage populations increased in wild-type and CCR2-deficient mice, and EdU labeling suggested in situ proliferation of resident macrophages. Finally, sucrase deficiency correlated with activation of $CD8^+$ T cells. Together these data indicate that the intestinal microbiota contribute to immune cell activation during infections and suggest that the microbiota may contribute to different infection outcomes.

T92. Secretory Immunoglobulin Sensing Facilitates Infection of Norovirus

Holly Turula[1], Mariam Gonzalez-Hernandez[1], Alexandra Pry[1], Michael Elftman[2] and Chrisitane Wobus[1]. [1]University of Michigan, Ann Arbor, MI; [2]Central Michigan University College of Medicine, Mount Pleasant, MI

Early events in viral infection are a proven intervention point. While published studies indicate intestinal microbiota modulate virus infections such as murine norovirus (MNV), nothing is known whether natural, non-specific, secretory immunoglobulins (sIg) influence enteric viruses. MNV can hijack M cells to cross the intestinal epithelial barrier and infect the rich population of antigen presenting cells below. However, MNV must first navigate a complex sea of luminal contents including intestinal microbiota and sIg to reach the epithelium. SIgA is internalized into the host via M cells, after being secreted into the lumen following IgA transport via polymeric immunoglobulin receptor (pIgR). We determined that MNV receptor binding P-domain directly bound natural SIgA without affecting infectivity *in vitro*. To determine if natural SIgA enhances MNV infection *in vivo*, we used pIgR-deficient mice, which lack sIg in the intestinal lumen. Despite enhanced target cells (DC, Macrophages, B cells), the first round of viral replication was reduced in the ileum of pIgR-deficient mice compared to controls. We also determined that natural sIgA did not alter MNV epithelial binding or internalization into the Peyer's patch. Ileal mRNA analysis revealed that interferon gamma (IFNg), a cytokine known to inhibit MNV transcription, was enhanced in naive pIgR-deficient mice compared to WT mice, which could account for the reduced virus load. Taken together, these results suggest sIg sensing reduces interferon gamma levels, aiding acute MNV infection. In the future, we will further assess MNV IFNg modulation, as well as the role that enteric bacteria play in this process.

T93. A Neonatal Infection Model for Enteric Infection with *Giardia lamblia*

Ambre Riba, Kasra Hassani and Mathias Hornef. Uniklinikum RWTH Aachen, Aachen, Nordrhein-Westfalen, Germany

Giardia lamblia (G.l.) is a protozoan enteropathogen that colonizes the upper small intestinal mucosa and causes a non-invasive lasting diarrheic disease. It represents an import pathogen in travelers and is highly prevalent in endemic areas in many developing countries in particular in the infant population. Although some overlap in the host range between the different G.l. assemblages has been documented, no animal model has been reported. The lack of a suitable small animal model amenable to genetic modification has hampered progress in our understanding of the protozoan virulence factors and mechanisms of the host's antimicrobial response. Here we present a new intestinal G.l. infection model using neonatal mice. Oral exposure of 4-day-old mice to 2x10^5 G.l. trophozoites via intragastric gavage resulted in increased luminal fluid and fecal humidity and at 1 and 4 weeks post infection (p.i.) as compared to age-matched controls. Additionally, a transient mucosal host response and reduced gain in body weight was observed. In contrast, adult mice were highly resistant and cleared G.l. colonization in the absence of clinical symptoms within 1-2 weeks. Despite resistance of adult mice, infected neonate animals remained positive for G.l. for > 20 weeks indicating a major influence of G.l. on the infected host. Together, we present the first neonatal animal

infection model for G.I. and highlight the importance of age-dependent factors in the susceptibility to infection.

T94. Unraveling Dietary and Host Immune Determinants of *Giardia*-Mediated Enteropathy in Gnotobiotic Mice

Luther Bartelt[1], Rose Viguna Thomas Becket[1], Jeremy Herzog[1], Maureen Bower[1], Stacey Burgess[2], Aki Oka[1], Bo Liu[1] and Balfour Sartor[1]. [1]University of North Carolina, Chapel Hill, NC; [2]University of Virginia, Charlottesville, VA

Endemic pediatric giardiasis associates with growth impairment and impaired gut function, but unlike many other enteropathogens, *Giardia* does not associate with increased fecal markers of inflammation or diarrhea in these children. In our murine malnutrition model of chronic giardiasis, *Giardia* combines with resident microbiota to impair growth and microbial host co-metabolism despite an apparent absence of mucosal inflammation, and even attenuation of intestinal inflammatory markers during co-infections and *ex vivo* TLR4-stimulated BMDCs. To advance mechanistic understandings of nutrient, microbial, and host-mediated determinants of *Giardia*-associated growth impairment, we developed a new model of chronic giardiasis in ex-GF *Rag2*$^{-/-}$ mice using purified *G. lamblia* H3 cysts. These mice develop a similar intestinal burden and chronicity of viable *Giardia* trophozoite infection regardless of diet, even when conventionalized with murine-derived microbiota. Nourished infected mice are resilient to growth impairment, however, *Giardia* combines with protein-malnutrition to result in 10% weight loss through 10-15 days post-challenge (Figure). Although *Giardia* leads to significantly increased FITC-Dextran, histological sections of duodenum do not demonstrate consistent morphometric changes in villus length or crypt hyperplasia, paneth cell morphology, or acute inflammation. Unlike prior models in nourished mice that demonstrate *T cell*-dependent mechansims of malabsorption (dissacharidase deficiency and microvillus blunting), this is the first demonstration of diet-dependent growth faltering independent of *T cell*s during giardiasis. Future investigations will clarify whether specific *T cell* or B-cell transfers can overcome *Giardia*-mediated growth faltering and whether direct parasite-host interactions or metabolic dysregulation are primary mechanisms of *Giardia*-mediated growth faltering.

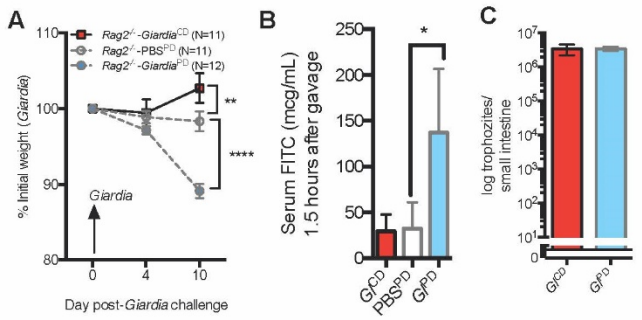


Figure. *G. lamblia* (*Gl.*, H3, cyst challenge) combines with protein malnutrition to A) promote weight loss ($p<0.001$) and B) increase intestinal permeability ($p<0.05$) in protein deficient (PD)-fed previously germ free (GF) *Rag 2*$^{-/-}$ without significant differences in *Giardia* small intestinal burden.

T95. Pathogen-Mediated Inhibition of Anorexia Promotes Survival and Transmission During Acute Illness

Sheila Rao, Alexandria M. Palaferri Schieber, Carolyn P. O'Connor, Mathias Leblanc, Daniela Michel, and Janelle S. Ayres. Salk Institute, Jolla, CA

Traditionally, we have understood host-pathogen interactions in the context of antagonism; that is, to maintain a fitness status, the pathogen must evolve mechanisms that have a negative impact on host health and vice versa. However, pathogen fitness is not solely dependent on the ability to grow within the host. Indeed, some of the most unsuccessful pathogens are those with excessive replication, resulting in pathology that ultimately kills the host and, thus, the pathogen's niche and ability to transmit to a new host. To maintain a replicative niche, a pathogen must achieve the proper balance of microbial growth and pathology inflicted on the host. Therefore, we propose that regulation of virulence, the ability to cause disease, is one means by which pathogen fitness can be enhanced. Here we describe how a bacterial intestinal pathogen, *Salmonella Typhimurium*, regulates its virulence and transmission by manipulating the sickness induced anorexic response of the host via manipulation of the gut-brain axis. Inhibition of inflammasome activation by the *Salmonella* effector, SlrP, prevented anorexia caused by IL-1b-mediated signaling on the hypothalamus via the vagus nerve. Rather than compromising host

defense, pathogen-mediated inhibition of anorexia increased host survival by reducing bacterial virulence. A reduction in bacterial virulence resulted in increased *Salmonella* transmission to new hosts, suggesting that there are trade-offs between transmission and virulence. Our results suggest that microbes have evolved mechanisms to modulate sickness-induced behavior to promote host health and pathogen transmission at the expense of virulence.

T96. IL-10 Production by Neutrophils is a Key Determinant for Host Survival During *Klebsiella pneumoniae* ST258 Infection in Mice

Hernan Penaloza, Loreani Noguera, Francisco Salazar-Echegarai, Alexis M. Kalergis and Susan M. Bueno. Millennium Institute on Immunology and Immunotherapy, Pontificia Universidad Católica de Chile, Santiago, Region Metropolitana, Chile

Carbapenem-resistant *K. pneumoniae* sequence type 258 (KPN-ST258) are an increasing cause of hospital-associated infection worldwide, due their antimicrobial resistance and their resistance to neutrophil-mediated clearance. During infection, the production of the anti-inflammatory cytokine Interleukin-10 (IL-10) modulates the inflammatory response, establishing an equilibrium between a successful pathogen clearance and a limited tissue damage. Since in humans there are several polymorphisms that affect IL-10 production we evaluated the effect of IL-10 production on survival after a KPN-ST258 infection. IL-$10^{-/-}$ and C57BL6-WT mice were infected intranasally with 10^8 of a highly prevalent strain of KPN-ST258 strain (KP35). At day 7, IL-$10^{-/-}$ mice had 90% mortality, whereas WT mice had 10% mortality, suggesting that the modulatory effect of IL-10 is key for host survival during KP35 infection. Since one of the highlights of KPN-ST258 pathogenesis is their resistance to neutrophil-mediated killing, we evaluated the hypothesis that during KP35 infection, neutrophils acquire an anti-inflammatory profile characterized by the production of IL-10. To evaluate this, we infected IL-10/GFP transgenic mice with KP35 for 12, 24, 48, 72, 96 and 240 hours, and measured the production of IL-10 by flow cytometry. Bacterial burden in lungs and spleen were evaluated to correlate the cellular infiltrate with the presence of bacteria. After infection, there was a decrease of alveolar macrophages, but an increase of monocytes and neutrophils in lungs. Importantly, neutrophils not only were the most abundant cells in lungs after KP35 infection, but they also were the most important source of IL-10 over time. Our data support the hypothesis that infection with KP35, promotes IL-10 production by neutrophils, driving the acquisition of an anti-inflammatory phenotype that modulates lung inflammation which is unable to clear KP35 infection.

T98. Gut Mucosal δ1 γδ T Cells Maintain Th1/Th7 Responses and Cytotoxic Potential in SIV-Infected Rhesus Macaques

Namita Rout[1], Adrienne Woods[1,2], Faith Schiro[1,2], Pyone Aye[1,2], Stephen Braun[1,2], Ronald Veazey[1,2], Marcelo Kuroda[2] and Andrew Lackner[1,2]. [1]Tulane National Primate Research Center, Covington, LA; [2]Tulane University, Covington, LA

γδ T cells have important functional roles in a range of diseases including infectious diseases and cancer. It has been reported that HIV/SIV infection results in a loss of δ2 subsets and expansion of δ1 subsets of γδ T cells in the periphery. In this study, we evaluated δ1 and δ2 γδ T cells from blood, gut mucosa, lung and liver of rhesus macaques with or without SIV infection. The γδ T cell phenotype is more skewed towards Th17 function in the gut mucosa in contrast to a more Th1 and cytotoxic functionality in the liver of SIV-naive macaques, suggesting tissue-specific immune functions. Macaque γδ T cells are predominantly δ1 in the peripheral blood as well as mucosal tissues regardless of SIV infection. Gut mucosal δ1 and δ2 subsets display similar Th17 cytokine responses to mitogen stimulation; however, the δ1 subsets produce significantly higher Th1 cytokines TNFα and IFNγ. Although, there is a trend towards reduced frequency of γδ T cells in tissues of SIV-infected macaques at 3-4 weeks post-infection, no significant change in the Vδ1/Vδ2 ratio was observed either in blood or mucosal tissues. The δ2 subsets in SIV-infected macaques display reduced Th17 cytokine responses to mitogen stimulation. However, there is no significant change in the Th1 or Th17 cytokine producing ability and Granzyme B expression of the δ1 subsets, suggesting that the immune functions of δ1 subsets are unaffected in early SIV infection and may be targeted for cytotoxic killing of HIV/SIV-infected cells, particularly in tissues.

M.02. Batf3-dependent Classical Dendritic Cells are Required for Mounting Optimal Rotavirus-Specific IgA Immune Responses

Julia Hütter[1], Joy Nakawesi[2], Katharina Lahl[3]. [1]Bayer Pharma AG, Berlin, Germany [2]Lund University, Lund, Sweden, [3]Technical University of Denmark, Lyngby, Denmark.

Rotavirus (RV) infects the small intestine causing severe dehydrating diarrhea in young children. Based on correlative studies, RV-specific intestinal IgA is one of the principle effectors of long term immunity. Dendritic cells (DCs) have been shown to facilitate both T cell-dependent and -independent secretory IgA. However, whether DCs can induce intestinal anti-RV antibody responses during RV infection is not known. Here, we show that a specific subset of DCs, which depends on the transcription factor BATF3 and is commonly referred to as cDC1, is required for generating an optimal RV-specific IgA immune response upon infection. Batf3-deficient mice (Batf3-/-) shed RV longer than heterozygous littermate controls. This correlated with fewer RV-specific CD8 T cells and a lower fecal RV-specific IgA titer. We used rotavirus like particles (VLP2/6) bound to GFP in order to identify RV-specific B cells in orally infected Batf3-/- mice on a single cell level. Compared to littermate controls, Batf3-/- mice had significantly fewer RV-specific IgA+ and IgM+ B cells in mesenteric lymph nodes and Peyer's Patches, while total B cell numbers were not affected. Together, our results show a previously unappreciated and essential role for cDC1 DCs in the generation of a virus-specific IgA immune response at the intestinal wall.

Mucosal Tolerance

OR.74, T119. Goblet Cells and Goblet Cell Associated Antigen Passages are Required for the Induction and Maintenance of Tolerance to Dietary Antigen

Keely G. McDonald[1], Jenny Gustafsson[1], Devesha Kulkarni[1], Kathryn Knoop[1], Rachel Kassel[2], Mark Miller[1] and Rodney Newberry[1]. [1]Washington University School of Medicine, Saint Louis, MO; [2]University of Alabama Birmingham Children's of Alabama, Birminham, AL

Tolerance to dietary antigens is fundamental to gut homeostasis. Oral tolerance requires the acquisition of luminal antigens by small intestine (SI) lamina propria (LP) dendritic cells (DCs) and their trafficking to the mesenteric lymph node (MLN) to induce regulatory T cells (Tregs), which subsequently home to the LP and is maintained by continued antigen encounters. How luminal antigens are acquired by the immune system for induction and maintenance of oral tolerance is an important but incompletely understood process. *In vivo* imaging of $CD11c^{YFP}$ reporter mice revealed that LP-DC extension of trans-epithelial dendrites (TEDs) were rare, ~0.004 TEDs/villus in the steady state, but were induced by removal of the luminal contents and mucus layer. In contrast, the formation of goblet cell associated antigen passages (GAPs) were ~1000 fold more common in the steady state. Deletion of goblet cells (GCs) and GAPs resulted in a ~30-fold increase in intestinal leak, but abrogated LP-DCs acquisition of gavaged fluorescent Ovalbumin (Ova) and the ability of LP-DCs to stimulate Ova specific T cells in *ex vivo* assays and in the MLN and following Ova gavage. Deletion of GCs/GAPs or inhibition of GAPs prevented the induction of Tregs in the MLN in response to dietary antigen and abrogated tolerance to dietary Ova. Deletion of GCs/GAPs resulted in a rapid reduction in the pre-existing LP Treg population and loss of aldehyde dehydrogenase activity in LP CD103+ DCs. These observations indicate that GCs and GAPs are essential for tolerance to dietary antigens.

OR.76, T120. Oral Administration of Hsp65-Producing *Lactococcus lactis* Prevents Collagen-Induced Arthritis in an IL-10-Dependent Manner

Guilherme Gusmao, Mariana C. Goncalves, Mauro A. Guimaraes, Samara R. Medeiros, Sarah F. Aguiar, Luisa Lemos, Rafael P. Oliveira, Denise C. Cara, Vasco A. Azevedo, Anderson Miyoshi and Ana Maria C. Faria. Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

Rheumatoid arthritis (RA) is a systemic autoimmune disease in which inflammation of the joints determines their destruction. Some auto-antigens such as collagen and heat shock proteins are targets of immune response during arthritis development. Hsp65 is a chaperonin essential to protein folding and response to cellular stress. This protein is also a regulator of the immune response and inflammatory process. Studies in animal models have shown that the loss of tolerance resulting in pathogenic autoimmunity can be restored by oral administration of the target antigen. This procedure induces oral tolerance and the continuous feeding of antigen is the most efficacious way to induce it. However, this feeding regimen is hardly feasible as an alternative therapy. To overcome this difficulty, a recombinant *Lactococcus lactis* was constructed to secret *Mycobacterium leprae* Hsp65 providing a continuous and direct delivery system for Hsp65 to the gut mucosa. Our study showed that oral treatment with Hsp65-producing *Lactococcus lactis* prevented the development of murine collagen-induced arthritis. This effect included inhibition of paw edema and joint inflammation associated with reduced levels of inflammatory cytokines (IFN-gamma, IL-17, TNF-alfa) and pathogenic autoantibodies (anti-HSP65, anti-collagen, rheumatoid factor). Treatment with HSP65-*L.lactis* induced augmented frequencies of IL-10-producing B cells and TGF-beta-producing CD4+LAP+ T cells. The preventive effect was dependent on IL-10 since it did not occur in IL-10-deficient mice. Therefore, our study demonstrated that efficient oral tolerance to collagen-induced arthritis can be induced by oral administration of HSP65-producing *L.lactis* and the suppressive effect was IL-10 dependent.
Financial support: FAPEMIG, CNPq (Brazil)

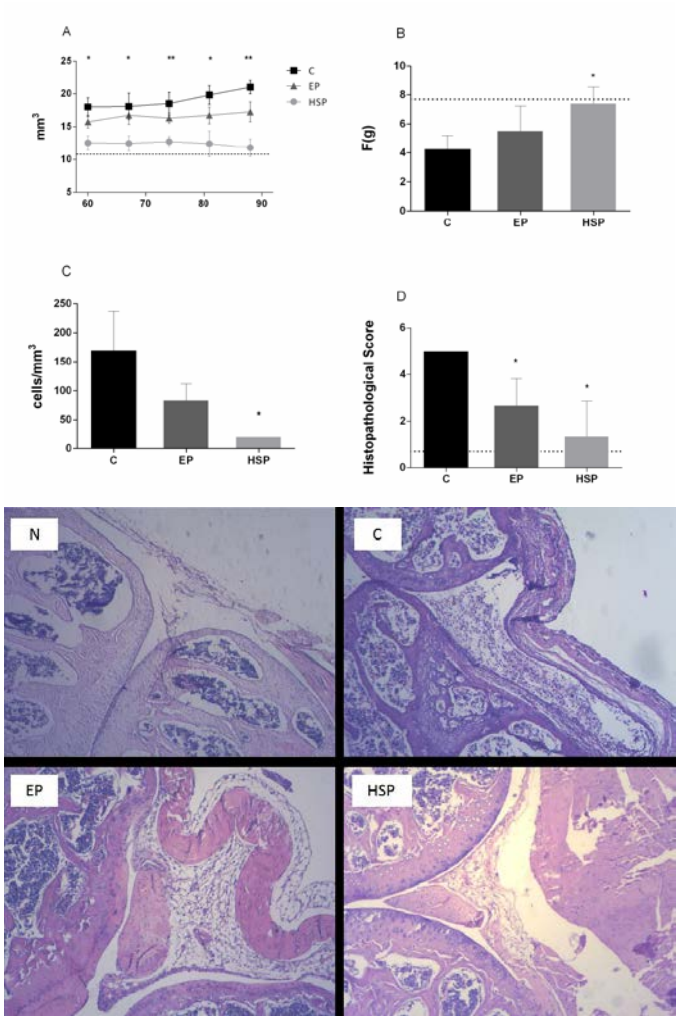


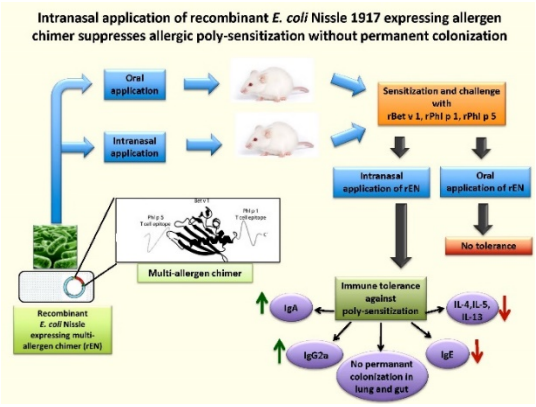
Figure 1. Treatment with *Lactococcus lactis*-HSP65 prevents collagen-induced arthritis in mice. BALB/c mice were fed either medium (C group), medium with *L.lactis* bearing an empty vector (EP group) or Hsp65-producing *L.lactis* (HSP group) for four days. Non-manipulated naïve animals were used to obtain basal levels (N group). Ten days after treatment, arthritis was induced by injection of chicken type II collagen + ovalbumin + CFA. (A) Edema of both hind paws were measured with a pletysmometer (Ugo Basile, Italy). Measurements were taken weekly after the third booster immunization. The group treated with Hsp65-Ll presented lower edema than the control groups at all verified times. (B) Mechanical nociceptive threshold was quantified using an Analgesy-meter (Ugo Basile, Italy) at the day 90. Hsp65-Ll treated animals presented the higher thresholds compared to the control groups. (C) Leucocytes in synovial cavity. The cavity of the left knee was washed and the number of total leucocytes counted. Inflammatory cells were found only in one animal in the Hsp65-Ll treated group. (D) Histopathological score based on inflammatory infiltrate, pannus, cartilage, and bone erosion. (E) At day 95 animals were euthanized and the right knee was used for histological sections stained with HE. Magnification of 10X. Values plotted represent mean ± SEM. *p<0,05 compared to control C group. **p<0,005 compared to control C group. ***p<0,0005.

T113. Intranasal Application of Recombinant *E. Coli* Nissle 1917 Expressing Allergen Chimer Suppresses Allergic Poly-Sensitization without Permanent Colonization

Priya Sarate[1], Stefan Heinl[2], Catherine Daniel[3], Sabine Poiret[3], Mirjana Drinic[1], Christian Zwicker[1], Irma Scabussova[1] and Ursula Wiedermann[1]. [1]Medical University of Vienna, Austria, Wien, Austria; [2] University of Natural Resources and Life Sciences, Wien, Austria; [3] Institute Pasteur de Lille, France, Lille, Nord-Pas-de-Calais, France

Poly-sensitization is becoming an increasing health issue in Western countries, since poly-sensitized individuals are difficult to treat by conventional therapeutic measures. Recently, probiotic bacteria such as non-pathogenic *Escherichia coli* Nissle 1917 are increasingly used to treat allergies and other diseases. It is therefore of interest to test if recombinant probiotics expressing specific allergens may represent the proper tool for prevention of poly-sensitization. In this study, we demonstrate the effect of mucosal application of recombinant *E. coli* Nissle expressing an allergic chimer (rEN-chi-m) of birch and grass pollen allergens in adult mice. Mice were pre-treated either orally or intranasally with rEN-chi-m before allergic poly-sensitization. Particularly after intranasal treatment with rEN-chi-m the mice showed a significant reduction of lung inflammation (eosinophils, IL-5, IL-13 in BAL) along with reduction in allergen specific IgE and Th2 cytokines in spleen and lung cell cultures. In contrast, allergen specific IgA in lungs and gut and serum IgG2a were significantly increased in these mice. Using *in vivo* imaging techniques, we further demonstrated that intranasally applied *E. coli* Nissle were detected in lungs and gut but no longer than 2 days after application, indicating that the bacteria are not colonizing at mucosal surfaces. In confocal imaging system, we proved that the bacteria are taken up by epithelial cells and dendritic cells and both cells seems to be responsible for initiation of regulatory immune responses. In conclusion, we demonstrate that intranasal

application of rEN expressing allergen chimers can be a safe and effective strategy to prevent allergic poly-sensitization.



T114. Role of Alarmin IL-33 on TH17 Cells in the Small Intestine

Enric Esplugues[1] and Anna Pascual[2]. [1]Yale School of Medicine, Guilford, CT; [2] DRFZ, Berlin, Germany

TH17 cells have been associated with several autoimmune diseases and inflammation. The process of tissue inflammation could have a positive effect as a host response to infection, but it can also contribute to autoimmunity. The crosstalk between a tissue and the immune system during an inflammatory response is key for preserving tissue integrity and restoring physiological processes. However, how the inflamed tissue regulates the magnitude of an immune response by controlling pro-inflammatory T cells is not well characterized. Here we show that TH17 cells in the small intestine during inflammation are able to express the IL-33 receptor (ST2). We also show that intestinal epithelial cells (IEC) are the main source of the alarmin interleukin-33 (IL-33). We have observed that pro-inflammatory TH17 cells acquire a regulatory phenotype with immunosuppressive properties in response to IL-33. The lack of ST2 signaling promotes the secretion of pro-inflammatory cytokines by TH17 cells and reduces the secretion of IL-10. Our results provide new insights into the mechanisms by which IEC, via IL-33/ST2 axis, may control pro-inflammatory TH17 cells in the small intestine to sustain homeostasis.

T115. Unique Invariant Natural Killer T Cells Promote Intestinal Polyps by Suppressing TH1 Immunity and Promoting Regulatory T Cells

Ying Wang[1], Saikiran Sedimbi[2], Linda Löfbom[1], Steven A Porcelli[3] and Susanna Cardell[1]. [1]University of Gothenburg, Gothenburg, Vastra Gotaland, Sweden; [2]Karolinska Institute, Stockholm, Stockholms Lan, Sweden; [3]Albert Einsten College of Medicine, Bronx, NY

CD1d-restricted invariant natural killer T (iNKT) cells are known as potent early regulatory cells of immune responses. Besides the established roles in the regulation of inflammation and autoimmune disease, studies have shown that iNKT cells have important roles in tumor surveillance and the control of tumor metastasis. Here we found that absence of iNKT cells dramatically decreased the total number of intestinal polyps in $APC^{Min/+}$ mice, a model for colorectal cancer. Polyp iNKT cells were enriched for IL-10 and IL-17 producing cells, showed a distinct phenotype being $CD4^+$, $NK1.1^-$ $CD44^{int}$ and $PD\text{-}1^{lo}$, and they were negative for the NKT cell transcription factor PLZF. Absence of iNKT cells was associated with a reduced frequency of Treg cells and lower expression levels of FoxP3 protein and transcript uniquely in the polyps, and a switch to an inflammatory macrophage phenotype. Moreover, in iNKT cell deficient $APC^{Min/+}$ mice, expression of T helper (TH) 1-associated genes, such as *IFN-g* and *Nos2*, was increased in polyps, concomitantly with elevated frequencies of conventional $CD4^+$ and $CD8^+$ T cells in this tissue. The results suggest that a population of regulatory iNKT cells locally promote intestinal polyp formation by enhancing Treg cells and immunosuppression of anti-tumor TH1-immunity.

T116. Common and Differential Effects of TGF-b1 and Lactoferrin on the Expression of Regulatory T Cell Phenotypes

Young-Saeng Jang, Ha-Eon Song, Da-Hyun Go, Goo-Young Seo and Pyeung-Hyeun Kim. Kangwon National University, Chuncheon, Kangwon-do, Republic of Korea

It is well established that TGF-b1 stimulates naïve $CD4^+$T cells to differentiate into regulatory T cells. We first found that lactoferrin (LF) enhanced Foxp3 expression by itself and further increased TGFb1-induced Foxp3 expression. Although both molecules

maintained expression of CD44 (T cell activation marker), TGF-b1 augmented shedding of CD62L while LF strongly inhibited its shedding. Thus, addition of TGF-b1 and LF resulted in differentiation of $CD62L^{-}$ $Foxp3^{+}$T cells and $CD62L^{+}Foxp3^{+}$ T cells, respectively. Interestingly, LF actually diminished TGFb1-caused CD62L shedding. Nevertheless, we observed, through a subsequent experiment including time-lapse reciprocal addition of two molecules, that both molecules can induce Foxp3 expression independent of CD62L phenotype. We next examined the involvement of TbRIII which is a candidate receptor for LF, as recently shown by us in B cell differentiation. Pretreatment of LF with soluble TbRIII caused a marked decrease of LF-induced Foxp3 expression but not CD62L expression. Taken together, these results suggest that LF mostly induces Foxp3 expression through TbRIII while it inhibits CD62L shedding through an unknown receptor.

T117. Regulator of G Protein Signaling 10: A Novel Regulator of Intestinal Inflammation Pertinent to Parkinson's Disease

Madelyn Houser, Jianjun Chang, and Malú Tansey. Emory University, Atlanta, GA

Sustained intestinal inflammation has been implicated in the development and progression of numerous diseases, including neurodegenerative disorders such as Parkinson's disease (PD). Our recent evidence confirms indicators of inflammation and oxidative stress in colonic biopsies from PD patients, including drastically elevated levels of NFκB. This suggests that modulators of intestinal immune activity could influence PD-associated pathology. Regulator of G protein Signaling 10 (RGS10) is a GTPase-activating protein that is protective in animal models of PD pathology. It has been found to regulate proinflammatory activity in microglia, macrophages, and T cells from central nervous system tissues, lymph nodes, and spleen, but its role in the intestine had not previously been investigated. We discovered that RGS10 deficiency in murine lamina propria and intrepithelial immune cells drives significant increases in levels of NFκB. RGS10-deficient mice exhibit greater baseline intestinal inflammation, altered immune cell populations with evidence of increased activation, compromised tight junction integrity, and dysbiotic microbiota composition compared to WT mice, with differing effects in males and females. These changes are physiologically relevant, as RGS10-deficient animals exhibit intestinal shortening, decreased gut motility, and impaired resolution of dextran sodium sulfate-induced intestinal inflammation and injury compared to their WT counterparts. This study identifies RGS10 as a novel regulator of mucosal immune homeostasis and suggests that modulation of levels of this protein may affect pathology in Parkinson's disease and potentially other disorders associated with gastrointestinal inflammation. [Funding by NSF and NIH/NINDS]

T118. Dendritic Cell Expression of the Signaling Molecule TRAF6 is Required for Immune Tolerance in the Lung

Daehee Han[1] and Charles D. Surh[2]. [1]Institute for Basic Science, Pohang, Republic of Korea, Korea; [2]Postech, Pohang, Kyongsang-bukto, Republic of Korea

Immune tolerance in the lung is important for preventing hypersensitivity, such as allergic asthma. Maintenance of tolerance in the lung is established by coordinated activities of poorly understood cellular and molecular mechanisms, including participation of dendritic cells (DCs). We have previously identified DC expression of the signaling molecule TRAF6 as a non-redundant requirement for the maintenance of immune tolerance in the small intestine of mice. Because mucosal tissues share similarities in how they interact with exogenous antigens, we examined the role of DC-expressed TRAF6 in the lung. As with the intestine, we found that the absence TRAF6 expression by DCs led to spontaneous generation of Th2-associated immune responses and increased susceptibility to model antigen-induced asthma. To examine the role of commensal microbiota mice deficient in TRAF6 in DCs were treated with broad-spectrum antibiotics and/or re-derived on a germ-free (GF) background. Interestingly, we found that antibiotics-treated specific pathogen-free (SPF), but not GF, mice showed restored immune tolerance in the absence of DC-expressed TRAF6. We further found that antibiotics mediate microbiota-independent effects on lung T cells to promote immune tolerance in the lung. This work provides both a novel tool for studying immune tolerance in the lung, and an advance in our conceptual understanding of potentially common molecular mechanisms of immune tolerance in both the intestine and the lung.

T121. ICOSL-ICOS Signaling Promotes Regulatory T Cell Stability at the Host-Microbial Interface

Ashley E. Landuyt and Craig L. Maynard. University of Alabama, Birmingham, AL

Production of immunosuppressive cytokine IL-10 by regulatory T cells (T_{reg} cells) is critical for immune tolerance toward commensal microbiota. The binding of inducible T cell costimulator (ICOS) to its cognate ligand (ICOSL) potently induces such IL-10 secretion and both *ICOSLG* and *IL10* are susceptibility loci for inflammatory bowel disease (IBD). Utilizing the 10BiT mouse (in which IL-10 induction is reported by Thy1.1 expression), we have found that IL-10 induction by colonic T_{reg} cells is independent of ICOS expression. In fact, we find *Icos*$^{-/-}$ mice eventually possess more IL-10^{+} CD4 T cells than wild-type mice, due to an intestinal Foxp3^{-}IL-10^{+} population that increases with age. This compensatory mechanism does not rescue the reduced Foxp3^{+} compartment in *Icos*$^{-/-}$ mice, however. We used a fate-mapping reporter system to probe the cause of diminished Foxp3expression and found *Icosl*$^{-/-}$ mice, while similar in Foxp3 induction, had more "ex-Foxp3" cells at barrier sites. We employed the T cell transfer model of murine colitis to investigate functional stability. While both *Icos*$^{+/+}$ and *Icos*$^{-/-}$ thymically-derived T_{reg} cells could prevent disease onset, ICOS-deficient T_{reg} cells were incapable of reversing ongoing inflammation and preferentially lost Foxp3 expression. Collectively, these data indicate a role beyond IL-10 induction for ICOS in the phenotypic resilience of T_{reg} cells encountering proinflammatory signals.

T122. Unique PD-L1 Expression in Oral Mucosae and Trans-Coinhibition by PD-L1-Expressing Keratinocytes in CD4^{+} T Cell-Mediated Tissue Inflammation

Miyuki Azuma, Siwen Kang, Worawalun Hirunwidchayarat and Tatsukuni Ohno. Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan

The PD-1:PD-L1 pathway regulates immune responses and maintains homeostasis. Here, we identified a unique expression of PD-L1 on masticatory mucosae in the oral cavity. PD-L1 was physiologically expressed on the dorsal surface of the tongue, gingiva, and hard palate. Other squamous epithelia and other structures of the epithelia did not express PD-L1 in the steady state. Physiological PD-L1 expression on masticatory mucosae was limited on prickle cells, and its expression on basal keratinocytes (KCs) was strictly regulated. PD-L1 on prickle cells was upregulated by external topical stimuli, and PD-L1 on basal KCs was induced only by internal stimuli via infiltrating cells. The blocking of KC-associated PD-L1 or the lack of PD-1 on tissue effector CD4^{+} T cells in mice lacking B7-H1 on immune cells drastically exacerbated the tissue inflammation induced by topical OVA painting as an exogenous antigen, indicating direct interaction with KCs and CD4^{+} T cells. Trans-coinhibitory signals by KCs may modulate local *T cell*/dendritic cell activation, resulting in inhibition of *T cell* responses in both peripheral and secondary lymphoid tissues. Careful control of PD-L1 induction in KCs may play a crucial role in the protection from CD4^{+} *T cell*-mediated tissue inflammation by exogenous antigens delivered from the mucosal surface.

T123. Anti-Allergic Effect of Intranasal Vitamin D Treatment by Inhibiting Dendritic Cell Activation in the Allergic Rhinitis Mouse Model

Dong-Young Kim, Sung-Woo Cho and Yu-Lian Zhang. Seoul National University College of Medicine, Seoul, Seoul-t'ukpyolsi, Republic of Korea

The role of Vitamin D as a potential immune-modulator has been recently elucidated. Dendritic cell, a key regulator driving towards Th2 immune response in allergic diseases is known to be affected by vitamin D. However, the role of vitamin D in the pathogenesis of allergic rhinitis is unclear and its anti-allergic effect has not been established yet, especially in the mouse model. The aims of this study are to evaluate 1) the anti-allergic effect of topically applied vitamin D in the allergic rhinitis mouse model, and 2) the effect of vitamin D on dendritic cell activation. BALB/c mice were intraperitoneally sensitized with ovalbumin (OVA) and alum, and they were intranasally challenged with OVA. Intranasal 1, 25-dihydroxyvitamin D3 was given to treatment group and solvent was given intranasally to sham treatment group. Allergic symptom scores, eosinophil infiltration, cytokine mRNA levels (IL-4, IL-5, IL-10, IL-13, IFN-γ) in the nasal mucosa, serum total and OVA-specific IgE, IgG1, and IgG2a were analyzed and compared with negative and positive controls. Cervical lymph nodes were harvested for flow cytometry analysis. In the treatment group, allergic symptom scores, eosinophil infiltration, and the mRNA levels of IL-4 and IL-13 were significantly reduced compared to positive control. IL-5 mRNA level, serum total IgE, and OVA-

specific IgE and IgG1 levels showed a tendency to decrease in the treatment group, but did not reach to a significant level. IL-10 did not show a significant difference between groups. $CD11c^+$, MHCIIhi, $CD86^+$ activated dendritic cells were significantly reduced in the treatment group. $CD4^+$, $CD25^+$, $FOXP3^+$ Treg cells tended to increase in the treatment group, however it was not significant. The intranasal instillation of 1, 25-dihydroxyvitamin D3 has an anti-allergic effect in the allergic rhinitis mouse model. We believe that the anti-allergic effect of vitamin D is mediated by the inhibition of dendritic cell activation, and therefore decreased Th2 mediated inflammation.

T124. Monocyte-Dependent Expansion of Intestinal IFNγ-producing Natural Killer Cells Precedes the Type 2 Immune Response During Parasitic Helminth Infection

Marilena Gentile, Yue Li, Ghislaine Fontes and Irah King. McGill University, Montreal, PQ, Canada

Intestinal helminths infect over 2 billion people worldwide and can cause significant morbidity. However, in many cases, these tissue-invasive pathogens do not cause significant illness, indicating that mammalian hosts have evolved unknown strategies to tolerate helminth infections. To understand the cell types that are critical for mediating pathogen tolerance, we characterized the earliest stages of infection by the small intestine-dwelling murine helminth *Heligmosomoides polygyrus bakeri* (*Hpb*), a model of human roundworm infection. Unexpectedly, we observed a rapid and robust type 1 inflammatory response characterized by induction of IL-1b, TNFa and iNOS expression that preceded the expected type 2 response. This early response was associated with activation and expansion of IFNg-producing Eomesodermin+ Natural Killer (NK) cells and a decrease of other innate lymphoid cell subsets. Confocal microscopy of infected tissue revealed that NK cells surround the larvae in the small intestine and co-localize with gut-infiltrating Ly-6C+ monocytes in infected mice. Notably, depletion of CCR2-expressing cells during *Hpb* infection eliminated both intestinal NK cells and monocytes, and led to severe pathology including weight loss and intestinal bleeding. Our work provides new insight into the cellular dynamics required for protection during intestinal helminth infection and will help inform strategies to maximize host fitness in the context of tissue injury and repair.

T125. C1q Induce Regulatory T Cell Development and Attenuate Emphysema

Xiaoyi Yuan[1], Ran You[2], Hui-Ying Tung[2], Ming Shan[3], Bon-Hee Gu[2], Matthew Madison[2], Yi Xu[4], Rick Wetsel[5], Holger Eltzschig[1], David Corry[2] and Farrah Kheradmand[2]. [1]University of Texas Health Science Center Houston, Houston, TX; [2]Baylor College of Medicine, Houston, TX; [3]Merck, Boston, MA; [4]IBT – Texas A&M Health Science Center, Houston, TX; [5]IMM, UT Health Science Center at Houston, Houston, TX

Smoking-induced chronic obstructive pulmonary disease (COPD), which encompasses chronic bronchitis and emphysema, is a progressive inflammatory lung disease with no known effective treatment. Sterile inflammation induced by cigarette smoke can activate lung antigen presenting cells (APCs) expressing CD11b/CD11c markers that can differentiate T helper type 1 (Th1) and Th17 cells. Maturing APCs are associated with expression of complement components because a large number of their proteins and receptors have been detected in mice and humans. Interestingly deficiency of C1q in humans and mice is associated with a lack of immunoregulatory response that results in inflammation and autoimmunity characteristic of systemic lupus erythematosus (SLE). Thus, we hypothesized that C1q serves a regulatory role in the inflammation and autoimmunity observed in emphysema. We show here that C1q mRNA level is reduced in lung APCs from emphysema patients, which correlates with decreased lung function, and mice exposed to cigarette smoke. SiRNA knock down of C1q in human lung $CD1a^+$ APCs renders increased ability to induce Th17 responses. $C1q^{-/-}$ mice exposed to cigarette smoke exhibit increased lung inflammation when compared with wild type counterparts. Interestingly, relative to vehicle control, intranasal C1q treated mice show attenuated smoke-induced emphysema. Furthermore, we identified regulatory T cells as the key player in C1q mediated immune regulation. These findings suggest a critical role for C1q in the inhibition of smoke-induced lung inflammation, and should be further explored to develop specific new therapeutics for the treatment of emphysema.

T126. Dissecting the Tissue-Specific Mechanisms that Support the Maintenance of Intestinal Regulatory T Cells

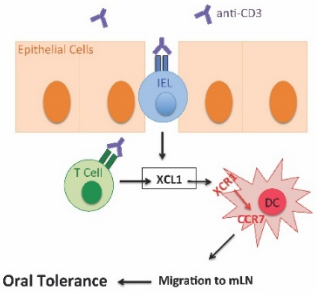
Elisa Cruz-Morales and Terri M. Laufer. University of Pennsylvania, Philadelphia, PA

Intestinal immune tolerance critically relies on regulatory T cells (Tregs). Thus, understanding the mechanisms that sustain the intestinal Treg niche will guide the development of Treg-based immunotherapies for inflammatory intestinal disorders. It has been suggested that the specificity of intestinal lamina propria (siLP) Tregs is biased towards recognition of food and commensal antigens. However, we previously utilized a transgenic mouse model (K14) lacking peripheral TCR-MHCII interactions and the peripheral differentiation of inducible Tregs to demonstrate that the siLP contains a niche for Tregs that can be filled and maintained independently of MHCII-TCR interactions. After thymic egress, central Tregs receive TCR signals in the periphery and become effector Tregs. Although antigen-specific signals appear to maintain the phenotype and function of effector Tregs in lymphoid organs, we find that siLP Tregs express Nur77 and IRF4 (typically induced by TCR signaling) and maintain an effector Treg transcriptional signature despite the absence of local MHCII. These results suggest that the siLP contains MHCII-independent pathways to generate and sustain a local population of effector Tregs. Treg suppression of dendritic cell (DC) function may be mediated via cell-cell adhesion that is MHCII-independent. Indeed, in the K14 siLP, Tregs in isolated lymphoid follicles contact MHCII-deficient B cells and DCs. Short term costimulation blockade decreases Nur77 expression on K14 siLP Tregs, suggesting that MHCII-independent effector Tregs may be generated and maintained via costimulatory signals, adhesion molecules and/or paracrine cytokine stimulus. These results suggest that the intestinal milieu can support the homeostasis of effector Tregs independently of MHCII-TCR signaling.

T127. Involvement of Conventional Dendritic Cells in the Oral Tolerance Induced by Anti-CD3

Rafael Rezende[1,2], Chantal Kuhn[2], Thais Moreira[2], Stephen Rubino[2] and Howard Weiner[2]. [1]Harvard Medical School, Boston, MA; [2] Brigham and Women's Hospital, Boston, MA

Oral tolerance (OT) is defined as the specific suppression of immune response to an antigen by prior administration of the same antigen by the oral route. Although the investigation of OT has classically involved the administration of antigens, we have found that oral administration of anti-CD3 monoclonal antibody induced OT and ameliorated experimental autoimmune encephalomyelitis (EAE), the murine model for multiple sclerosis. Anti-CD3-induced OT is related to the increase of regulatory T (Treg) cells. Even though Tregs are the executors of the regulatory activity of oral administered anti-CD3, antigen presenting cells (APCs), such as dendritic cells (DCs) may play a crucial role in inducing Tregs after oral anti-CD3 treatment. We investigated whether OT induced by anti-CD3 required different APCs in the context of EAE, by specifically depleting either plasmacytoid (pDCs) or conventional DCs (cDCs). We found that depletion of cDCs, but not pDCs, completely abrogated the protective effect of oral fed anti-CD3 on EAE. Moreover, adoptive transfer of cDCs, but not pDCs, from mice fed anti-CD3, conferred protection to recipient EAE-induced mice. Transcriptome analysis of cDCs from mice fed anti-CD3 showed high upregulation of the chemokine receptors *Xcr1* and *Ccr7*. It has been shown that gut activated T cells release XCL1, which binds to XCR1 expressed on tolerogenic DCs leading to the upregulation of CCR7 and their migration to the mesenteric lymph node (MLN), where OT is induced. Thus, we postulate that oral anti-CD3 induces tolerance via activation of XCL1-XCR1-CCR7 pathway.



Mucosal Vaccines

F108. Recombinant Norovirus P Domain Protein Fused with the Mucosal Adjuvant FlaB Induces Protective Conformer Antibody Response

Wenzhi Tan, Sao Puth, Seol Hee Hong, Kwangjoon Jeong, Shee Eun Lee and Joon Haeng Rhee. Chonnam National University, Hwasun, Cholla-namdo, Republic of Korea

Noroviruses (NoVs) are a major cause of childhood gastroenteritis and foodborne diseases worldwide. VP1 is the major capsid protein of the NoVs that acts as a binding motif to human histo-blood group antigens (HBGAs) through its protruding 2 (P2) domain and can serve as a protective antigen candidate for vaccine development. In the present study, we show that recombinant NoV P domain (Pd) polypeptide formed small particles and induced a robust humoral immune response when administered through intranasal route. Moreover, the mixture of the Pd with the mucosal adjuvant FlaB (Pd+FlaB) significantly enhanced the antibody response that was further enhanced when Pd was fused with FlaB (Pd-FlaB) as a fusion protein vaccine. Pd-FlaB, as well as Pd+FlaB induced a mixed T_H1/T_H2 type of immune response with a significant induction both of IgG1 and IgG2a antibodies in serum, and also induced strong IgA responses in serum and feces. FlaB-mediated antibody responses were toll like receptor 5 (TLR5) dependent, which was abrogated in $TLR5^{-/-}$ mice. Interestingly, we found that the antisera induced by Pd-FlaB bound only native form of Pd not the denatured conformation in the SDS-PAGE gel, while the antisera induced by Pd only or Pd+FlaB reacted both with native and denatured forms of Pd. The FlaB-Pd fusion vaccine preferentially stimulated Pd-specific conformational antibody production. These results suggest that Pd-FlaB fusion protein would serve as an effective anti-norovirus mucosal vaccine, providing protective immune responses in mucosal and systemic compartments neutralizing the native virus.

F110. C5a Receptor Signaling in Peyer's Patch Dendritic Cells Enhances the Antigen-Specific $CD8^+$ T Cell Response in Dengue Mucosal Vaccine Model

Sae-Hae Kim, Yu-Na Kim, Jisang Park, Ju Kim and Yong-Suk Jang. Chonbuk National University, Jeonju, Korea, jeonju-si, Cholla-bukto, Republic of Korea

Chemoattractant complement 5a (C5a) receptor (C5aR) is closely associated with mucosal protective immunity. In this study, we found that $CD11c^+CD11b^-CD8^-$ PP DCs expressed the C5aR where stimulation of C5aR with its cognate ligand, C5a, or a specific peptide (Co1) effectively induced antigen-specific IFN-γ^+ Th1 cells through the induction of pro-inflammatory cytokines. To confirm the role of C5aR activation *in vivo*, a model antigen, partial-nonstructural 3 (NS3) protein of dengue virus serotype 2 (DENV-2) was conjugated with Co1 peptide (p-NS3-Co1) and M cell-targeting of the antigen and co-localization with C5aR on M cells were confirmed. As we assumed, oral prime and boost immunization with p-NS3-Co1 effectively induced the NS3-specific IFN-γ^+ effector $CD8^+$ T cells. In addition, challenge with DENV-2 at 4 wks post immunization with p-NS3-Co1 induced not only the functional restimulation of memory effector $CD8^+$ T cells but also proliferation of $CD107a^+$ cytolytic effector $CD8^+$ T cells in mucosal and systemic compartments. Collectively, we concluded that C5aR plays a role as mucosal immune modulator in PPs and Co1 peptide ligand-mediated C5aR activation contributes to develop the $CD8^+$ T cell immune response induction. (This study was supported by 2014K1B1A1073861 through the National Research Foundation (NRF) funded by the Korean Ministry of Science, ICT, & Future Planning and by HI15C3039 through the Korea Health Industry Development Institute (KHIDI) funded by the Korean Ministry of Health and Welfare. Ms. Y. N. Kim and Mr. J. Park were supported by the BK21 PLUS program in the Department of Bioactive Material Sciences.)

F111. The Cathelicidin LL-37 Modulates the Peyer's Patch Follicular Helper T Cell Responses via P2X7 Receptor in Oral Porcine Epidemic Diarrhea Virus Vaccine Model

Sae-Hae Kim, Byeol-Hee Cho, Yu-Na Kim, Kyung-Yeol Lee and Yong-Suk Jang. Chonbuk National University, Jeonju, Korea, Jeonju-si, Cholla-bukto, Republic of Korea

Follicular helper $CD4^+$ T (T_{FH}) cells are closely associated with germinal center (GC) formation and the regulation of GC B cell differentiation into plasma cells or memory B cells. Recent report has shown that T_{FH} cells express ATP-gated ionotropic P2X7 receptor which regulates the number of T_{FH} cells, and dysregulation of T_{FH} cells caused by the lack of P2X7 receptor evokes the increased susceptibility to polymicrobial sepsis. Based on the report that P2X7 receptor interacts with LL-37 and then induces the IL-1β release in monocytes, we explored whether LL-37 modulates the T_{FH} cell response in Peyer's patch (PP). Our findings have shown that T_{FH} cells activated by LL-37 showed the decreased frequency of apoptosis compared with that by ATP and the increased IL-21 expression. We confirmed this observation *in vivo* by using N-terminal domain (NTD) of S1 spike protein of porcine epidemic diarrhea (PED) virus (PEDV) as a model antigen. Targeting function of LL-37 was confirmed by co-localization of LL-37-conjugated NTD (N-LL-37) with P2X7 receptor on M cells. In addition, the GC formation and T_{FH} activation was observed in the mice orally immunized with N-LL-37. Collectively, we conclude that the LL-37 plays a role as modulator of mucosal T_{FH} cells in PP and LL-37-mediated P2X7 receptor activation. (This work was supported by a grant from the Next-Generation BioGreen 21 Program, Project No. PJ011801, Rural Development Administration of Korea. Ms. B.-H. Cho and Y. N. Kim were supported by the BK21 PLUS program in the Department of Bioactive Material Sciences.

F112. *In vivo* Efficacy of Combining the M Cell-Targeting Molecules with MERS-CoV Antigen as an Adjuvant and Delivery Platform for Mucosal Vaccine Development

Ju Kim, Ye Lin Yang, Sae-Hae Kim and Yong-Suk Jang. Chonbuk National University, Jeonju, Korea, jeonju-si, Cholla-bukto, Republic of Korea

The mucosal epithelium is a major portal for pathogen invasion and M cells are able to shuttle the luminal antigen into the MALT for initiating mucosal immune responses. Consequently, strategies to activate this network can be applied to develop the effective mucosal vaccines against the pathogen infection. In this study, we have constructed three RBD fragments of MERS-CoV fused with previously defined M cell-targeting molecules and further investigated the antigen delivery to MALT. In addition, immunogenicity of the antigens conjugated with various M cell-targeting ligands was confirmed by comparing the ability to induce potent neutralization antibody responses after oral/intranasal administration. The results showed that the delivery of fusion proteins to mucosal immune inductive site was quite effective and that fusion proteins elicited higher antigen-specific mucosal IgA/systemic IgG responses than original RBD fragment without ligand conjugation. In addition, elevated expression of both IFN-α and IFN-β, which have been known to be required for MERS-CoV control was observed in PP lymphocytes from the mice immunized with fusion proteins compared with that of the mice immunized with RBD fragments without ligand conjugation. We will discuss the functional characterization of the efficacy of M cell-targeting molecules in a transgenic mouse model for MERS-CoV infection after mucosal immunization of the ligand-conjugated RBD antigens. (This study was supported by HI15C3039 through the Korea Health Industry Development Institute (KHIDI) funded by the Korean Ministry of Health and Welfare. Ms. Y. L. Yang was supported by the BK21 PLUS program in the Department of Bioactive Material Sciences.)

F113. *In vivo* Characterization of the Immune Response Towards the Pathogenic *Escherichia coli* Antigen SslE and Modulation of the intestinal Microbiota

Ilham Naili[1], Juliette Vinot[2], Barbara Baudner[2], Annick Bernalier-Donadille[1], Mariagrazia Pizza[2], Mickael Desvaux[1], Ugo D'Oro[2], Grégory Jubelin[1] and Cecilia Buonsanti[2]. [1]French National Institute of Agronomical Research, Saint-Genès Champanelle, Auvergne, France; [2]GlaxoSmithKline Vaccines, Siena, Toscana, Italy

Pathogenic *E. coli*, both intestinal (InPEC) and extraintestinal (ExPEC), account for a wide range of diseases, which can be fatal across developing and developed countries. They can acquire a vast array of virulence factors which may dramatically increase the severity of the infection. Emergence of resistant strains often renders antibiotics inefficient; in the case of Shiga toxin-producing *E. coli*, antibiotics therapy can even be detrimental. Hence, an *E. coli* vaccine with broad coverage could be a promising alternative to prevent the spread of such diseases, while offering the potential for protection against several InPEC and ExPEC at once. Using the «reverse vaccinology» approach on ExPEC strains, nine antigens were identified as protective against a murine sepsis model. With 82% protective efficacy, SslE (Secreted and Surface-associated Lipoprotein of *E. coli*) was the most potent candidate. Additional models showed SslE to also be cross-protective against other ExPEC strains. Functional assays have demonstrated *in vitro* and *ex vivo* that SslE is a mucinase which plays an important role in colonization and virulence of *E. coli*. To better understand the mechanism behind such protection, we investigated the intestinal and systemic immune responses obtained after immunization with SslE using different routes of administration. Analysis of each regimen has allowed us to determine the most efficient method of immunization for SslE to be protective, and we are currently evaluating the potential effects of SslE immunization on the murine intestinal microbiome. Together, these findings will be important to help us optimize the development of a potential broad-spectrum *E. coli* vaccine.

F114. Attenuation of Disease Severity Following Experimental H10407 Enterotoxigenic *E. coli* (ETEC) Challenge is Associated with $CD4^+$ T Cell Responses Against Heat-Labile Toxin LT, Fimbrial Tip Adhesin CfaE, and Major Subunit CfaB

Stefanie Trop, Glomil Corbin, Ramiro Guitierrez, Mark Riddle, Chad Porter, Michael Prouty and Milton Maciel. Naval Medical Research Center, Silver Spring, MD

ETEC infection is a leading cause of travelers' diarrhea and vaccine development is a priority for the US military. We assessed antigen-specific $CD4^+$ T cell responses to LT, CfaE, and CfaB among naïve controls in a Phase 2b ETEC vaccination-challenge trial. PBMCs from forty-two subjects who were orally challenged with $2x10^7$ CFU ETEC strain H10407 were peptide-stimulated *in vitro* pre-challenge and 28 days post-challenge, and evaluated by flow cytometry. Cell culture supernatant was analyzed by multiplex cytokine assays. Highest post-challenge responder rates observed by flow cytometry were IL-17 production in $CD4^+CCR6^+$ T cells, following stimulation with CfaB (69.0% subjects), CfaE (61.9%), and LT (54.8%). Cytokines most frequently detected in cell culture supernatant were to anti-CfaB: IL-13 (90%), IFNgamma (75%), IL-12, IL-17, and IL-2 (72.5%), IL-10 and IL-7 (70%); anti-LT: IL-13 (70%), IFNgamma (67.5%), and anti-CfaE: G-CSF (65%) and IFNgamma (62.5%). Moderate-to-severe diarrhea (MSD) was observed in 23/42 subjects. Those without MSD had higher antigen-specific $CD4^+$ responses within several parameters: MSD-negative subjects more frequently generated anti-LT, CfaE, and CfaB IL-17 and IL-4, most notably within $\alpha4^-\beta7^+CD4^+$ T cells (Fisher $P < 0.05$). Anti-LT and -CfaE IFNgamma response rates (Fisher $P < 0.05$), and net percentage of positive cells (Wilcox $P < 0.05$), were significantly higher in MSD-negative subjects as well. This response, particularly within potentially gut-homing $\alpha4^-\beta7^+$ $CD4^+$ cells, may suggest a cellular immune response was associated with protection from MSD. While humoral immunity has a primary role in protection from ETEC, optimal immunity may be multifaceted, and benefit from support of $CD4^+$ responses.

F115. Pneumococcal pep27 Mutant Immunization Activates Adaptive Immune Response via Tfh Proliferation and Memory Response in Spleen

Gyu Lee Kim and Dong-Kwon Rhee. Sungkyunkwan University, Su-Won, Kyonggi-do, Republic of Korea

Streptococcus pneumoniae, a known commensal and respiratory bacterial pathogen residing in the human nasopharynx is responsible to cause pneumonia for over 1 million deaths worldwide annually. It is characterized into more than 90 serotypes based on the composition of antigenic differences in capsular polysaccharides, however only 13-23 serotypes are effectively targeted by injectable vaccines currently available, and fail to protect initial colonization against various serotypes including non-typeable strains in the nasopharynx. Previously, we reported that intranasal Δpep27 immunization without any adjuvant effectively protected encompassing a wide array of serotypes with increased secretion of IL-4, IL-10, INF-γ, IL-17, and s-IgA in BALF. Here, we investigated immune response activation in spleen by Δpep27 vaccination. Δpep27 vaccination enriched specific sub-population of CD4 T cells, activated memory T cell response, and induced Tfh proliferation that ultimately lead to differentiation of plasma cells in splenocytes. Taken together, Δpep27 via activation of adaptive immune response in spleen is an important consideration in the current era of mucosal vaccination targeting a subset of capsule types that causes serotypes replacement.

F117. Oral Administration of Poly-γ-Glutamic Acid (γ-PGA) Induces Locally Antigen-Specific Cellular Responses through Mobilization and Maturation of Mucosal Dendritic Cells by Inducing Intestinal Chemokines and Cytokines

Jihyun Yang and Haryoung Poo. Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea

Poly gamma glutamic acid (γ-PGA) is a safe and edible biomaterial naturally secreted by *Bacillus subtilis sups*. *Chungkookjang* (Korean traditional soybean paste). Recently, it has been proposed that protective effects against tumor or viral infection are induced by oral administration of the γ-PGA. Although γ-PGA is considered as one of the mucosal adjuvants, little is known about their effects on mucosal immune system. This study demonstrated that oral administration of γ-PGA can enhance locally T cell activation through both up-regulation of chemokines and activation of dendritic cells. When γ-PGA was orally administered in mice, uptake of γ-PGA was observed in subepithelial dome region in peyer's patch (PP). Oral administration of γ-PGA increased various chemokines and IL-1α in the intestine. In addition, percentage of $CD8^-$ dendritic cells (DCs) were decreased in the PP but increased in the mesenteric lymph node (MLN) by oral administration of γ-PGA. Reversely, percentage of $CD8^+$ DCs were increased in the PP but decreased in the MLN. Oral administration of γ-PGA induced weakly maturation of mucosal DCs in the PP and MLN. The γ-PGA indirectly modulated activation of mucosal DCs. Finally, oral administration of γ-PGA plus ovalbumin antigen enhanced production of IFN-γ by T cells in MLN but not spleen. Conclusively, γ-PGA might play the role of a mucosal adjuvant capable of inducing locally antigen-specific cellular responses through mobilization and maturation of mucosal dendritic cells by inducing intestinal chemokines and cytokines.

F118. Mucosal Vaccine Adjuvant Cyclic Di-GMP Activates Pulmonary $CD11b^+$ Dendritic Cells in a TNFα-Dependent Manner *in vivo*

Samira Mansouri[1], Steven Blaauboer[1] and Lei Jin[2]. [1]Albany Medical College, Albany, NY; [2]University of Florida, Gainesville, FL

The mucosal surface is the major entry site for many human pathogens, however there are only a dozen of mucosal vaccines approved for human use. 3', 5'-Cyclic di-GMP (CDG) is an attractive mucosal vaccine adjuvant candidate that activates STING (stimulator of interferon genes) in pulmonary dendritic cells when administered intranasally. Pulmonary dendritic cells consist of two functionally distinct subsets: $CD103^+$ DC and $CD11b^+$ DC. How CDG targets these pulmonary DC subsets to promote its mucosal adjuvant activity is not known. Here, we investigated the role of different pulmonary DCs subsets in mediating the mucosal adjuvant activity of CDG. Using $Batf3^{-/-}$ mice, we found that $CD103^+$ DCs do not mediate CDG-induced antibody production. The population of pulmonary $CD11b^+$ DCs is still intact in the $Batf3^{-/-}$ mice, suggesting that $CD11b^+$ DCs are sufficient for CDG-induced humoral immunity. Furthermore, deleting $CD11b^+$ DCs in the $IRF4^{fl/fl}CD11c^{cre}$ mice demonstrated that $CD11b^+$ DCs are needed for CDG-induced antibody production and Th1/Th2/Th17 responses in

the lung. In addition, we found that CDG adjuvant activity completely depends on TNFα signaling and not type I interferon. Using $TNFR^{-/-}$ mice, we found that CDG-induced TNFa signaling is required for the activation of pulmonary $CD11b^{+}$ DCs. Our results demonstrate that CDG primarily targets and activates $CD11b^{+}$ pulmonary DCs to promote its mucosal adjuvant activity *in vivo*. Understanding the role of different pulmonary DC subsets will reveal insight in the mode of action of CDG *in vivo* and advance the development of CDG as an efficacious mucosal vaccine adjuvant.

F119. Oral Administration of Poly-γ-Glutamic Acid (γ-PGA) and Human Papillomavirus 16 E7-Expressing *Lactobacillus casei* Enhances Antitumor Efficacy Against Tumor Growth in a Murine Cancer Model

Eunjin Kim, Jihyun Yang and Haryoung Poo. Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea, South

Human papillomavirus (HPV) infection is closely associated with cervical cancer that is the second leading cause of cancer death among women worldwide. We previously reported that oral administration of HPV16 E7-expressing *Lactobacillus casei* induces antitumor effects in murine cancer model, but more efficacious antitumor strategies are needed. Recently, poly-gamma-glutamate (γ-PGA), a safe and edible biomaterial naturally secreted by *Bacillus subtilis*, has been reported to increase antitumor activity by activating dendritic cells and natural killer (NK) cells. Here we investigated the antitumor effect of γ-PGA in combination with HPV16 E7-expressing *L. casei* in a murine cancer model seeded with the E7-expressing tumor cell line (TC-1). When mice were administered orally with γ-PGA plus HPV16 E7-expressing *L. casei* in a mouse cancer model, the combined treatment markedly suppressed tumor growth and increased survival compared to oral administration of γ-PGA or HPV16 E7-expressing *L. casei* alone. Notably, in combined treatment of γ-PGA and HPV16 E7-expressing *L. casei*, pre-treatment of γ-PGA inhibited effectively tumor growth compared to pre-treatment of HPV16 E7-expressing *L. casei*. Collectively, these results indicate that the oral administration of γ-PGA induces a synergistic antitumor effect in combination with HPV 16 E7-expressing *L. casei*.

F120. Enhancement of Protective Mucosal Immunity Against Influenza Virus Infection by a Combination of Influenza Antigen Plus G9.1 (CpG ODN) as Nasal Adjuvant

Hideki Asanuma[1], Kohichiro Tateishi[1], Hideki Hasegawa[1], Norio Yamamoto[2], Kayoko Sato[1], Junichi Maeyama[1], Sumiko Iho[3], Saburo Yamamoto[4], Takato Odagiri[1] and Kohtaro Fujihashi[5]. [1]National Institute of Infectious Diseases, Musashi-Murayama-shi, Tokyo, Japan; [2]Juntendo University, Chiyoda-ku, Tokyo, Japan; [3]University of Fukui, Fukui-shi, Fukui, Japan; [4]Japan BCG Laboratory, Bunkyo-ku, Tokyo, Japan; [5]University of Alabama, Birmingham, AL

The majority of current licensed influenza vaccines are administered through systemic routes, and thus induce exclusively plasma IgG but not mucosal secretory IgA (SIgA) antibody (Ab) responses. Therefore, vaccine-induced Abs fail to directly block virus-cell attachment in the upper respiratory tract (URT). In addition, during the vaccine manufacturing process, substitution of viral nucleic acids leads to amino acid substitutions in the viral hemagglutinin (HA) which subsequently leads to lower vaccine efficacy. In order to overcome these disadvantages, we have developed a novel vaccine consisting of HA-expressing, virus-like particles (HA-VLP) and CpG ODN G9.1 (G9.1) as nasal adjuvant. BALB/c mice were nasally immunized with HA-VLP (HA from A/California/7/2009, Cal7; H1pdm) plus G9.1 or HA-VLP alone two times at two week intervals. Further, mice were given nasal Cal7 split vaccine (X179A) with or without G9.1. Two weeks after the last immunization, HA-specific Ab responses in the URT were determined and mice were nasally infected with live Cal7 influenza virus. Mice given nasal HA-VLP plus G9.1 revealed significantly higher levels of HA-specific SIgA Ab responses in the URT when compared with those mice given nasal HA-VLP alone, X179A plus G9.1 or X179A alone. Thus, mice vaccinated with nasal HA-VLP plus G9.1 showed complete protection from Cal7 challenge. Of importance, mice given nasal HA-VLP alone failed to provide protective immunity against the Cal7 challenge. These results showed that nasal vaccination with HA-VLP plus G9.1 is an effective strategy to induce HA-specific protective SIgA Ab responses in the URT against influenza virus infection.

F121. Peyer's Patch B Cells Play Distinct Roles for Induction of Antigen-Specific Intestinal IgA Antibody Responses Against Orally Administered Recombinant *Salmonella*

Tomomi Hashizume-Takizawa[1], Kohtaro Fujihashi[2], Jerry R. McGhee[2], Yosuke Kurashima[3], Naoko Shibata[4], Hiroshi Kiyono[4] and Tomoko Ochiai[1]. [1]Nihon University, Matsudo, Chiba, Japan; [2]University of Alabama, Birmingham, AL; [3]Chiba University, Minato-ku, Tokyo, Japan; [4]University of Tokyo, Minato-Ku, Tokyo, Japan

We have previously shown that Peyer's patch (PP) lymphoid structures and derived lymphocytes were critical for induction of antigen (Ag)-specific mucosal secretory (S)IgA antibody (Ab) responses against orally administered recombinant *Salmonella* expressing the fragment C of tetanus toxin (r*Salmonella*-Tox C). In this study, we have further defined the major cell types involved in the induction of tetanus toxoid (TT)-specific intestinal SIgA Ab responses. Our results showed that when wild-type PP cells were adoptively transferred into lethally irradiated, recipient mice, donor-derived PP cells were preferentially accumulated in recipient PPs. When $CD4^+$ T cells and $B220^+$ B cells isolated from PPs, mesenteric lymph nodes (MLNs) and spleen of naïve mice were cultured with $CD11c^+$ dendritic cells (DCs) purified from PPs of mice given oral r*Salmonella*-Tox C, significantly elevated levels of TT-specific IgA Ab responses were only induced in the cultures containing PP B cells. Importantly, when splenic $CD11c^+$ DCs purified from mice given r*Salmonella*-Tox C were cultured with $CD4^+$ T cells and $B220^+$ B cells from PPs, MLNs, or spleen, increased levels of TT-specific IgA Ab responses were only seen in the cultures containing PP B cells. Furthermore, surface IgA^+, but not IgA^-, PP B cells produced significantly higher levels of anti-TT IgA Abs in this culture system. These results suggest that surface IgA^+ PP B cells play distinct roles for induction of Ag-specific intestinal SIgA Ab responses against orally administered r*Salmonella*-Tox C.

F122. Novel Adjuvants for Mucosal and Systemic Immunity against Amebiasis

Mayuresh M. Abhyankar[1], Christopher B. Fox[2], Suraju Mohammed[1] and William A. Petri[1]. [1]University of Virginia, Charlottesville, VA; [2] Infectious Disease Research Institute, Seattle, WA

Diarrheal diseases are the second leading cause of death in children under the age of five. *Entamoeba histolytica* is an enteric protozoan pathogen that causes amebiasis in children from low income countries and is responsible for up to 100,000 deaths annually. The disease presentation ranges from mild diarrhea to severe dysentery and rarely liver abscess. Human immunity to amebiasis is associated with the production of IFN-g and stool IgA, however, no vaccine exists. We evaluated a liposomal adjuvant system containing two synthetic TLR ligands to trigger a protective immune response against the *E. histolytica* recombinant antigen LecA. We used a two-step approach comprising optimization of the adjuvant composition followed by that of immunization regimen. Liposome formulation containing a longer length PEG (MW 2000) generated 2-fold higher stool IgA ($p=0.02$) and 3-fold higher plasma IgG2a ($p=0.01$) response than that containing a shorter PEG (MW 750). Preliminary data suggested complementary functions for the TLR ligands: TLR4 ligand favored stool IgA production whereas TLR7/8 ligand favored a systemic TH1 response. Immunization using the optimal formulation and an intranasal only regimen elicited 8-fold higher stool IgA ($p<0.0001$), 3-fold higher plasma IgG2a ($p<0.0001$) as well as 3-fold higher IFN-g and IL-17A response ($p=0.0007$) over a subcutaneous only regimen. Finally, intranasal immunization resulted in superior protection efficacy compared to subcutaneous (56% Vs 33%, $p=0.03$) in a mouse model of intestinal amebiasis. Such an adjuvant system capable of eliciting a multifaceted immune response is a promising prospect for the development of vaccines against enteric pathogens.

F124. Roles of Variable Domain-Matched IgA and IgG Monoclonal Antibodies in Preventing Invasion of Gut-Associated Lymphoid Tissues by *Salmonella typhimurium*

Angelene F. Richards[1], John J. Varrone[2], Jennifer E. Westfall[2] and Nicholas J. Mantis[2]. [1]University at Albany, Albany, NY; [2]New York State Department of Health, Albany, NY

There is a desperate need for an effective vaccine against *Salmonella enterica* serovar Typhimurium (ST), an emerging cause of invasive non-typhoidal *Salmonella* (NTS) in sub-Saharan Africa. Because ST invades the intestinal mucosa through Peyer's patches before spreading to the liver and spleen, it is unclear to what degree secretory and serum antibodies collaborate to limit ST infection. In this study, we demonstrate that two different IgA monoclonal antibodies (mAb), Sal4 and PeA3, directed against different O-antigen epitopes on lipopolysaccharide (LPS) are effective at blocking ST uptake into mouse Peyer's patches. *In vitro*, the same two IgA mAbs inhibited ST flagellum-based motility and blocked type three secretion-mediated uptake into epithelial cells, suggesting a possible mechanism by which IgA interferes with ST entry into gut-associated lymphoid tissues. However, grafting for Sal4's variable domains (Fv) onto an IgG1 framework altered the antibody's biological activity. While Sal4 IgG inhibited ST motility and invasion into epithelial cells to a similar degree *in vitro* as Sal4 IgA, it was not effective at blocking ST entry into Peyer's patch tissues. Sal4 IgG did passively protect mice against systemic infection, as evidenced by a reduction in bacterial burden in the liver and spleen following intraperitoneal (i.p.) antibody treatment followed 24 hours later by ST challenge by the same route. These results highlight important biological differences associated with IgG and IgA antibodies that must be considered in vaccine design.

F125. Attenuated Oral Typhoid Vaccine Ty21a Elicits Tissue-Resident Memory CD8$^+$ T (CD8$^+$ TRM) Responses in Human Terminal Ileum

Jayaum Booth, Seema Patil, Robin Barnes, Bruce D. Greenwald, and Marcelo B. Sztein. University of Maryland School of Medicine, Baltimore, MD

Cell mediated immunity elicited by the oral typhoid vaccine Ty21a has been extensively characterized in human peripheral blood. However, following Ty21a-immunization, it is unknown whether tissue resident memory T cells (T_{RM}; $CD8^+CD69^+CD103^+$) located at the site of infection (terminal ileum (TI)) contribute towards protection against *S. Typhi* infection. Here we examined the response of $CD8^+$-T_{RM} subsets elicited by Ty21a-immunization in TI-lamina propria mononuclear cells (LPMC) obtained from volunteers undergoing routine screening colonoscopy. No significant differences were observed in the frequencies of LPMC $CD8^+$-T_{RM} subsets following Ty21a-immunization; however, ex-vivo total LPMC $CD8^+$-T_{RM} producing single cytokines (IFNg, IL-17A, or TNFa) were significantly higher in Ty21a-vaccinated than in unvaccinated volunteers. We next evaluated LPMC $CD8^+$-T_{RM} *S. Typhi*-specific responses using *S. Typhi*-infected autologous EBV-B cells as stimulators. These cells exhibited significantly higher levels of *S. Typhi*-specific IL-17A and IL-2 while LPMC $CD8^+CD69^+CD103^-$ T cells produced significantly higher IFNg and IL-17A. *S. Typhi*-specific IL-17A was significantly higher in $CD8^+$-T_{RM} than $CD8^+CD69^+CD103^-$ T cells, suggesting that TI-LPMC $CD8^+$-T_{RM} are primarily T_H17. Finally, we assessed $CD8^+$-T_{RM} in intraepithelial T lymphocytes (IEL) and observed that the frequency of IEL-$CD8^+$-T_{RM} is significantly lower following Ty21a-immunization. However, ex-vivo unstimulated IEL-$CD8^+$-T_{RM} elicited by Ty21a-immunization produced significantly higher levels of cytokines (IFNg, IL-17A, IL-2 and TNFa) than unvaccinated. This study provides the first demonstration of the effect of Ty21a-vaccination on $CD8^+$-T_{RM} (non-specific and *S. Typhi*-specific) responses in the LPMC and IEL compartments in the human TI mucosa and contributes to our understanding of the generation of mucosal immunity following oral Ty21a-immunization.

OR.84, F109. Activation and Induction of T Follicular Helper Cells in Nasopharnx-Associated Lymphoid Tissue of Children and Adults and its Critical Role in Anti-Influenza Response

Abdullah Aljurayyan[1], Muhammad S Zhang[1], Ravi Sharma[2], Paul McNamara[1], Steve Gordon[3] and Qibo Zhang[1]. [1]University of Liverpool, Liverpool, England, United Kingdom; [2] Alder Hey Children's Hospital, Liverpool, England, United Kingdom; [3] Liverpool School of Tropical Medicine, Liverpool, England, United Kingdom

Recent studies suggest a critical role for T follicular helper cells (Tfh) in vaccine-induced immunity against infection, although direct evidence from humans is limited. We have studied the frequency and function of Tfh in nasopharynx-associated lymphoid tissue (NALT) from children and adults, the activation/induction of Tfh by influenza vaccines and its role in antibody response. Frequencies of Tfh in NALT and their counterpart (pTfh) in peripheral blood from children and adults were analysed by flow-cytometry, and their role in anti-influenza HA antibody response examined following stimulation by influenza vaccines. Results: Abundant Tfh were shown in NALT mononuclear cells (MNC) compared to a modest number of "peripheral Tfh" (pTfh) in PBMC. Stimulation by either a live-attenuated influenza vaccine(LAIV) or an inactivated H1N1 vaccine antigen induced an increase in Tfh, with a stronger response in the former. The increase in Tfh number correlated well with the magnitude of anti-HA antibody production. In the cell co-culture with purified Tfh and B cells, significant anti-HA responses were observed only in the presence of Tfh following stimulation. Activation of IL-21-producing Tfh cells following the stimulation correlated with anti-HA antibody production, and IL-21 receptor blocking reduced the Tfh number and the antibody production. Induction of Tfh by the vaccine stimulation with/without adjuvant CpG-DNA was demonstrated by flow-cytometry and antigen-specific Tfh confirmed by CD154 expression. Conclusion: We demonstrated a critical role of Tfh in human anti-influenza antibody response. Promoting Tfh to enhance antigen-specific antibody responses in NALT may have important implications in future vaccination strategies.

OR.85, F116. Priming in the Mesenteric Lymph Nodes Licenses Tissue-Resident Memory CD8$^+$ T Cell Development in the Intestine

Zhijuan Qiu, Camille Khairallah, Jessica N. Imperato, Pedro E. Perez, Galina Romanov, and Brian S. Sheridan. Stony Brook University, Stony Brook, NY

CD8$^+$ tissue-resident memory T (T_{RM}) cells are a subset of memory T cells that reside in tissues and serve as the frontline defense to pathogen re-encounters. However, mechanisms regulating their development are not completely understood. Priming in specific lymphoid organs dictates the migration of effector CD8$^+$ T cells to appropriate tissues, whereas factors existing in the tissue determine *in situ* CD8$^+$ T_{RM} cell differentiation. Whether priming also regulates *in situ* CD8$^+$ T_{RM} cell differentiation has not been clearly addressed. Oral immunization with *Listeria monocytogenes* induced rapid and robust CD8$^+$ T_{RM} cells in the intestinal mucosa whereas intravenous (i.v.) immunization did not. To study the impact of priming location and environment on intestinal CD8+ T_{RM} cell development, effector CD8$^+$ T cells isolated from the MLN of oral-immunized mice or the spleen of i.v.-immunized mice were adoptively transferred into orally infected mice providing the same intestinal environment for donor cells primed in distinct locations. CD8$^+$ T cells primed in the MLN differentiated into CD69$^+$CD103$^+$ T_{RM} cells efficiently after entering the intestine. In surprising contrast, CD8$^+$ T cells primed in the spleen failed to differentiate into CD69$^+$CD103$^+$ T_{RM} cells after entering the same intestinal environment. We further demonstrated that pathogen-induced inflammation and pathogen-derived antigen present in the intestine were dispensable for CD8$^+$ T_{RM} cell differentiation. These data suggest that priming in the MLN licenses *in situ* CD8$^+$ T_{RM} cell development in the intestine regardless of the intestinal inflammatory state. Our study provides significant insights on rational vaccine design for gastrointestinal infections and cancers.

OR.87, F123. ATP Released by Bacteria in the Intestine Limits IgA Secretory Response and Protective Immunity

Fabio Grassi. Institute for Research in Biomedicine, Bellinzona, Ticino, Switzerland

In the small intestine, T cell dependent secretory IgA originates in PPs within the ileal mucosa. We previously demonstrated that T follicular helper (Tfh) cells in Peyer's patches (PPs) are sensitive to extracellular ATP and undergo cell death via ATP-gated ionotropic P2X7 receptor. In the small intestine of mice housed in our specific pathogen free (SPF) facility we detected micromolar concentrations of endoluminal ATP that was barely detectable in germ-free mice. ATP releasing bacteria were identified both in humans and mice. Accordingly, we detected extracellular ATP in cultures from different bacterial strains isolated from *ilea* of our mouse colony. Treatment of ileal bacteria with vancomycin/ampicillin/metronidazole (VAM) as a bactericidal antibiotic association resulted in bacterial damage and prominent ATP release. As expected, *in vivo* VAM administration resulted in acute significant increase in intestinal ATP with concomitant enhanced phosphatidyl-serine (PS) exposure in Tfh cells from PPs of WT but not *P2rx7*$^{-/-}$ mice, indicating that release of ATP by bacterial death affected Tfh cells abundance via P2X7. We show that lowering endoluminal ATP release by bacteria elicits more potent secretory IgA response and can confer enhanced protection to *Salmonella* infection in vaccination protocol with attenuated *Salmonella Thyphimurium*.

OR.88, F126. Evaluation of Novel Nanoemulsion Adjuvants (NanoStatTM) to Prevent Genital Chlamydia Infection and Associated Pathology

Kenneth Beagley[1], Logan Trim[1], Vira Bitko[2] and Ali Fattom[2]. [1]Queensland University of Technology, Brisbane, Queensland, Australia; [2]NanoBio Corporation, Ann Arbor, MI

Globally, 113 million new chlamydial infections occur each year. Antibiotics have not halted this epidemic and an effective vaccine is recognized to be the best means of reducing the incidence of pelvic inflammatory disease, ectopic pregnancy and infertility in women. Using the mouse model of chlamydial infection, the potential of NanoStat™, an oil-in-water nanoemulsion adjuvant, in combination with the chlamydial major outer membrane protein (MOMP) was investigated. Female BALB/c mice were vaccinated intranasally and then challenged with *C. muridarum* to determine vaccine effectiveness against infection and pathology. Intranasal immunization (IN) resulted in high MOMP-specific IgG and IgA antibody titers in serum and vaginal lavage as well as strong splenocyte proliferation as determined by CFSE assay. Splenocytes from immunized mice secreted high levels of IFNg, TNF-a and IL-17A, which are essential for protective immunity against chlamydial infection. Mice that were immunized with NanoStat™ and 20ug MOMP cleared infection faster and had 80% reduced incidence of oviduct occlusion (Hydrosalpinx) compared to PBS immunized controls. Using a unique combination of 3 antigens, targeting different stages of the chlamydial life cycle, IN immunization reduced oviduct occlusion by 90%, even though infectious burden was only reduced by 25-30%. Interestingly, intramuscular immunization with the same antigen combination mixed with a second generation nanoemulsion adjuvant reduced infectious burden by >80% but only reduced oviduct occlusion by 50%. These data highlight the potential of NanoStat™ adjuvants for preventing genital chlamydial infections and further support our findings that inflammatory damage is not related to infectious burden.

Respiratory Virus Infections

OR.14, W125. Mucosal GM-CSF Overexpression Limits Lung Immunopathology during Severe Influenza Infection by Altering Airway Macrophage M1/M2 Polarization

Eric Scott Halstead, Todd Umstead, Michael Davies, Yuka Imamura, Judie Howyrlak, Linlin Yang, Weichao Guo, Sanmei Hu, Roland Myers, Timothy Cooper and Zissis Chroneos. Penn State University, Hershey, PA

Influenza A viruses (IAV) cause life-threatening pneumonia and lung injury in the lower respiratory tract. GM-CSF has been shown to reduce morbidity and mortality from pathogenic influenza infection but the mechanisms of protection have not been fully delineated. Using an inducible airway GM-CSF over-expression model starting at 3 days post-infection we determined that supra-physiologic airway levels of GM-CSF could confer protection against influenza A virus infection-induced mortality as compared to wild-type levels. GM-CSF overexpression caused the significant preservation of several lung mechanic parameters and decreased broncho-alveolar fluid protein levels. RNA-seq analysis of FACS-sorted alveolar macrophage and exudative macrophage at 8 dpi gene sets revealed that enhanced levels of GM-CSF led to the preferential expression of genes usually characterized as M2 rather than M1-macrophage related transcripts. Unbiased RNA-seq analysis revealed significant activation of tripartite motif-containing 24 (TRIM24), and de-activation of STAT1 and multiple downstream interferon transcripts. Our data indicate that, contrary to canonical theory, high levels of GM-CSF in the lung lead to M2 polarization of macrophages and blockade of macrophage interferon responses. These changes in macrophage polarization decreased lung immunopathology during IAV infection. These data suggest a possible role for GM-CSF as a potential therapeutic agent in clinical lung diseases dominated by Tc1/Th1 responses such as acute respiratory distress syndrome (ARDS).

OR.15, W126. Inhibition of Necroptosis Ameliorates the Severity of *Pneumovirus bronchiolitis* and the Subsequent Development of Asthma in Mice

Jennifer Simpson[1], Zhixuan Loh[2], Vivian Zhang[1], Jason Lynch[1], Rhiannon Werder[1], Antoon Van Oosterhout[3], Peter Gough[4], John Upham[5], Kirsten Spann[6] and Simon Phipps[1]. [1]University of Queensland, Brisbane, Queensland, Australia; [2]Institute for Molecular Bioscience, Brisbane, Queensland, Australia; [3]University of Groningen, Groningen, Netherlands; [4]GlaxoSmithKline, Collegeville, PA; [5]Princess Alexandra Hospital, Brisbane, Queensland, Australia; [6]Queensland University of Technology, Brisbane, Queensland, Australia

Pneumovirus-induced bronchiolitis, characterised by neutrophilic inflammation and bronchial epithelial cell (BEC) death and sloughing, causes significant mortality in developing nations and is a major risk factor for subsequent asthma in the developed world. In a preclinical model, we have previously established that interferon regulatory factor 7 (IRF7) deficiency increases susceptibility to *Pneumovirus*-associated bronchiolitis and subsequent asthma, however the molecular processes that link these diseases remains poorly understood. Here, we assessed whether the mode of BEC death that occurs during viral bronchiolitis contributes to the later development of asthma. In both wild-type and IRF7$^{-/-}$ mice, *Pneumovirus* infection did not increase pro-apoptotic caspase-3 gene or protein expression, however in IRF7$^{-/-}$ mice there was a significant up-regulation in gene expression of the necroptosis-associated proteins, receptor-interacting protein kinase-1 (RIP1K) and mixed lineage kinase domain-like protein (MLKL). This response was mirrored by an increase in immunoreactive pRIPK1 and MLKL localised to BECs, and coincided with the release of several tissue alarmins: dsDNA, HMGB1, IL-33. Genetic or pharmacological inhibition of pRIP1K or MLKL significantly decreased necroptosis and increased apoptosis in BECs. This switch of cell death pathway significantly decreased BEC sloughing, alarmin release, neutrophilic inflammation, and viral load. Inhibition of

necroptosis in early-life prevented the development of asthma-like pathologies (airway hyperreactivity, airway remodelling and type-2 immunity) induced in response to viral challenge in later-life. Our findings suggest that viral bronchiolitis is causally linked to asthma and that inhibition of necroptosis may be a viable strategy to limit viral bronchiolitis and break its nexus with asthma.

W123. Long-Term Alteration of the Immune System Following Early-Life Respiratory Syncytial Virus Infection

Carrie Malinczak, Catherine Ptaschinski and Nicholas Lukacs. University of Michigan, Ann Arbor, MI

Respiratory syncytial virus (RSV) is often the first pathogen encountered in life and may alter the immune system, affecting future immune responses, such as those leading to chronic childhood asthma. Here we examine the effect of early-life RSV infection on the immune system and whether it remains altered later in life. Mice were infected at 7 days of age and tissues collected 4 weeks following infection to observe long-lasting changes. When comparing responses between sexes, bone marrow-derived dendritic cells (BMDC) from male mice infected with RSV exhibit lower expression of MHC II as well as co-stimulatory molecules than females and do not induce interferon-beta (IFN-β), IL-12p35 or IL-10 but do produce high levels of TNF. In contrast, females show increased expression of IFN-β, IL-12p35, IL-10 but lower TNF expression. Histology of the lungs revealed the presence of mucus in the male lungs but not the female lungs, at 4 weeks following infection. These data indicate that early-life infection with RSV leads to systemic and long-lasting alteration of the immune system, which may affect the immune response to allergen/pathogens later in life and that males may be more susceptible. These latter data are consistent with clinical data in RSV-infected infants.

W124. Respiratory Syncytial Virus Infection Elicits Enriched CD8$^+$ T lymphocyte Responses in Lung in African Green Monkeys

Hualin Li, Cheryl Callahan, Michael Citron, Zhiyun Wen, Sinoeun Touch, Morgan Monslow, Kara Cox, Daniel DiStefano, Kalpit Vora, Andrew Bett and Amy Espeseth. Merck & Co., Inc., West Point, PA

Respiratory syncytial virus (RSV) is a leading cause of serious lower respiratory tract disease in young children and older adults throughout the world. Prevention of severe RSV disease through active immunization is optimal but no RSV vaccine has been licensed so far. Immune mechanisms of protection against RSV infection in humans have not been fully established, thus a comprehensive characterization of virus-specific immune responses in a permissive animal model will be beneficial in defining correlates of protection. In this study, we infected juvenile naive African green monkeys (AGM) with RSV A2 strain and longitudinally assessed virus-specific humoral and cellular immune responses in both peripheral blood and the respiratory tract. RSV viral loads at nasopharyngeal surfaces and in the lung peaked from day 3 to 7 following infection, and then largely resolved by day 10. Low levels of neutralizing antibody titers were detected in serum, with similar kinetics as RSV fusion (F) protein-binding IgG antibodies. RSV infection induced CD8$^+$, but very little CD4$^+$, T lymphocyte responses in peripheral blood. Virus-specific CD8$^+$ T cell frequencies were ~10 fold higher in bronchoaveolar lavage (BAL) compared to peripheral blood and exhibited effector memory (CD95$^+$CD28$^-$) / tissue resident memory (CD69$^+$CD103$^+$) T cell phenotypes. The kinetics of virus-specific CD8$^+$ T cells emerging in peripheral blood and BAL correlated with declining viral titers, suggesting that virus-specific cellular responses contribute to the clearance of RSV infection. RSV-experienced AGMs were protected from subsequent exposure to RSV infection. Additional studies are underway to understand protective correlates in these seropositive monkeys.

W127. The Effects of Chronic Vapor Exposure on the Lung Mucosa and its Anti-Viral Defenses

Matthew Cameron Madison, Cameron Landers, Hui-Ying Tung, Bon-Hee Gu, Ran You, Nima Baghaei, Li-Zhen Song, Gretchen Diehl and Farrah Kheradmand. Baylor College of Medicine, Houston, TX

eCigarettes or electronic nicotine delivery systems (ENDS) have emerged as a common alternative to cigarette smoking and are promoted by advocates as a safe option. Regulations on these devices, however, have been minimal, and the solutions used in such systems have been found to contain potentially harmful compounds. It is, therefore, imperative that the health risks associated with the long-term use of ENDS be investigated. Our laboratory sought to address the consequence of chronic exposure to ENDS vapor on the recruitment and function of innate and adaptive immune subsets in the lung. Mice were exposed to ENDS, cigarette smoke, or room air, 5 times per week for a 4-month period. Markedly, mice receiving ENDS vapor did not exhibit the robust lung inflammation and emphysema that is characteristic of smoke-exposed mice. ENDS-exposed mice had no significant recruitment of immune cells to the airway, and computed tomograpical analysis of the lungs revealed no increase in overall lung volume. To further define the effects of vapor on mucosal defenses, mice were exposed to ENDS vapor and subsequently infected with a sublethal dose of the influenza A virus. Infected, ENDS-exposed mice exhibited augmented weight loss and impaired recovery when compared to air-exposed controls. Additionally, vapor-exposed mice had decreased recruitment of innate immune cells to the lung parenchyma and reduced influenza-neutralizing antibody titers. Together, our results suggest that ENDS vapor does not elicit an overt inflammatory response in the lung, but rather, may act to impede the appropriate immune responses to microbial insults.

W128. Immunological Changes in the Upper and Lower Respiratory Tract of Pigs Vaccinated with a Candidate Universal Swine Influenza Vaccine

Emily Porter[1], Sophie Morgan[2], Hanneke Hemmink[2], Ross Harley[1], Alain Townsend[3], Bryan Charleston[2], Elma Tchilian[2] and Mick Bailey[1]. [1]University of Bristol, Bristol, England, United Kingdom; [2]The Pirbright Institute, Woking, England, United Kingdom; [3]University of Oxford, Oxford, England, United Kingdom

Swine influenza virus (SIV) is a zoonotic pathogen circulating in pig herds. A universal vaccine would provide protection against multiple strains and avoid the need for annual novel vaccine development. S-flu is a candidate vaccine with a suppressed haemagluttinin (HA) signal sequence; it expresses viral proteins whilst remaining un-infective. Pigs were vaccinated with S-flu expressing H1 or H5 HA. Pigs were given H1 S-flu ($6.85x10^6 TCID_{50}$ by aerosol, $2x10^{7}$-$TCID_{50}$ intra-tracheally, $2x10^7 TCID_{50}$ intra-nasally) or H5 S-flu at $8x10^7 TCID_{50}$ in 4ml intra-tracheally and boosted 28 days later. All pigs were infected with $1.5x10^5$PFU/ml A/Sw/Eng/1353/09 31 or 45 days post boost. Three days post-infection, pigs were euthanised and samples collected for immunofluorescent analysis. Cryosections from tonsil and lung were stained for CD4, CD8, MHC II and MHC I. Images were analysed using ImageJ and R statistical package. Analysis of the tonsil revealed a significant difference between non-interacting $CD8^+$ and $MHCI^+$ cells in groups given homologous and heterologous vaccines; an increased number of $CD8^+$ and $MHCI^+$ cells was observed following H1 S-flu vaccination. S-flu vaccination alters the partition of $CD4^+$ and $CD8^+$ *T cell*s in *T cell* zones and B-cell follicles in tonsil. Further changes in the lung may distinguish between responses in vaccinated and unvaccinated animals, in addition it will allow comparisons between responses to infection in the upper and lower respiratory tract. S-flu has an impact on the immune response to infection with H1N1 and appears to reduce clinical signs and viral shedding (Morgan et al, 2016), suggesting that it may be a potential universal candidate vaccine.

W129. Complement C3 Plays a Key Role in Conferring Protection by the M2e-Based Influenza Vaccine by Regulating Both Humoral and Cellular Immunity

Yu-Jin Kim[1], Young-Tae Lee[1], Min-Chul Kim[1], Yu-Na Lee[1], Ki-Hye Kim[1], Jae-Min Song[2] and Sang-Moo Kang[2]. [1]Georgia State University, Atlanta, GA; [2]Sungshin Women's University, Seoul, Republic of Korea

The classical complement pathway is activated to eliminate antigen-antibody immune complexes, subsequently followed by complement-dependent cytotoxicity in addition to Fc receptor-mediated antibody-dependent cell-mediated cytotoxicity. However, the role of complement system remains largely unknown in influenza virus M2e-mediated cross protective immunity. We found that complement protein C3 is essential in inducing immune responses to influenza M2e vaccination and influenza virus infection, which include M2e-specific isotype-switched antibody production, M2e-specific effector CD4 and CD8 T cells, and the recruitment of dendritic cells. C3 knock-out (C3 KO) mice showed lower levels of M2e-specific IgG isotype antibodies after M2e vaccination, and no control of lung viral replication and no recovery from weight loss upon challenge infection compared to those in wild type (WT) mice. Whereas, C3 KO mice were protected against homologous virus after immunization with neutralizing antibody inducing hemagglutinin-based vaccine despite lower levels of antibodies than those in WT mice. In addition, C3 KO mice showed impaired recruitment of different subsets of dendritic cells after M2e vaccination and virus challenge. The findings in this study suggest that C3 is a key regulator in developing protective immunity by non-neutralizing antibody-based vaccination.

W130. LTbR Signaling Promotes Influenza-induced Lung Damage by Regulating Mucin Production

Cody M. Spencer[1], Ekaterina O. Gubernatorova[2], Ekaterina Koroleva[3], Wayne T. Muraoka[3], Marcia A. Blackman[1], Christopher M. Evans[4] and Alexei V. Tumanov[3]. [1]Trudeau Institute, Saranac Lake, NY; [2]Lomonosov Moscow State University, Moscow, Russia; [3]University of Texas Health, San Antonio, TX; [4]University of Colorado, Aurora, CO

Lymphotoxin beta receptor (LTbR) has been shown to play a critical role for the development and maintenance of lymphoid organs and protective immune responses to various pathogens. Surprisingly, we found that genetic or biochemical inhibition of LTbR signaling confers protection against highly pathogenic H1N1 influenza virus infection in spite of reduced influenza-specific IgG responses and $CD8^+$ T cells in the lung. LTbR-deficient mice showed attenuated viral loads during the peak of viral replication associated with marked goblet cell hyperplasia, increased MUC5AC mucin secretion and increased Th2 responses in the lung. Further, treatment of infected wild type mice with an agonistic anti-LTβR antibody inhibited MUC5AC expression in the lung and reduced the severity of disease. Mucin-containing BAL fluid from influenza-infected LTbR-deficient mice inhibited virus replication *in vitro*. Consistently, genetic inactivation of MUC5AC in LTbR-deficient mice promoted viral burden in the lung. Taken together, our data suggest that LTbR signaling inhibits mucin production in response to respiratory viral infection leading to increased viral burden, lung pathology, and death.

Abstract Index by Author

F

G

H

I

J

K

L

M

N

O

P

W

X

Y

Z

Abstract Index by Topic

Aging and Mucosal Immunology
W01, W4

Antigen Uptake
W05, W06

Celiac Disease
OR.56 (T04), OR.57 (T03), OR.58 (T07), PR.04 (T05), T01, T02, T06

Dendritic Cells
OR.21 (W14), OR.22 (W08), OR.23 (W07), OR.24 (W16), OR.25 (W12), PR.03 (W18), M.07, W09, W10, W11, W13, W15, W17

Effector T Cells and Cytokines
OR.86 (F12), OR.89 (F20), OR.90 (F07), OR.91 (F18), OR.92 (F06), OR.93 (F09), M.01, F01, F02, F03, F04, F05, F08, F10, F11, F13, F14, F15, F16, F17, F19, F21, F22

Epithelial Cells and Innate Immunity
OR.45 (T38), OR.46 (T20), OR.47 (T37), OR.48 (T13), OR.49 (T09), OR.78 (T24), OR.94 (F26), OR.95 (F25), OR.96 (F27), OR.97 (F23), OR.98 (F24), T08, T10, T11, T12, T14, T15, T17, T18, T19, T21, T22, T23, T25, T26, T27, T28, T29, T30, T31, T33, T34, T35, T36, T39, T40, T41, T42

Food Allergy
OR.75 (T49), OR.77 (T46), L.03 (F28), T43, T44, T45, T48

HIV
OR.11 (W19), OR.50 (T51), OR.51 (T50), OR.52 (T56), OR.54 (T53), M.05 (F29), T52, T54, T55, T57

Host-Microbiota Interactions
OR.01 (W25), OR.02 (W22), OR.03 (W21), OR.05 (W24), OR.100 (F70), OR.101 (F55), OR.103 (F52), OR.31 (W23), OR.34 (W20), OR.41 (T60), OR.43 (T61), OR.44 (T59), OR.55 (T58), OR.72 (T62), OR.99 (F42), L.04 (F59), F30, F31, F32, F33, F34, F35, F36, F37, F38, F39, F40, F41, F43, F44, F45, F46, F47, F48, F49, F50, F51, F53, F54, F56, F57, F58, F60, F61, F62, F63, F64, F65, F66, F68, F69, F71, F72, F73, F74, F75, F76, F77, F78

Immune Cell Migration
OR.53 (T65), T63, T64

Immunology of Asthma – Basic
OR.79 (F83), OR.81 (F90), OR.82 (F89), OR.83 (F92), F79, F80, F81, F82, F84, F85, F86, F87, F88, F91

Immunology of Asthma – Clinical
OR.80 (F83)

Immunology of the Eye
OR.42 (T66)

Immunology of the UG Tract
OR.26 (W32), OR.28 (M.06, W30), OR.29 (W26), W27, W28, W29, W31

Inflammatory Bowel Disease – Basic
OR.04 (W44), OR.102 (F96), OR.16 (W70), OR.17 (W53), OR.18 (W81), OR.19 (W84), OR.20 (W80), OR.36 (W55), OR.37 (W49), OR.38 (W64), OR.39 (W68), OR.40 (W72), OR.62 (T71), OR.70 (T67), OR.71 (T68), OR.73 (T69), T128, L.01 (F94), M.03 (F95), M.04, W35, W36, W37, W38, W39, W40, W41, W42, W45, W46, W47, W48, W50, W51, W52, W54, W56, W57, W58, W59, W60, W61, W62, W63, W65, W66, W67, W69, W71, W73, W74, W75, W76, W77, W78, W79, W82, W83, W85, W86, W87, W131

Inflammatory Bowel Disease – Clinical
OR.69 (T72), PR.02 (T70), T32, T47, T73

Innate Lymphoid Cells
OR.06 (W97), OR.07 (W106), OR.08 (W105), OR.09 (W103), OR.10 (W104), PR.01 (W94), W100, W101, W102, W88, W89, W90, W91, W92, W93, W95, W96, W98, W99

Monocytes and Macrophages
OR.59 (T78), OR.60 (T81), OR.61 (T16), OR.63 (T79), T74, T75, T76, T77, T80, T82, T83

Mucosal B Cells
OR.104 (F99), OR.105 (F103), OR.106 (F102), OR.107 (F101), OR.108 (F105), F97, F98, F100, F104, F106

Mucosal Immunology in the Neonate
OR.32 (W117), OR.33 (W107), OR.35 (W113), PR.05 (W116), W02, W03, W43, W108, W109, W110, W111, W112, W114, W115, W118, W119

Mucosal Infections
OR.12 (W121), OR.13 (W122), OR.30 (W120), OR.64 (T87), OR.65 (T97), OR.66 (T91), OR.67 (T110), OR.68 (T99), L.02 (F107), M.02, T84, T85, T86, T88, T89, T90, T92, T93, T94, T95, T96, T98, T100, T101, T102, T103, T104, T105, T106, T107, T108, T109, T111, T112

Mucosal Tolerance

OR.74 (T119), OR.76 (T120), T113, T114, T115, T116, T117, T118, T121, T122, T123, T124, T125, T126, T127

Mucosal Vaccines

OR.84 (F109), OR.85 (F116), OR.87 (F123), OR.88 (F126), F108, F110, F111, F112, F113, F114, F115, F117, F118, F119, F120, F121, F122, F124, F125

Respiratory Virus Infections

OR.14 (W125), OR.15 (W126), W123, W124, W127, W128, W129, W130