

# ICMI

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ABSTRACT  
SUPPLEMENT

### **A conserved population of MHC II-restricted, innate-like, commensal-reactive T cells (Tmic) in the gut of humans and mice**

Emily Thornton - University of Oxford, Carl-Philipp Hackstein, Linnea Drexhage, Claire Pearson, Samuel Bullers, Nicholas Ilott, Hossain Delowar Akther, Yisu Gu, Dana Costigan, Michael E.B. FitzPatrick, Oliver J. Harrison, Lucy C. Garner, Elizabeth H. Mann, Matthias Friedrich, Nicholas M. Provine, Emanuele Marchi, Paul Klenerman, Fiona Powrie

Interactions with commensal microbes shape host immunity on multiple levels and are recognized to play a pivotal role in health and disease. MHC Class II-restricted helper T cells are a sizeable and diverse cell population in the gut, and their role in orchestrating immune responses places them at the balance point between protection and pathogenesis. In this study, we show that MHC-II restricted, commensal-reactive T cells in the colon of both humans and mice acquire transcriptional and functional characteristics typically associated with innate-like T cells, including the expression of the key transcription factor PLZF and the ability to respond to cytokines including IL-12, IL-18 and IL-23 in a TCR-independent manner. These MHC-II restricted, innate-like, commensal-reactive T cells (Tmic) are endowed with a polyfunctional effector potential spanning classic Th1- and Th17-cytokines, cytotoxic molecules as well as regulators of epithelial homeostasis and represent an abundant and conserved cell population in the human and murine colon. T cells with the Tmic phenotype were increased in ulcerative colitis patients and their presence aggravated pathology in DSS-treated mice, pointing towards a pathogenic role in colitis. Our findings add Tmic cells to the expanding spectrum of innate-like immune cells positioned at the frontline of intestinal immune surveillance, capable of acting as sentinels of microbes and the local cytokine milieu.

### **A Gata3 enhancer necessary for ILC2 development and function**

Shan Kasal - University of Washington

The type 2 helper effector program is driven by the master transcription factor GATA3 and can be expressed by subsets of both innate lymphoid cells (ILCs) and adaptive CD4+ T helper (Th) cells. While ILC2s and Th2 cells acquire their type 2 differentiation program under very different contexts, the

distinct regulatory mechanisms governing this common program are only partially understood. Here we show that the differentiation of ILC2s, and their concomitant high level of GATA3 expression, are controlled by a Gata3 enhancer, Gata3 +674/762, that plays only a minimal role in Th2 cell differentiation. Mice lacking this enhancer exhibited defects in several but not all type 2 inflammatory responses, depending on the respective degree of ILC2 and Th2 cell involvement. Our study provides molecular insights into the different gene regulatory pathways leading to the acquisition of the GATA3-driven type 2 helper effector program in innate and adaptive lymphocytes.

### **A high-fat diet activates the BAs-FXR axis and triggers cancer-associated fibroblast properties in the colon**

Tae-Young Kim - University of Ulsan College of Medicine/Asan Medical Center, Seungil Kim, Yeji Kim, Yong-Soo Lee, Sohyeon Lee, Su-Hyun Lee, Mi-Na Kweon

**Background & Aims:** Dietary signals are known to modulate stemness and tumorigenicity of intestinal progenitors; however, the impact of a high-fat diet (HFD) on the intestinal stem cell (ISC) niche and its association with colorectal cancer remains unclear. Thus, we aimed to investigate how a HFD affects the ISC niche and its regulatory factors. **Results:** We found that expression of CD44 and Wnt signal-related genes was higher in the colonic crypts of HFD-fed mice than in those fed a purified diet. Within the ISC niche, mesenchymal stromal cells (MSCs) were expanded and secreted predominant levels of Wnt2b in the colon of HFD-fed mice. Of note, increased energy metabolism and cancer-associated fibroblast (CAF)-like properties were found in the colonic MSCs of HFD-fed mice. Moreover, colonic MSCs from HFD-fed mice promoted the growth of tumorigenic properties and accelerated the expression of cancer stem cells (CSCs)-related markers in colon organoids. In particular, production of primary and secondary BAs was increased through the expansion of bile salt hydrolase-encoding bacteria in HFD-fed mice. Most importantly, BAs-FXR interaction stimulated Wnt2b production in colonic CAF-like MSCs. **Conclusions:** HFD-induced colonic CAF-like MSCs play an indispensable role in balancing the properties of CSCs through activation of the BAs-FXR axis.

### **A mucin-degrading gut bacterium modulates food allergy sensitization in a diet-dependent manner**

Marie Boudaud - Luxembourg Institute of Health, Amy L. Parrish, Marie Boudaud, Erica T. Grant, Stéphanie Willieme, Mareike Neumann, Oliver Hunewald, Markus Ollert, Mahesh S. Desai

Alterations in the gut microbiome, including diet-driven changes, are commonly linked to the rising prevalence of food allergy, yet little is known about mechanisms of how gut bacteria are involved in the breakdown of oral tolerance. Our previous work showed that depriving mice of dietary fiber leads to a microbiota-driven mucus barrier erosion. Here, we hypothesized that the microbiota-mediated mucus barrier disruption contributes to the breakdown of oral tolerance, thereby leading to exacerbated allergic sensitization. Specific-pathogen-free (SPF) and gnotobiotic mice with functionally characterized synthetic human gut microbial communities were fed a fiber-deprived diet. Broad immunophenotyping was performed by using time-of-flight mass cytometry, alongside ELISA-based assays and IgE-coating of the gut bacteria was evaluated by flow cytometry. Depriving mice of dietary fiber led to the microbiota-mediated colonic mucus barrier disruption, a surge in IgE-coated commensals and an increase in the colonic type 2 immune cells. Consistently, fiber deprivation exacerbated anaphylaxis symptoms of ovalbumin and peanut-sensitized mice in both SPF and gnotobiotic models. By removing *Akkermansia muciniphila* from the synthetic gut microbiota, fiber-deprived mice displayed decreased severity upon challenge, with reduced proportions of T<sub>H</sub>2 cells, Gata3 + regulatory T cells, CD8 + Gata3 + T cells, ILC2, M2 macrophages and eosinophils in the colon, as well as reduced IgE-coating of commensals. These results support a role for a commensal mucin-degrading gut bacterium and the microbiota-mediated mucus barrier disruption in the sensitization to food allergens. Our study highlights a mechanistic link between diet and the gut microbiome in food allergy, which has important therapeutic implications.

### **A single genetic locus regulates mouse strain-specific tuft cell differentiation and innate type 2 immunity in the small intestine.**

Marija Nadsombati - University of Washington, Natalie Niepoth, Lily Webeck, Andrés Bendesky, Jakob von Moltke

Epithelial tuft cells are critical initiators of the small intestinal type 2 immune response. During helminth infection or protist colonization, tuft cell-derived IL-25 activates group 2 innate lymphoid cells (ILC2s) in the lamina propria. IL-13 from ILC2s signals on intestinal stem cells promoting their differentiation into tuft cells, thereby completing a feed-forward tuft-ILC2 circuit. The importance of this circuit has been demonstrated in numerous contexts, but how epithelial progenitors become tuft cells remains poorly understood. Here we report a novel genetic locus that regulates tuft cell differentiation and the threshold of tuft-ILC2 circuit activation. C57BL/6J mice treated with succinate, a known intestinal tuft cell ligand, develop tuft cell hyperplasia whereas Balb/cJ mice do not. Priming Balb/cJ mice with recombinant IL-25 restores succinate responsiveness, suggesting all components of the tuft-ILC2 circuit are functional, yet differentially regulated. This regulation is not dependent on the microbiome and can be recapitulated in epithelial organoids. Quantitative trait loci mapping of succinate responsiveness revealed a single peak on chromosome 9. Congenic Balb/cJ mice carrying the C57BL/6J chromosome 9 locus (Balb.Chr9 B6/B6) have elevated baseline numbers of tuft cells and respond to succinate. The chromosome 9 locus is adjacent to *Pou2f3*, a transcription factor required for tuft cell differentiation, but does not contain any genes associated with tuft cell function. In sum, Balb.Chr9 B6/B6 mice demonstrate how baseline tuft cell frequencies determine the threshold of activation for type 2 immune responses in the small intestine and will facilitate discovery of a novel genetic regulator of tuft cell differentiation.

### **A subset of CD4+ effector memory T cells limit immunity to pulmonary viral infection and prevent tissue pathology via activation of latent TGFβ**

Mark Travis - University of Manchester, Stephanie Houston, Stefano Rossi, Gang Liu, Tovah Shaw, Joshua Casulli, Mark Fife, Catherine Smedley, Thomas Griffith, Marion Pepper, Tracy Hussell, Philip Hansbro, Jean-Marc Schwartz, Helena Paidassi

A rapid immune response upon pathogen re-exposure underpins immunological memory, with cross protection against divergent pathogens such as heterologous or novel viral strains requiring cross-reactive memory T cells. Understanding the pathways which control memory T cell formation and function is therefore crucial for the rational

design of viral vaccines, and will aid the discovery of therapies to boost anti-viral immunity. The cytokine TGF $\beta$  is a key regulator of mucosal homeostasis by restraining effector T cell function. TGF $\beta$  is always secreted as a latent complex which must be activated extracellularly. However, the role of TGF $\beta$  activation in regulating memory T cell function remains poorly understood. Here, we show that a population of CD4+ effector memory T (T EM ) cells activate latent TGF $\beta$  via expression of an integrin,  $\alpha\beta 8$ . Integrin  $\alpha\beta 8$  expression marks a transcriptionally distinct sub-population of CD4+ T EM , enriched for anti-inflammatory pathways. Loss of  $\alpha\beta 8$  on murine CD4+ T EM , but not Foxp3+ Tregs, led to exacerbated virus-specific CD8+ T cell responses following secondary influenza infection, which was associated with enhanced viral clearance. However, although accelerating viral clearance, loss of  $\alpha\beta 8$  expression on CD4+ T EM resulted in enhanced lung pathology following secondary influenza infection, which was completely reversed by adoptive transfer of  $\alpha\beta 8$ + CD4+ T EM . These data highlight a novel pathway by which a distinct CD4+ memory T cell subset restrains anti-viral immunity to prevent host tissue damage during secondary viral infection. Such pathways could be targeted therapeutically to either boost memory T-cell-mediated immunity, or restrain host tissue damage during viral infection.

### Activated CD4+ T cells induce epithelial cell death in an IFN-gamma dependent mechanism

Michelle Cruz, Satoru Fujii, Thaddeus Stappenbeck, Alan Levine

Ulcerative colitis (UC) affects the mucosal epithelial layer with prominent chronic mucosal inflammation of the distal colon, which spreads proximally. Although there is evidence of multiple T cell subsets causing damage to the mucosa, the specific pathophysiology remains unclear. This study aims to define the bi-directional communication of T cells subsets with epithelial cells in the context of health and diseased states such as UC. Blood-derived CD4+ or CD8+ T cells were isolated from healthy human donors using negative magnetic separation. These cells were co-cultured with colonic epithelial stem cell spheroids in Matrigel from either healthy donors or UC patients (latter from inflamed lesions and non-inflamed areas). A subset of T cells was activated with anti-CD3/CD28 tetramer for 24 h prior to coculture. Activated CD4+ T cells co-cultured with spheroids from healthy donors caused significant

epithelial cell death, using brightfield microscopy (Fig 1A and B) and flow cytometry. Other T cells (CD8 with and without activation and non-activated CD4) had no effect. In contrast, epithelial spheroids from involved and uninvolved UC tissue co-cultured with any T cell subset were resistant to cell death (Fig 1B). UC spheroids cocultured with non-activated T cells and activated CD8+ T cells, in fact, were protected or proliferated. These findings demonstrate the complex effects that T cells have on intestinal epithelial cells. Further studies are needed to define the mechanism of cell death in healthy tissue and resistance to T cell-mediated death in UC epithelial cells.

### Akkermansia muciniphila influences adaptive immune responses to the microbiota in early life

Sarah Maddux - University of Pennsylvania, Jamal Green, Jean-Bernard Lubin, Tereza Duranova, Michael Silverman

The mucus-degrading microbe *Akkermansia muciniphila* is a common human commensal that is correlated with better outcomes for conditions ranging from autoimmune diabetes to cancer immunotherapy; it is an immunostimulatory microbe that strengthens the intestinal epithelial barrier and is capable of inducing antigen-specific T cell and antibody responses. However, little is known about the mechanisms by which *A. muciniphila* influences host immunity and barrier function. Using a defined murine pediatric microbial community our lab developed, we have found that in addition to inducing peripheral Tregs and stimulating a strong systemic antibody response to itself, *A. muciniphila* enhances systemic antibody responses to other commensal microbes. These effects only appear when exposure happens prior to weaning in NOD mice, emphasizing the importance of early life microbial exposure in long term immune development. Using a mutant strain of *A. muciniphila* that cannot degrade mucus, we have also recently found that the mucus-degrading capacity of *A. muciniphila* is essential for *A. muciniphila* to elicit a humoral response. Collectively, this data elucidates many of the host and microbe parameters that are necessary for *A. muciniphila* to influence adaptive immunity.

### An unexpected role for cDC1s in Th1 responses to *Cryptosporidium*



Ian Cohn - University of Pennsylvania, Bethan Wallbank, Jodi Gullicksrud, Jennifer Dumaine, Breanne Haskins, Keenan O'Dea, Ryan Pardy, Boris Striepen, Christopher A. Hunter

Cryptosporidium is a protozoan parasite that is a major cause of diarrhea and death in immunocompromised individuals and malnourished children. There is no vaccine, and the single FDA-approved therapy is ineffective in those most at-risk. Protective immunity to Cryptosporidium requires CD4<sup>+</sup> T cells and interferon gamma (IFN- $\gamma$ ). Analyses of CD4<sup>+</sup> responses have been limited, as no MHCII-restricted parasite antigens are known, and most of the CD4<sup>+</sup> T cells in the gut possess an "activated but resting" phenotype," preventing the use of activation markers for identifying cells responding to infection. Therefore, few studies have investigated antigen-specific T cell responses to Cryptosporidium. Transgenic *C. parvum* was engineered to express the 2W1S or LCMV-gp 61-80 (gp61) MHCII-restricted epitopes ( Fig 1A ). Mice infected with these parasites showed expanded populations of endogenous (2W1S-specific) and adoptively transferred (SMARTA TCR transgenic) CD4<sup>+</sup> T cells when infected with 2W1S- or gp61-expressing *C. parvum* , respectively ( Fig 1B ). Transfer of parasite specific CD4<sup>+</sup> T cells from WT mice into IFN- $\gamma$ -/- mice provided protection, expressed T-bet, and produced IFN- $\gamma$ , confirming a role for Th1 cells in resistance to Cryptosporidium ( Fig 2 ). CD4<sup>+</sup> T cells required type 1 conventional dendritic cells (cDC1s), as mice lacking these cells were unable to generate parasite-specific CD4<sup>+</sup> T cells. We are now able to track parasite-specific T cells which allows us to uncover the cellular and molecular mechanisms of CD4<sup>+</sup> T cell priming and activity during Cryptosporidium infection.

### Analysis of Dectin-1 expression and its impact on recurrence in nasal polyp

Dong-Young Kim - Seoul National University College of Medicine

Dectin-1 is traditionally known as a pattern recognition receptor related to fungal immunity, and it can induce several other immune responses. There was a previous report that the expression of Dectin-1 was increased in nasal polyp (NP). In order to evaluate its effect on NP, we compared the expression levels of mRNA and protein by real-time PCR and Western blot analysis, respectively, among 4 tissue types (uncinate tissues (UT) from normal control, chronic rhinosinusitis (CRS) without

NP (CRSsNP) patient, and CRS with NP (CRSwNP) patient, and NP tissue from CRSwNP patient). As a result of the analysis, Dectin-1 mRNA expression was significantly higher in NP of CRSwNP patient than that in UT of normal control (  $p = 0.0069$  ). On the contrary, it was significantly lower in UT of CRSwNP group, compared with those in UTs of the control (  $p = 0.0055$  ) and CRSsNP groups (  $p = 0.0013$  ), and NP of CRSwNP group (  $p < 0.0001$  ). The expression of Dectin-1 protein was higher in NP than those in UTs of 3 groups, however, it was not significant. The patients who had a relapse of CRSwNP showed significantly lower expression of Dectin-1 mRNA in NP than those without recurrence. Taken altogether, the expression of Dectin-1 can be utilized as a predictive factor for the recurrence of NP, and the role of Dectin-1 agonist as a therapeutic agent can be considered.

### Analysis of the mechanisms that sustain regulatory T cells in the small intestine

Elisa Cruz-Morales - University of Pennsylvania, Andrew Hart, Terri M. Laufer

Regulatory T cells (Tregs) are important for maintaining intestinal homeostasis. In steady state, the pool of small intestine lamina propria (siLP) Tregs includes two subpopulations: peripheral Tregs that are microbiota specific and express ROR $\gamma$ t and a Helios<sup>+</sup> subpopulation of natural Tregs that developed in the thymus. We previously showed that Helios<sup>+</sup> thymic Tregs filled the siLP of K14 transgenic mice lacking peripheral TCR-MHCII and the differentiation of ROR $\gamma$ t<sup>+</sup> peripheral Tregs. We now ask how the Helios<sup>+</sup> and ROR $\gamma$ t<sup>+</sup> siLP Treg subpopulations are maintained in the siLP with a specific focus on the MHCII-dependence of costimulatory signals. We used blocking reagents to examine the differential requirements for CD28, CTLA4, and ICOS in the proliferation, maintenance, and survival of siLP Tregs. We found that the relative proportion of ROR $\gamma$ t<sup>+</sup> and Helios<sup>+</sup> Tregs in siLP is differentially modulated by blockade of ICOS or CD28. Helios<sup>+</sup> Tregs rely only on CD28 and require neither ICOS nor MHCII signaling for their expansion; MHCII, CD28, and ICOS signaling contribute to ROR $\gamma$ t<sup>+</sup> Treg maintenance. We find that the composition of the Treg pool is dynamic; both subpopulations expand to compensate for the loss of the other to maintain total siLP Treg numbers. Together, our data highlight the intricate signaling network that regulates the intestinal Treg homeostasis.

### Androgen receptor regulates ILC2s during influenza infection

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Influenza virus (IAV) infection can cause severe morbidity due to infiltration of inflammatory immune cells into lungs. While males show worse outcomes when very young or elderly, females suffer increased morbidity during the reproductive period. Female mice show increased weight loss and a decrease in lung function and recovery. Androgens have a protective effect in respiratory infections through modulation of the inflammatory response in the lung; however, direct effects of androgens on immune cells are understudied. We have noted high levels of androgen receptor (AR) expression in pulmonary ILC2s compared to other lymphocytes, and AR was reported to bind genes important for ILC2 development and function. Female mice have an increased number of lung resident ILC2s during homeostasis and IAV infection compared to male mice. However, female ILC2s show preferential suppression during IAV, noted by a decrease in expression of GATA3 and higher levels of IFNGR. Using a lymphocyte-restricted AR knockout model (ARKO), we showed that male ARKO and WT female mice have similar total ILC2 numbers and share the same suppressed phenotype during infection. These data lead us to hypothesize that AR signaling regulates the sex disparate differences in ILC2 function during both homeostasis and IAV infection. We are now elucidating the role of AR signaling in ILC2s by analyzing immune cells in the lung using flow cytometry, immunohistochemistry, and RNA analysis during the peak and recovery phases of IAV infection.

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### Antibiotic-induced *Malassezia* spp. expansion in infants elicits intestinal immune dysregulation and increases airway inflammation in mice

Erik van Tilburg Bernardes - University of Calgary, Mackenzie W. Gutierrez, Carolyn A. Thomson, Kathy D. McCoy, Stephen B. Freedman, Marie-Claire Arrieta

Antibiotics deleteriously impact the gut microbiome and can increase the risk of childhood asthma. The impact of antibiotics on the fungal mycobiome has been largely unexplored. We conducted an observational, prospective clinical study to investigate how antibiotics impact the mycobiome of young infants. We compared the bacterial and fungal microbiome in feces collected before and after antibiotics via shallow shotgun and ITS2 sequencing, as well as qPCR for fungal and bacterial DNA. Antibiotic use increased fungal detection, and resulted in the expansion of *Malassezia* spp. To determine how *Malassezia* spp. impacts host immune development, germ-free mice were colonized with a consortium of bacteria, and/or human associated yeasts. Four groups of germ-free mice were colonized with consortia of (i) 12 mouse-derived bacteria (Oligo-MM12), (ii) Oligo-MM12 and *M. restricta*, (iii) Oligo-MM12 and *Candida albicans* and *Saccharomyces cerevisiae*, or (iv) Oligo-MM12 and all three fungi. *M. restricta* colonization increased Th1 and Th2 cells, eosinophils, and delayed macrophage maturation in the colonic lamina propria. *M. restricta* also increased migratory dendritic cells, eosinophils, Th2, Th17 and ILC3 cells in mesenteric lymph nodes, suggesting increased movement of microbiome-derived

antigens to immune priming sites, and elevated immune responses deemed critical in atopy development. *Malassezia* colonization also increased house dust mite-induced airway inflammation, with marked eosinophilia in the bronchoalveolar lavage. This translational work shows that fungal overgrowth and expansion of *Malassezia* spp. are previously overlooked collateral effects of infant antibiotic use, which causally contribute to immune dysregulation and increased susceptibility to allergic airway inflammation in mice.

### Antibody functionality orchestrates intestinal tissue homeostasis

Biana Bernshtein - Ragon Institute of MGH, MIT and Harvard, Esther Ndungo, Deniz Cizmeci, Meagan Kelly, Edward T. Ryan, Karen Kotloff, Marcela F. Pasetti, Galit Alter

Antibodies mediate protection against enteropathogenic infection while constantly maintaining and refining the composition of gut commensal bacteria. These complementary roles of antibodies are mediated not only by antigen binding, but also by leveraging the innate immune system to respond appropriately. We aim to decipher the mechanisms antibodies employ to maintain homeostasis and fight infection within the intestinal tissue. As a first step, we applied Systems Serology to study antibody mediated protective immune responses against *Shigella* infection. Shigellosis is the second leading cause of death due to diarrheal disease in young children worldwide. Efforts to develop an effective *Shigella* vaccine have been hindered by the limited understanding of immunological correlates of protection against shigellosis. We performed comprehensive analysis of *Shigella* specific antibody responses in sera obtained from a series of controlled human challenge studies collected both prior to and after challenge. Polysaccharide specific antibody responses were infrequent prior to infection and evolved concomitantly with disease severity. In contrast, pre-existing antibody responses to Type 3 secretion system proteins were consistently associated with clinical protection from disease. Linked to particular Fc-receptor binding patterns, IpaB specific antibodies leveraged neutrophils, monocytes, and complement and were strongly associated with protective immunity. IpaB antibody mediated functions improved with a subsequent re-challenge resulting in complete clinical protection. We further expand our findings in an endemic cohort of *Shigella* infection of young children living in Peru. Our data uncover a novel

protective role for antibodies against shigellosis that may contribute to the successful design of an efficacious *Shigella* vaccine.

### Arresting microbiome development limits immune system maturation and resistance to infection

Michael Silverman - The Children's Hospital of Philadelphia, Jean-Bernard Lubin, Jamal Green, Tereza Duranova, Matthew Lanza, Meghan Wynosky-Dolfi, Igor E. Brodsky, Paul J. Planet

Disruptions to the intestinal microbiome during weaning lead to long-term negative effects on host immune function. However, the critical host-microbe interactions occurring during weaning required for healthy immune system development remain poorly understood. We find that restricting microbiome maturation during weaning leads to stunted immune system development and increased susceptibility to enteric infection. We developed a gnotobiotic mouse model of the early-life microbiome designated as Pediatric Community (PedsCom). This nine-member consortium of microbes derived from intestinal microbiomes of preweaning mice stably colonized germfree adult mice and was efficiently transmitted to offspring for multiple generations. Unexpectedly, the relative abundance of PedsCom microbes were largely unaffected by the transition from a milk-based to a fiber rich solid food diet. PedsCom mice developed less peripheral regulatory T cells and Immunoglobulin A, hallmarks of microbiota-driven immune system development. Consistent with long-term defects in immune maturation, adult PedsCom mice retain high susceptibility to *Salmonella* infection, which is characteristic of young mice and humans. Altogether, our work illustrates how the post-weaning transition in intestinal microbiome composition contributes to normal immune maturation and protection from enteric infection. Accurate modelling of the pre-weaning microbiome provides a window into the microbial requirements of healthy immune development and suggests an opportunity exists to design microbial interventions at weaning to improve immune system development in human infants.

### Attenuated *Salmonella typhimurium* targets proliferating cells in human colorectal tumour organoids during bacterial cancer therapy

Gillian Mackie - University of Birmingham, Alastair Copland,  
Lisa Scarfe, Andrew Beggs, Kendle Maslowski

Treatment options for colorectal cancer (CRC) remain limited, and advances in immunotherapies are only effective in a small proportion of CRC patients. Attenuated *Salmonella typhimurium* home to and colonise tumours. Specificity for tumour tissue makes bacterial cancer therapy (BCT) an attractive prospect, but the therapeutic mechanism remains unclear. Our lab has shown oral delivery of attenuated *Salmonella typhimurium* (S Tm  $\Delta$ aroA) significantly reduced tumour burden and size in two autochthonous models of intestinal cancer. We observed altered transcriptomic and metabolomic profiles of the tumours, via a direct impact on the tumour epithelium. We are investigating response of human colorectal tumour organoids to treatment with S Tm  $\Delta$ aroA. We have identified that the bacteria preferentially invade proliferating cells in patient-derived organoids (PDOs), recapitulating observations in mouse models. This targeting of proliferating tumour cells leads to a reduction in growth capacity of PDOs. It has been shown previously that cell-surface cholesterol is maximal during mitosis, and we have identified that the invasion of dividing cells is dependent on the cholesterol binding component of the type-3 secretion system in *Salmonella*. Thus, we postulate that preferential invasion of fast-dividing cancer stem cells leads to specific cell death; effectively diminishing the stem cell pool within the tumour. Future work will delineate the activation of cell death pathways further and investigate generation of immunogenic responses.

### The bacterial Lon protease degrades c-MYC and increases survival in two mucosal tumor models

Ines Ambite - Lund University, Daniel S.C. Butler, Caterina Cafaro, Johannes Putze, Murphy Lam Yim Wan, Thi Hien Tran, Shahram Ahmadi, Ulrich Dobrindt, Catharina Svanborg

MYC has been named "the quintessential oncogene" and is deregulated in the majority of human cancers. Still, finding c-MYC inhibitors for therapeutic use has been problematic and MYC itself has long been viewed as "undruggable". Here we present a novel strategy for achieving c-MYC inhibition, involving specific bacterial effector molecules. We made the surprising observation that uropathogenic *E. coli* activate c-MYC degradation and attenuate MYC expression in host cells and tissues and identify effector molecules responsible for this

effect. The bacterial Lon protease is shown to rapidly degrade c-MYC and therapeutic efficacy is demonstrated in two murine cancer models. In mice, intravesical or peroral delivery of Lon protease delayed tumor progression of bladder cancer and colon cancer, respectively. Long-term protection, defined by delayed tumor progression, increased survival and low toxicity further supports the therapeutic potential of Lon. The results suggest that bacteria have evolved strategies to control c-MYC tissue levels in the host, which can be exploited to target c-MYC therapeutically in different cancers. Butler DSC et al. A bacterial protease depletes c-MYC and increases survival in mouse models of bladder and colon cancer. *Nature Biotechnology* 39: 754-764 (2021).

### The Bacterial Metabolite Indole Promotes Collagen Induced Arthritis through Enhanced Th17 Immunity

Brenda Seymour - University of Colorado, Brandon Trent, Sabrina Fechtner, Brendan Allen, Jimmy Tangchittsumran, Kristine Kuhn

Altered tryptophan catabolism by the intestinal microbiota has been observed in autoimmunity, but the mechanisms by which tryptophan catabolites alter immune cell function are unclear. We found that mice fed a tryptophan-deficient (TD) diet are protected from collagen-induced arthritis (CIA) by alteration of the Th17:Treg ratio, and that levels of the bacterial-derived tryptophan metabolite indole correlate with CIA severity. Thus, we hypothesized that indole promotes CIA by skewing intestinal Th17 cell differentiation. To assess our hypothesis, mice were fed a TD diet starting at day -1. Indole (10mM) or vehicle was added back by oral gavage every other day. CIA was induced by immunization with bovine type II collagen in complete Freund's adjuvant at days 0 and 21. We found that indole-gavaged mice had a significant increase in disease severity and an expansion of Th17 cells at day 35 compared to vehicle-gavaged mice. Next, we evaluated if intestinal antigen presenting cells (APCs) would skew towards Th17 differentiation during CIA. We observed an expansion of IL-6, IL-23p19, and TNF-producing CD11c + MHCII + CD11b hi cells in the mesenteric lymph nodes of CIA mice at day 7, suggesting that cytokines important for Th17 cell function are upregulated early in CIA. To test whether indole promotes cytokine production in APCs, bone marrow derived dendritic cells were stimulated with indole +/- LPS for 24hr. Stimulation with indole + LPS resulted in significantly higher levels of IL-6,



IL-23, and TNF compared to LPS alone. In conclusion, our initial data suggests that indole promotes CIA through enhanced Th17 cell differentiation.

### Bacterial suppression of intestinal fungi via activation of human gut $\gamma\delta$ T-cells

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Symbiont-derived molecules induce host immunity against gut pathogens, but microbiota composition and the mucosal responses elicited vary between animal species. *Candida albicans* does not naturally colonise mouse gut but is a commensal yeast in human intestine, where transition to tissue-invasive filamentous growth can be triggered by epithelial barrier damage such as occurs in Crohn's disease (CD). In higher primates, sensing of bacterial phosphoantigens (PAG) is restricted to  $V\delta 2+$  T-cells, which we now report can potentially suppress growth of endogenous fungi in human colonic organ cultures upon PAG stimulation in the presence of epithelial damage-associated cytokine IL-15.  $V\delta 2$ -mediated fungal suppression required barrier protectant cytokine IL-22 and was sufficient to restrict the growth of multiple fungal species, including archetypal pathobiont *C. albicans*. In CD patients with reduced numbers of  $V\delta 2+$  T-cells, mucosal stimulation with PAG failed to control fungal growth, and the *C. albicans* strains obtained displayed rapid filamentation and triggering of neutrophil extracellular traps *ex vivo*. When hypoxic *C. albicans* was compared with oxygenated cultures that mimic the inflamed intestine, a representative healthy control strain displayed proteomic hallmarks of symbiosis, whereas a CD isolate displayed characteristic features of invasive growth (e.g. IHD1), and rapidly translocated across the gut barrier in an 'intestine-on-a-chip' model. Together, these data suggest that commensal bacteria can activate host  $V\delta 2+$  T-cells to suppress fungal invasion of the gut epithelium via an IL22-dependent mechanism that is deficient in CD patients.

### Basophils support optimal intestinal Th2 cell responses

Lauren Webb - University of Washington, Pavithra Sundaravaradan, Oyebola Oyesola, Macy Matheson, Elia Tait Wojno

Type 2 inflammation is characterized by production of the cytokines IL-4, IL-5 and IL-13 and promotes clearance of gastrointestinal helminths, which infect over 2 billion people worldwide. During infection with the helminth *Trichuris muris*, a mouse model of human whipworm infection,  $CD4+$  T cells are an essential source of type 2 cytokines that promote parasite clearance. However, the mechanisms that support  $CD4+$  T cell anti-helminth function in the intestine during type 2 inflammation are unknown. Basophils are a rare innate immune cell population that can interact with  $CD4+$  T cells during type 2 inflammation. Basophils are not critical antigen presenting cells for Gata3+ T helper 2 cell priming *in vivo*. Instead, our previous work shows that perturbation of Notch-dependent basophil activation led to reduced frequencies of Th2 cells at the site of infection, decreased type 2 effector responses following *T. muris* infection and impaired worm clearance. How basophils influence the function of  $CD4+$  T cells resident in the intestine remains undefined. We now show that blockade of basophil Notch signaling leads to defective type 2 cytokine production by  $CD4+$  T cells but is not associated with reduced Th2 cell survival or proliferation during infection. Further, *in vitro* coculture experiments demonstrate that basophils interact with intestinal  $CD4+$  T cells from either naïve or *T. muris*-infected mice to directly promote Th2 phenotype and function independently of antigen. Thus, we are now actively investigating the function of basophils in promoting and maintaining a Th2 cell fate in the intestine, which will inform our understanding of how Th2 cells function at mucosal surfaces.

### B-cell-intrinsic MHCII Signaling Promotes Microbiota Diversity

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T-cell-dependent (TD) antibody responses generated against the microbiota (mainly immunoglobulin A (IgA)) are primarily produced within gut Peyer's patches (PPs). Within these sites, the expression of major histocompatibility complex class II (MHCII) molecules on B cells is thought to be necessary for

the development of germinal center (GC) reactions in lymphoid follicles. However, this assumption has not been adequately addressed, and its relevance to the generation of anti-commensal IgA and the management of microbiota composition has not been described. Here, we use a RAG1  $\Delta/\Delta$  adoptive transfer model where RAG1  $\Delta/\Delta$  mice are either reconstituted with naive CD4 + T cells and MHCII + B cells or naive CD4 + T cells and MHCII - B cells to address these gaps in our knowledge. Results from these experiments demonstrate that B-cell-intrinsic MHCII signaling is a strict requirement for the development of GC responses. Consequently, we show that B-cell-intrinsic MHCII signaling promotes the generation of high-affinity, anti-commensal IgA responses in the gut that is associated with increased species richness within the small intestinal microbial community. Collectively, our data suggest that B-cell-intrinsic MHCII signaling is crucial for the generation of high-affinity anti-commensal IgA responses generated against the gut microbiota, and that this response favors a more diverse bacterial community.

### **Bcl6 deficiency in dendritic cells affects intestinal cDC2 subset abundance and Th17 priming in response to *Citrobacter rodentium* infection**

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Dendritic cells (DCs) comprise two main subsets (type 1 and type 2 DCs, called cDC1 and cDC2) with distinctive transcriptional dependencies. Subset identity and their transcriptional fine-tuning dictate the outcome of the functional differentiation of primed effector T cell subsets. We found that while cDC1 and cDC2 deficient in the transcriptional factor Bcl6 (CD11c.Bcl6 KO) maintain their core lineage-specific identity, both DC subsets exhibit substantial transcriptional and phenotypical changes across tissues. Within the cDC2 subset, we found a significant and selective loss of ESAM<sup>hi</sup> CD11b + cDC2s in the spleen and their CD103 + CD11b + counterpart in the intestine, a phenotype reminiscent of DC-specific Notch2-deficiency. Intestinal Notch2-dependent cDC2s were previously shown to drive Th17 responses towards extracellular bacteria. To characterize the functional consequences of Bcl6-deficiency in DCs specifically in the intestinal compartment, we infected CD11c.Bcl6 KO mice with *Citrobacter rodentium*. We found that DC-targeted Bcl6-

deficient mice display reduced levels of CD4 + T cells producing IL-17A and IL-22 in the colon and draining lymph nodes 9 days after infection. These findings mimic the well-established phenotype observed upon Notch2 deficiency in cDC2s, and suggest a non-redundant role of Bcl6 in DCs with consequences for the response to bacterial pathogens in vivo. Together, this introduces Bcl6 as a new player to the list of transcription factors governing cDC2 subset heterogeneity.

### **Beyond Immunosuppression – Unraveling the complex features of tumor-associated Tregs in colon cancer**

Alexandra Adamczyk - University Hospital Essen, Eva Pastille, Jan Kehrmann, Stefan Kasper, Philippe Krebs, Astrid M. Westendorf

One hallmark of cancer immunoescape mechanisms is the recruitment of suppressive regulatory T cells (Tregs) into the tumorous tissue. Colorectal cancer (CRC) is one of the most frequent malignancies worldwide. Despite considerable progress in early detection and treatment, there is still an unmet need for novel antitumor therapies. Given that colonic Tregs are potent suppressors of inflammation but also inhibit anti-tumor immunity, insights into specific features and the localization of tumor-associated Tregs are of great importance, when considering targeted antitumor therapies in CRC. In a murine model of inflammation-induced CRC, we detected specific migratory properties of tumor-associated Tregs. Particularly, a dense infiltration of Tregs in mouse and human colorectal cancer lesions correlated with increased expression of the orphan chemoattractant receptor GPR15. Despite, GPR15 expression was associated with elevated IL17 and TNF- $\alpha$  secretion. Finally, we demonstrated that GPR15 facilitated the recruitment of Tregs into the colon of tumor-bearing mice, thereby modifying the tumor microenvironment and promoting intestinal tumorigenesis. Interestingly, preliminary 3D immunofluorescence scans of the tumorous colon hint for a potential gatekeeper function of tumor-associated Tregs, in which they seem to control the infiltration of anti-tumoral T cells into the tumor. In future, we aim at dissecting this specific localisation, a potential co-localisation with other immune cells and the resulting consequences in more detail.

### Bladder RNAseq identifies mediators of Gardnerella covert pathogenesis

Nicole Gilbert - Washington University School of Medicine,  
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Gardnerella are frequently present in the microbiota of the vagina and urinary tract. Although a rare cause of symptomatic urinary tract infection (UTI), Gardnerella has often been reported in urine samples collected in studies aimed at profiling the urinary microbiome, or urobiome. Although the presence of Gardnerella in urine samples could reflect peri-urethral or vaginal colonization, many studies have cultured Gardnerella in urine collected directly from the bladder by transurethral catheterization or suprapubic aspiration. This suggests that Gardnerella gains access to the bladder, at least transiently, in some women. We recently reported that transient bladder exposures to Gardnerella trigger uropathogenic Escherichia coli (UPEC) recurrent (r)UTI from quiescent intracellular reservoirs in a mouse model. Here we performed whole bladder RNA-seq to further examine the host response to Gardnerella, both in naive mice and in mice with latent UPEC reservoirs. In both models, gene set enrichment analysis identified many host pathways differentially expressed following Gardnerella exposure, including ones involved in inflammation/immunity and epithelial turnover. In naive mice, pre-exposures to Gardnerella resulted in heightened UPEC titers acutely during an initial experimental UTI. In mice with UPEC reservoirs, Gardnerella activated immediate early response genes, including Nur77 (aka Nr4a1). Data from Nur77<sup>-/-</sup> mice suggest that Nur77 is necessary for Gardnerella exposure to trigger rUTI from intracellular reservoirs. Together these findings demonstrate that even short-lived exposures to urogenital bacteria such as Gardnerella can affect gene expression in the bladder and impact UPEC UTI in multiple contexts.

### Blood myeloid cells differentiate into lung tissue resident cells in a 3D-human tissue engineered lung model to study immune response to respiratory syncytial virus infection

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Respiratory syncytial virus (RSV) leads to a large global health burden especially in neonates and older adults. How myeloid cells contribute to the age disparities associated with severe RSV infection is not completely understood. We developed a 3D-human tissue-engineered lung model (3D-HTLM), which consists of layers of primary human pulmonary fibroblasts in a collagen gel, pulmonary microvascular endothelial cells, and small-airway epithelial cells grown at air-liquid interface to study human myeloid cell response to RSV in a tissue environment. Enriched blood monocytes consisting mostly of CD14<sup>+</sup> monocytes and minor subsets of CD1c<sup>+</sup> cDC2s and Mo-DCs, and CD16<sup>+</sup> non-classical monocytes were added to the 3D-HTLM. Myeloid cells recovered from the 3D-HTLM demonstrated adaptation to the lung environment with an increased proportion of cells expressing lung tissue-resident markers CD206, CD169, and CD163. Expression of HLA-DR and co-stimulatory molecules CD86 and CD40 were increased in all subsets. A small population of cells with surface marker and morphologic characteristics of alveolar and interstitial macrophages were recovered from the 3D-HTLM. Transcript analyses of myeloid cell subsets demonstrated marked gene expression differences between the input and recovered cells, including PPARG and MARCO, and further distinguished the recovered myeloid subsets as unique cell types. RSV challenged models increased secretion of CXCL10, IFN $\beta$ , and IL-10 compared with uninfected models. Together these data demonstrate the 3D-HTLM provides a suitable environment for myeloid cell differentiation into lung tissue resident cells that respond to RSV challenge. Ongoing experiments compare newborn, adult, and elderly myeloid cell responses to RSV in the 3D-HTLM.

### Breakdown of the gut barrier in dengue virus infection may be a key factor precipitating severe disease

Jaehyeon Kim - University of Queensland, Adriana Pliego Zamora, Naphak Modhiran, Kate Bowerman, Helle Bielefeldt-Ohmann, Katryn J. Stacey

Dengue virus infections are seen in over 100 countries, with an estimated 390 million annual infections. A minority of symptomatic cases progress to severe dengue disease which occurs very abruptly at the time when fever and viremia are abating. Severe disease is associated with thrombocytopenia and vascular leak and can manifest as hemorrhage, shock, and organ damage. Our earlier work showed that dengue

patients had elevated circulating bacterial lipopolysaccharide, and gut leak correlating with severe disease has recently been demonstrated. Thus, we hypothesized that dengue disease-associated gut pathogenesis can be accompanied by gut microbe translocation, exacerbating disease severity. According to our histological analysis, dengue virus (DENV) infection in mice brings patchy but severe gut pathology by four days post infection (dpi) including inflammatory cell infiltrate and loss of goblet cell mucus. Quantitative RT-PCR analysis of the DENV-infected gut tissues showed that induction of pro-inflammatory cytokine interleukin-6 and appearance of DENV RNA both follow the trend of gut pathology. Simultaneously, the gut became leaky, and bacteria translocated to the liver at 4 dpi. Reduction of gut bacteria with an antibiotic cocktail treatment attenuated gut pathology and cytokine expression, suggesting that influx of bacterial products is partially responsible for gut inflammation. In conclusion, we propose the connection between bacterial product influx that accompanies leaky gut and dengue disease exacerbation. Ongoing work focuses on microbiome change and what factors originating in the gut exacerbate dengue disease. Treatments to reinforce the gut barrier may provide a new approach to limiting dengue severity.

### Breastmilk IgG imprints host-microbiota mutualism in neonates

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Meghan A Koch

To establish and maintain intestinal homeostasis, the immune system must mount sterilizing inflammatory responses to pathogens yet remain tolerant to beneficial or innocuous commensal microbiota. This process is particularly complicated in early life when the neonatal immune system encounters new antigens. As such, defining the mechanisms that regulate host-microbiota interactions in early life is of paramount importance. Using a mouse model of maternal antibody deficiency, we demonstrate that ingestion of maternal IgG in the first week of life restrains microbiota-dependent adaptive immune responses during the weaning transition (3-4 weeks of age). Compared to neonates fed IgG in the first week of life, neonates that only receive maternal IgG in utero or after the first week of life mount heightened gut-associated T follicular helper cell (Tfh) and germinal center B cell responses following weaning. This dysregulated weaning reaction is not

driven by alterations in gut microbiota composition or intestinal barrier function. Interestingly, in pups that do not receive maternal IgG, adoptive transfer of transgenic T cells and oral administration of their cognate antigen just prior to weaning leads to expansion and differentiation of transgenic Tfh cells, suggesting that maternal IgG ingested during early life restrains future mucosal T cell reactions regardless of T cell specificity. Here we identify a novel mechanism by which maternal IgG regulates nascent microbiota-reactive adaptive immunity. These exciting findings elucidate mechanisms by which maternal microbiota-reactive IgG directly programs neonatal immunity and shed light on how breastfeeding can have long-lasting consequences for host-microbiota mutualism.

### Bystander CD8 T cell memory responses partially protect mice against lethal vaginal HSV-2 challenge

Tanvi Arkatkar - University of Washington, Veronica Dave,  
Irene Cruz Talavera, Martin Prlic, Jennifer Lund

Herpes Simplex Virus type-2 (HSV-2) infection is one of the most prevalent sexually transmitted diseases, yet no vaccine is currently available. Detailed analysis of HSV-infected human tissue revealed tissue-resident CD8 T cells (TRM) limit the duration and severity of HSV-2 episodes. Interestingly, recent studies indicate that the sensing and alarming function of CD8 TRMs is not restricted to cognate antigen interaction, but CD8 TRM can mediate protection against antigenically unrelated pathogens, termed "bystander activation." Here, we extended our findings to determine if antigen non-specific CD8 T cells could provide some degree of protection in a bystander fashion in the context of HSV infection. To test this, we created antigen non-specific memory compartments through immunization of mice with *Listeria* expressing ovalbumin (OVA) (LM-OVA). Mice were then challenged with wild-type HSV-2 to assess the degree of vaccine-mediated protection. Immunization with LM-OVA delayed disease progression from lethal viral challenge, suggesting that bystander CD8 T cells (BA-CTL) may mediate protection despite the lack of antigen-specificity. Furthermore, we found lethal HSV-2 infection resulted in early infiltration of antigen-non-specific CD8 T cells (Ova-specific) in the vaginal tract. To gain mechanistic insights on activation of memory CD8 T cells, we locally administered type I interferons to create an antigen-free inflammation setting. We observed an increased abundance of Ova-specific CD8 T cells. Additionally,



assessment of memory CD8 compartment in human vaginal tissue upon in-vitro treatment with cytokines caused bystander activation of memory CD8 T cells. Finally, we found the in-vitro treatment of CD8 T cells with cytokines slightly delayed disease progression and reduced viral burden upon adoptive transfer.

### CCR5 and Pannexin-1 and its potential role in colon cancer progression

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CCR5 is a chemokine receptor showing increased expression in colon cancer (CC), and when blocking promotes antitumor responses. Pannexin-1 is a hemichannel allowing passage of small molecules, such as ATP, essential in proliferation and migration. The activation of CCR5 induces the release of ATP through Pannexin-1 (Panx-1) in CD4+ T lymphocytes, however, a linkage between these two molecules has not been described in cancer. The objective of this study is to determine the CCR5 and Panx-1 expression levels and its relationship with CC. CCR5 and Panx-1 content was analyzed in tumor and healthy mucosa biopsies from CC patients by immunohistochemistry, correlating these molecules with tumor progression. Moreover, to evaluate CCR5 effect on ATP production, we stimulated CCD481CoN cells with CCL3 in absence/presence of pharmacological inhibitors of Panx-1 and CCR5 in vitro. Preliminary results show a higher expression of CCR5 and Pannexin-1 in tumor cells compared to epithelium of healthy mucosa (n=27; Wilcoxon signed rank test p<0.05), without significant differences in stroma (tumor vs. healthy tissue). The higher expression of CCR5 and Panx-1 are associated with advanced stages of CC, with a correlation between CCR5 and Panx-1 (Spearman, p<0.0001). Furthermore, in vitro analysis suggests that activation of CCR5 induces ATP secretion in cell lines mediated by Panx-1 opening (n=3). Our results suggest that CCR5 and Pannexin-1 have a role in CC, as both express in tumor and stroma cells and are related with disease progression. Financing: FONDECYT 11190990.

### CD116+ fetal precursors migrate to the perinatal lung and give rise to human alveolar macrophages

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Despite their importance in lung health and disease, it remains unknown how human alveolar macrophages develop early in life. We previously discovered how human blood monocytes migrate into the lung and become different types of lung macrophages in adult life (Evren et al., Immunity 54:1, 2021). Here we define the ontogeny of human alveolar macrophages from embryonic progenitors in vivo, using a humanized mouse model expressing human cytokines (MISTRG mice). We identified alveolar macrophage progenitors in human fetal liver that expressed the GM-CSF receptor CD116 and the transcription factor MYB. Transplantation experiments in MISTRG mice established a precursor-product relationship between CD34 - CD116 + fetal liver cells and human alveolar macrophages in vivo. Moreover, we discovered circulating CD116 + CD64 - CD115 + macrophage precursors that migrated from the liver to the lung. Similar precursors were present in human fetal lung and expressed the chemokine receptor CX3CR1. Fetal CD116 + CD64 - macrophage precursors had a proliferative gene signature, outcompeted adult precursors in occupying the perinatal alveolar niche, and developed into functional alveolar macrophages. In contrast, human lung macrophages of adult origin had a gene signature characteristic of interferon-induced pro-inflammatory macrophages that are expanded in severe COVID-19. Taken together, we identify CD116 + fetal liver cells as precursors of human alveolar macrophages in early life and determine the impact of cell origin on lung macrophage identity and function in the human context with a unique in-vivo model. The discovery of the fetal alveolar macrophage progenitor advances our understanding of human macrophage origin and ontogeny.

### CD11c+ F4/80+ Macrophages are Major APC in Intrauterine Vaccination

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Mucosal immune responses rely on the mucosal-associated lymphoid tissue (MALT) to sample antigens from the mucosal surface and present them to localized lymphoid cells. Studied inductive sites of the mucosae include the gut-associated lymphoid tissue, bronchus-associated lymphoid tissue and the nasal-associated lymphoid tissue. In our previous research, vaccines administered to the uterus induced an immune response in distal and local mucosal sites as well as in the serum. The mechanism by which these animals were able to elicit an immune response remain unclear since the uterus lacks a MALT which is thought to be needed to induce an immune response. To explore how the uterus can act as an immune induction site, we examined uptake on antigen delivered directly into the uterus using BALB/c mice. In this study, we used flow cytometry and immunohistochemistry to identify the different antigen presenting cell (APC) population in the uterine tissue. We vaccinated mice with Alexa Fluor-Ovalbumin and tracked its uptake and migration by APC. We were able to show that CD11c+ F4/80+ macrophages are the main APC cells in response to an intrauterine vaccination formulated with poly I:C, anti-CD40 and OVA. The data also suggest that CD11c+ cells are responsible for trafficking of these antigens to the draining lymph nodes. Upon investigating different timepoints, significant amounts of OVA antigen were seen in cells after 24 hours but that OVA antigen was still present in the lumen of the uterus and being actively taken up by APCs. We are currently performing experiments to determine the site for antigen uptake in the uterus of pigs and mice during estrous. These finding provide new insights on the antigen processing in the uterus and thus will provide a framework for future works to understand the novel approach of intrauterine vaccination.

### CD8+ T cell priming in mandibular lymph nodes initiates systemic immunity during oral infection

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The digestive tract represents an essential barrier against systemic dissemination of ingested potential pathogens. While the immune response in the lower digestive tract has been extensively studied, the role of adaptive immunity in the upper

digestive tract with the oral cavity and the oesophagus remains poorly understood. Here, we investigated the relevance of the oral cavity-draining mandibular lymph nodes (mandLNs) in CD8 + T cell priming after *Listeria monocytogenes* (Lm) oral infection. We found that orally administered Lm is initially sampled in mandLNs draining the oral cavity, leading to an early local antigen-specific CD8 + T cell response that precedes their activation in spleen and gut-draining mesenteric lymph nodes (MLNs). In contrast to T cells activated in MLNs, CD8 + T EFF cells generated in mandLN did not acquire a defined gut-homing phenotype and instead contributed to systemic host protection. Accordingly, stromal and dendritic cells of mandLN showed a low gene expression of enzymes required for gut homing imprinting. Thus, the phenotype and homing profiles of mandLN antigen-specific CD8 + T cells resembled those acquired by T cells in peripheral lymph nodes and spleen. Accordingly, after transfer of mandLN derived antigen-specific CD8 + T cells to infection-matched mice, these cells were widely detected in lymphoid as well as non-lymphoid tissues (oral mucosa, submandibular salivary gland, small intestine, liver and lung). Collectively, our findings extend the concept of regional specialization of immune responses along the length of the digestive tract, with mandLN acting as oral cavity-draining counterparts of intestinal draining LN of the lower digestive tract.

### CD8+ T cells provide protection during *Cryptosporidium* Infection

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*Cryptosporidium* is a pathogen that resides in an intracellular, yet extracytoplasmic, location in intestinal epithelial cells and is an opportunistic pathogen in patients with defects in T cell-mediated immunity. To study the T cell response to *Cryptosporidium*, these parasites were engineered to express the model antigen SIINFELK. This allows us to use transgenic OT-I CD8 + T cells specific for SIINFELK to track *Cryptosporidium*-specific CD8 + T cells. The use of IFN $\gamma$ -Thy1.1 reporter mice showed these parasites induced a potent IFN $\gamma$  response from endogenous SIINFELK-specific CD8 + T cells and activated a CD8 + T cell response which enhanced

parasite control. To study how CD8 + T cells are primed, OT-I T cells that express a Nur77-GFP reporter of TCR activation were labeled with Cell Trace Violet and then transferred to infected mice. These studies revealed that OT-I cells were first exposed to antigen and proliferated (based on loss of CTV in the mesenteric lymph nodes (mLN)), while OT-I cells were not present in the small intestine until later during infection. To determine if cDC1s, the DC subset classically thought to prime CD8 + T cells, were important during *Cryptosporidium* infection, IRF8+32 -/- mice were infected and the CD8 + T cell response quantified. Mice that lacked cDC1s were more susceptible to infection and had decreased CD8 + T cell responses. Thus, CD8 + T cells are primed in the mLN and cDC1s are critical for the generation of robust CD8 + T cell responses required for resistance to *Cryptosporidium*.

### Cervical Goblet cell Diversity Ensures Immunotolerance in Pregnancy

Shanmugapriya Madhukaran - UTSW medical center, Anne Cooley, Gary Hon, Mala Mahendroo

Through pregnancy the cervix undergoes a remodeling process that collectively ensures preparation for birth while simultaneously providing immunoprotection against ascending pathogens and premature birth. The mouse cervix is a multi-layered squamous epithelia comprised of basal cells that can proliferate and expand into differentiated luminal cells. To comprehensively define the cervical epithelial cell types, that drive remodeling and immunoprotection during pregnancy, we performed single-cell RNA sequencing from mouse cervix on gestation day 6, 12, 15, 18, in labor (IL) and non-pregnant (NP) cervix using the 10x Genomics single cell platform. Analysis of 40,0086 cells identified 15 distinct epithelial clusters comprised of basal and luminal subtypes. We report that there is remarkable shift in epithelial cell phenotype during pregnancy compared to NP and IL datasets. Thus, NP and IL are transcriptionally similar to each other but distinct from pregnancy time points (days 6, 12, 15 and 18). During pregnancy, we identified the expansion of distinct epithelial subtypes that included KRT12 + luminal cells and a KRT 8 + mucin producing goblet cell population. The Krt8 + goblet subtypes could be further divided into PIGR + or RBP2 + populations that expanded in a temporally distinct manner. Both goblet subtypes had unique immunotolerant gene expression profiles compared to NP and IL. The diversity in

goblet subtypes provides distinct mucosal function depending on the gestational stage of pregnancy. Together, these data provide insight into the complexity and plasticity of mouse cervical epithelia during pregnancy that contributes to cervical remodeling.

### Characterising the Initial Events of Herpes Simplex Virus Infection in Human Anogenital Tissue

Hafsa Rana - Westmead Institute for Medical Research, Naomi Truong, Kerrie Sandgren, Kirstie Bertram, Blake Johnson, Jason Herbert, Heeva Baharlou, Andrew Harman, Anthony Cunningham

HSV is sexually transmitted through the anogenital mucosal epithelium and directly interacts with keratinocytes and antigen presenting cells, mainly Dendritic Cells (DCs), via the HSV entry receptor Nectin-1, causing infection. The cascade of events following initial infection cause inflammation in the affected region leading to the recruitment of immune cells, the degradation of the epithelial layer and increased susceptibility to HIV. We have successfully established a model of infection in human genital mucosal explants to investigate the events that occur during the early stages of HSV entry and infection that lead to co-infection with HIV. Microtrauma was essential for HSV entry into human foreskin and vagina, simulated by the application of microneedle Nanopatches (Vaxxas) to the mucosal surface. Epithelial infection only occurred in non-infant foreskin and vagina when Nanopatches were coated with virus prior to application, and penetrated one third of the thickness of the epidermis. HSV was detected in situ using the highly specific RNAScope (ACDbio) technique showing rapid lateral spread of the virus after 24 hours, and also interactions with epidermal DCs, recently discovered by our lab, and Langerhans Cells, visualized by IF. Nectin-1 was shown in a keratinocyte cell line to change its expression in response to HSV infection due to cytokines and chemokines that have been profiled via cytokine bead arrays. These results provide a detailed understanding of the initial events that occur during HSV infection that may help guide development of vaccines and immunotherapies against the sexual transmission of HSV and HIV.

### Chimeric secretory components neutralize *Clostridioides difficile* toxins and expand secretory IgA functions

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Sonya Kumar Bharathkar

Secretory (S) Immunoglobulin (Ig) A is the predominant human mucosa antibody, which mediates host interactions with commensal and pathogenic microbes. SIgA uses a polymeric structure to coat or crosslink antigens and can bind host and microbial proteins, carbohydrates, and receptors; however, SIgA structure-function relationships and therapeutic potential remain poorly understood. We recently reported the cryoelectron microscopy structure of SIgA, which revealed two IgA monomers connected through one joining-chain and bound by one secretory component (SC). The structure adopted a bent, asymmetric conformation; SC was solvent accessible, and its second Ig-like domain (D2) protruded from the center of the complex. This arrangement led us to hypothesize that D2 could be modified or replaced to confer alternative functions and allow us to explore SIgA effector mechanisms and build novel, bi-specific SIgAs. Accordingly, we replaced D2 with a single domain antibody (sdAb) that recognizes toxins produced by the opportunistic pathogen *Clostridioides difficile*, thereby creating chimeric (c) SC that we incorporated into recombinantly-produced cSIgA. The cSC and cSIgA bind and neutralize *C. difficile* toxins in vitro and in cell culture-based assays, and in the case of cSIgA, can be combined with antigen binding fragments targeting diverse antigens. Results demonstrate that SC can tolerate D2-replacement and suggest that virtually any sdAb could be incorporated into cSC and its function integrated into cSIgA. These studies open the door for using orally or intranasally administered cSIgA to study and/or treat *C. difficile* and other pathogenic infections, modulate host microbiota and/or investigate SIgA effector mechanisms in animal models.

### Circulating inflammatory protein and cellular profiles at time of diagnosis classify inflammatory bowel disease patients according to their underlying immune response and clinical disease course

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Chronicity of inflammatory bowel disease (IBD) is driven by inflammatory memory CD4 + T helper (Th) cells which activate an inflammatory cascade. Because of disease heterogeneity, novel treatment strategies tailored to target the patient's immune defect are required. We hypothesize that combined analysis of circulating inflammatory protein abundance and Th cells allows to dissect underlying immune pathogenesis. Therefore, we measured concentrations of 92 inflammatory proteins in plasma of pediatric Crohn's disease patients (CD), ulcerative colitis/IBD-unclassified patients (UC/IBD-U) and age-matched healthy controls (HC) using the Olink Proximity Extension Assay®. Th cell frequencies were monitored with flow cytometry. Blood was collected at diagnosis and after induction treatment to investigate response to therapy. Thirty-six plasma proteins discriminated IBD patients from HC. Increased abundance of interferon- $\gamma$  was strictly associated with CD while interleukin-17A was more abundant in UC/IBD-U. In CD, three patient clusters were identified. CD#3 patients had lower concentrations of inflammatory proteins, reflecting less severe disease, in agreement with lower clinical disease parameters. Clusters CD#1 and CD#2 had comparable clinical disease parameters. CD#1 patients had higher abundance of 14/36 proteins associated with neutrophil function and interferon- $\gamma$  signaling while CD#2 patients showed increase in frequencies of activated HLA-DR + memory Th cells. The CD clusters responded differently to therapy with CD#1 patients exhibiting more modulated proteins and greater fold changes, CD#2 patients showing intermediate modulation and CD#3 patients exhibiting only few changes. In conclusion, combined profiling of plasma immune proteins and circulating Th cells discriminates subgroups of pediatric IBD patients which differ in their IBD pathogenesis.

### *Clostridioides difficile* toxin B (TcdB) activates group 3 innate lymphocytes (ILC3s)

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Group 3 innate lymphocytes (ILC3s) are rare immune cells often localized in mucosal tissues. Upon activation, ILC3s are a major source of the cytokine interleukin-22 (IL-22). IL-22 modulates tissue responses during inflammation and minimizes dissemination of bacterial pathogens through barrier maintenance, making IL-22 protective in many infections, including *Clostridioides difficile*. *C. difficile* is a pathobiont in the GI tract of many healthy individuals, but after certain perturbations causes disease. In this study, we identified a mechanism of how *C. difficile* modulates host immune responses. A virulence factor, toxin B (TcdB), induced production of IL-22 in ILC3s. This was dependent on the glucosyltransferase activity of TcdB, which inhibits small GTPases. Pharmacological inhibition of Cdc42 phenocopied the TcdB effect and increased IL-22 production by ILC3s, suggesting Cdc42 is a negative regulator of ILC3 activation. Gene expression analysis revealed that TcdB modulated expression of many inflammation-related genes in ILC3s. *C. difficile* may perturb the immune system to increase levels of a cytokine that regulates its mucosal microenvironment.

### Colonic iNKT cells expansion is regulated by goblet cells in CD1d-dependent manner

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Invariant natural killer T (iNKT) cells are unique innate immune cells with adaptive immune cell properties. Upon recognition of self or microbial ligands presented by cells expressing CD1d, they secrete cytokines and immune mediators. These cells contribute to host protection or/and pathogenesis during intestinal inflammation. iNKT cells in the colon are established under the influence of the microbiota at an early stage and current literature suggests that iNKT cells are not manipulable in later life. Goblet cells or/and goblet cell-associated passages (GAPs) play a role in luminal antigen delivery and the induction and maintenance of peripherally induced T regulatory cells in the steady-state. However, GAPs in the colon are absent in adult mice due to goblet cell (GC) microbial sensing. In this study, we hypothesized, that when present, colonic GAPs may deliver glycolipids to stimulate colonic iNKT cells. We found that the glycolipids can be delivered through GAPs and that inducing GAPs in the colon in adult mice resulted in significant

iNKT cell expansion. Furthermore, deletion of CD1d on GCs inhibited iNKT cell expansion suggesting a role for colonic GCs in presenting glycolipids to iNKT cells. Single-cell RNA sequencing of FACS sorted colonic-iNKT cells showed significantly increased iNKT2 and iNKT1 subsets after colonic GAP induction. Moreover, iNKT cells expanding after inducing colonic GAPs were protective in DSS-induced colitis. Our findings suggest that the GAP function and CD1d expression by GCs play a role in modulating colonic iNKT cell subsets in adulthood and can be protective in some colitis models.

### Colonic MUC2 Mucin and FCGBP Together Stabilizes the Mucus Barrier in Innate Host Defense Against *Entamoeba histolytica*

Hayley Gorman - University of Calgary, France Moreau, Kris Chadee

MUC2 mucin and FCGBP are major goblet cell proteins that form the colonic mucus layer. The molecular interaction(s) and functional role of FCGBP with MUC2 are not well characterized. We hypothesized that MUC2/FCGBP are coordinately produced and interact by glycan-glycan binding in innate host defense. The aims are: 1) to determine if FCGBP alters the structural integrity of the mucus layer and 2) to determine the role of FCGBP in innate defense against *Entamoeba histolytica* (Eh). MUC2/FCGBP mRNA and protein expression induced by Eh in WT and FCGBP missense (MS) LS174T goblet cells were analysed by RT-PCR and Western blotting. Integrity of the mucus layer was quantified by fluorescent bead penetration and attachment and invasion of Eh. Eh degradation of MUC2 and FCGBP in the mucus layer and with purified MUC2 and recombinant FCGBP were quantified by Western blotting. In response to Eh, FCGBP/MUC2 mRNA and protein expressions were increased in a time-dependent manner. More fluorescent beads penetrated and Eh attached to the mucus layer of FCGBP-MS cells as compared to WT. Live Eh cysteine proteinase 5 cleaved FCGBP from purified mucins within 1 min and was essential for the cleavage of MUC2 at the C-terminus. This study revealed that FCGBP-MS cells exhibited significant loss in mucus barrier function as quantified by fluorescent beads penetration and increased Eh attachment and FCGBP/MUC2 degradation. Degradation of FCGBP by Eh was a prerequisite for MUC2 cleavage, providing direct evidence that FCGBP/MUC2 interactions conferred structural and

biophysical properties to the protective functions of the mucus gel.

### Colonoids from UC patients retain a phenotypic memory of the hallmark features of disease

Gerard Kaiko - The University of Newcastle Australia

Many of the hallmark pathological features of inflammatory bowel diseases (IBD) are observed in the intestinal epithelium yet compared to immune cell functions these disease-induced changes remain understudied in ex vivo models, including organoids. The relative contribution of cell programmed (i.e. genetic and epigenetic) versus microenvironment factors (inflammation, microbial dysbiosis, hypoxia) remains unclear. Given the importance of mucosal healing in long term remission, the modelling of these epithelial features is of key significance. We took a phenotype-specific approach to examine differences between ulcerative colitis (UC) and non-IBD patients based on functional colonoid assays designed to mimic the hallmark features of IBD, including repair, permeability, proliferation/apoptosis, differentiation, and epithelial-inflammation. Colonoids from UC patients retain a phenotypic memory of disease and demonstrate multiple defects in epithelial function compared to those from non-IBD patients. UC colonoids demonstrate functional disease-like features in vitro with increased levels of permeability, slower rates of repair and proliferation, decreased goblet cell differentiation. However, these colonoids show similar levels of inflammatory response to stimuli. Finally, we show that the colitis-like microenvironment including inflammatory stimuli and hypoxia re-programs the epithelium by altering barrier repair, cytokine responses, and differentiation. This study demonstrates that features of UC are programmed into colonic stem cells and thus these colonoid models re-capitulate many of the pathological features of the UC epithelium and are a useful system to both study disease pathogenesis and screen for therapeutics.

### Combination of Anti TNF $\alpha$ Agents with Agonistic Anti 4-1BB Antibodies for Treatment of Inflammatory Bowel Disease

Rachel Birnboim - Tel Aviv university, Professor Itai Benhar

Inflammatory bowel disease (IBD) is a group of chronic inflammatory disorders of the digestive tract. Although treatments for IBD have been extensively studied, treatment options remain disappointingly non-specific and include anti-inflammatory and immunosuppressive drugs. Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) is a pro-inflammatory cytokine. Anti-TNF $\alpha$  agents have been successfully applied in the clinic. CD137 is a member of the tumor necrosis factor receptor family and it is a co-stimulatory immune checkpoint. It was reported that although agonistic antibodies to CD137 can potentiate strong immune responses, it also has the potential to alleviate autoimmune diseases. For example, it has been shown that treatment with agonistic antibodies to CD137 increases regulatory T cells (Tregs) proliferation. Tregs can potentially suppress the activation of immune cells that are involved in the intestinal inflammation. Early studies suggested that combination therapy (CT) for IBD may improve the outcome of the treatment. The goal of my study is to test whether CT of anti TNF- $\alpha$  agents with anti CD137 antibodies outperforms monotherapy in a mouse DSS-colitis model. The motivating rationale for the CT is to reduce the severity of intestinal inflammation by combining two orthogonal anti-inflammatory mechanisms: the first mechanism is to reduce the levels of TNF- $\alpha$ , and the second mechanism is to increase the number of Tregs. Our preliminary results suggest that there is an advantage to the CT over monotherapy in several parameters that were evaluated in order to assess the severity of the colonic inflammation, such as body weight, colon length and disease activity index (DAI).

### Commensal bacteria promote type I interferon signaling to maintain immune tolerance

Adriana Vasquez Ayala - UCSD, Kazuhiko Matsuo, Chia-Yun Hsu, Marvic Carrillo Terrazas, Hiutung Chu

Commensal bacteria are critical to the development and education of the intestinal immune system. Although commonly associated with pathogenic infections, commensal microbes also elicit type I interferon (IFN) responses and are important in the maintenance of intestinal homeostasis. However, the relationship between tonic type I IFN driven by commensal bacteria and tolerogenic immune response is not well defined. Here, we show that commensal bacteria maintain basal expression of type I IFN in dendritic cells (DCs) to promote induction of regulatory T-cells (Treg) response. DCs

treated with the commensal bacterium, *Bacteroides fragilis*, showed increased expression of IFN related genes and induction of the cytokine IFN $\beta$ . Single cell RNA sequencing of gut Tregs also revealed a type I IFN gene signature in mice colonized with *B. fragilis* compared to germ-free controls. Collectively, these findings unveil the diverse role type I IFN play in the maintenance of intestinal homeostasis.

## The commensal microbiota sculpts T cell responses to Influenza A in newborns through circadian disruption

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Newborns have the highest infection-related mortality of any age group with viral pneumonia causing most of the estimated 1 million deaths per year. The perinatal environment profoundly affects lung health, which is an idea termed "the fetal origins of health and disease". Experiences early in life, such as antibiotic exposure, impact lung health in many ways, such as increasing susceptibility to viral pneumonia, including Influenza A virus (IAV). Using a model of perinatal antibiotic exposure, we explored mechanistic links between antibiotic-induced changes in gut microbiota, referred to as dysbiosis, and lung defenses against IAV. Infection with PR8, a type of IAV, generated a robust, lung-localized primary T cell response in newborn mice. Although peak T cell responses were similar between control (NABX) and antibiotic-exposed (ABX) newborns, the frequency of virus-specific CD8 + T cells was significantly reduced in ABX newborns during the phase immediately following viral clearance (~10 days post-infection). More importantly, frequencies of virus-specific CD8 + T cells remained diminished into adulthood in mice exposed to antibiotics as newborns. These data suggest that long-term persistence of virus-specific CD8 + T cells is compromised in ABX newborns. We utilized single-cell RNA and Assay for Transposase-Accessible Chromatin (ATAC) sequencing to build gene regulatory networks controlling pulmonary CD8 + T cells. Early-life antibiotic exposure strikingly disrupted several circadian rhythm networks in pulmonary CD8 + T cells, including *Bmal1* and *Nfil3*, suggesting a mechanism for early-life antibiotic disruption of mucosal CD8 + T cells. These data demonstrate the importance of intestinal microbiota in programming pulmonary immunity to respiratory viruses.

## Commensal protists remodel the intestinal epithelium and alter host antimicrobial production

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The gastrointestinal tract is home to trillions of microorganisms that contribute to host health. Most microbiome studies have focused on bacterial members, with emerging interest in how fungal and viral members impact host biology. In contrast, relatively little is understood about how commensal protists alter intestinal physiology. The murine commensal protist *Tritrichomonas musculus* (Tm) stimulates specialized chemosensory epithelial cells, called tuft cells, to activate type 2 immunity in the distal small intestine. This immune response results in a concomitant increase of tuft cells and mucus-producing goblet cells within the epithelium. To further characterize how Tm colonization alters host biology, we used spatial transcriptomics to evaluate changes in gene expression and cellular composition in situ. We found that Tm colonization increases the number of both goblet and Paneth cells, with dramatic changes to the antimicrobial peptide (AMP) landscape in the small intestine. These changes in AMP production result from Tm excretion of succinate, which stimulates tuft cells and subsequently activates type 2 immunity. Succinate-induced shifts in AMP production leads to compositional changes in the small intestinal microbiota, decreasing the abundance of bacterial species that are sensitive to AMPs specifically upregulated during protist colonization. Collectively, we demonstrate that Tm increases secretory-lineage cells in the epithelium, shifts host antimicrobial production, and alters the composition of the bacterial microbiota by stimulating tuft cells and activating mucosal type 2 immune responses. Our study illuminates the significant influence commensal protists exert on intestinal epithelial composition and how these microeukaryotes may influence host interactions with the microbiota.

### Complement C5a promotes antigen cross-presentation by Peyer's patch monocyte-derived dendritic cells and drives a protective CD8+ T cell response

Sae-Hae Kim - Chonbuk national university, Yong-Suk Jang

The complement fragment C5a is closely associated with adaptive immune induction in the mucosa. However, the mechanisms that control CD8 + T cell responses by C5a have not been extensively explored. This study reveals that C5/C5a in the Peyer's patch (PP) subepithelial dome increases upon oral *Listeria* infection. We hypothesize that C5aR + PP cells play an important role in the induction of antigen-specific T cell immunity. Using single-cell RNA sequencing, we identify C5aR- and lysozyme-expressing dendritic cells (C5aR + LysoDCs) in PP and examine their role in CD8 + T cell immune induction. Stimulation of C5aR + LysoDCs by C5a increases reactive oxygen species levels, leading to efficient antigen cross-presentation, which elicits an antigen-specific CD8 + T cell response. In C5-deficient mice, oral co-administration of C5a and *Listeria* enhances *Listeria*-specific cytotoxic T cell levels. Collectively, these findings suggest a role of the complement system in intestinal T cell immunity. This study was supported by the Basic Science Research Program through the National Research Foundation (NRF) funded by Korean Ministry of Science and ICT (NRF-2019R1A2C2004711).

### Coordination between NK1.1+ innate lymphocytes and neutrophils limits tissue damage caused by mucosal viral infection

Aisha Lee - Washington University in St. Louis, Ying Shiang Lim, Jason Scott, Wayne Yokoyama, Haina Shin

NK1.1+ innate lymphoid cells (ILC) provide key defense against pathogens in many mucosal tissues. However, characterization and protective functions of this population in the vagina during sexually transmitted infections have not been well-studied. Analysis of NK1.1+ cells in the murine vagina revealed a heterogeneous mixture of conventional NK cells (cNK), ILC1, and innate T cells at steady state. During genital HSV-1 infection, we found that this diverse vaginal NK1.1+ population was rapidly activated within the first day of HSV-1 infection and secreted large amounts of IFN $\gamma$  independently of the cNK transcription factor Eomes. In barrier tissues, NK1.1+

ILC IFN $\gamma$  typically confers host protection via rapid control of viral replication. However, depletion of NK1.1+ cells had no effect on mucosal HSV-1 titers in our model. Instead, we uncovered an unexpected role for NK1.1+ ILC in modulating disease outcomes, as depletion of NK1.1+ cells or neutralization of IFN $\gamma$  both markedly increased the severity of vaginal epithelial damage and genital skin inflammation. Pathology due to the absence of NK1.1+ ILC in both sites could be reversed by neutrophil depletion. However, in the presence of NK1.1+ ILC, neutrophils also played a key role in restricting epithelium damage caused by infection in the vagina, but not genital skin. Together, our data suggest that vaginal NK1.1+ ILC determine the function of neutrophils during viral infection and play a crucial role in determining disease outcomes at this important barrier site.

### The cryo-electron microscopy structure of teleost IgM reveals a tetrameric antibody that provides insights on polymeric antibody evolution, assembly, and function

Beth Stadtmueller - University of Illinois-Urbana Champaign, Mengfan Lyu, Andrey

Polymeric (p) immunoglobulins (Igs), including heavy-chain classes IgA and IgM, are critical for mediating host interactions with mucosal antigens and employ unique effector functions compared to monomeric Igs. Typically, plgs contain a protein called the joining-chain (JC) and between two and five Ig monomers, each with two antigen binding fragments (Fabs) and one fragment crystallization (Fc). However, plg assembly is poorly understood and JC incorporation and/or assembly into different-sized plgs varies with species, heavy-chain class and isoform. In 2020, our group and others published mammalian polymeric IgA and IgM structures, revealing JC-dependent plg assembly mechanisms and structure-function relationships. Here, we report the cryo-electron microscopy structure of the Fc region of IgM from teleost (t) fish, which do not encode a JC. Data revealed a donut-like structure comprising four Fc monomers linked through eight heavy-chain extensions, termed tailpieces (Tps), which adopt a single beta-sandwich-like domain located on one side of the complex between two Fcs. This arrangement is likely to polarize antigen interactions away from the Tp-domain and leave two putative Fc receptor binding sites accessible. The structure differs from mammalian plgs, in which Tps fold with the JC to form an asymmetric beta-sandwich that occupies a position near the



center of the complex. Results suggest all plgs evolved to assemble using a Tp-beta-sandwich scaffold; however the mechanism and resulting antibody structures differ markedly based on the presence or absence of the JC and are likely to correlate with differences in effector mechanisms such as antigen and Fc receptor binding.

### **Cryptosporidium parvum growth is inhibited by Microbiota-derived Metabolites**

Jan Mead - Emory University, Raheela Charania

Cryptosporidium is an important cause of diarrheal illness in young children and immunocompromised individuals. Since it is localized to the epithelial cells in the gut, it is likely that the microbiome and the metabolites from these bacteria play an important role in the ability of the parasite to infect and grow within the intestinal tract. We examined the effect of short chain fatty acids (metabolites of certain intestinal bacteria) on cryptosporidial growth in infected human ileocecal adenocarcinoma (HCT-8) cells. HCT-8 cells were infected with  $2 \times 10^5$  C. parvum oocysts. After 3 hours, different short chain fatty acids were then incubated with the infected cells. After 48 hours of incubation with the different SCFAs, cells were fixed and labelled with monoclonal antibody directed to all intracellular stages and the number of parasites were quantitated using a fluorescent microscope. Several short chain fatty acids (acetate, butyrate, propionic acid and valproic acid) significantly inhibited growth, with an EC50 between 4 and 10 mM. Additionally, when used together, certain short chain fatty acids (e.g. butyrate, acetate and propionic acid) showed increased efficacy. Butyrate also inhibited growth when incubated with sporozoites prior to infection of cell monolayer. Since short chain fatty acids are histone deacetylase inhibitors, they affect gene expression and several biological pathways. We therefore examined the role that autophagy, apoptosis and innate immune response play as mechanisms of inhibition against the parasite. We found that in addition to SCFAs having direct inhibitory activities against C. parvum in host cells, butyrate can also increase apoptosis and cytokines in C. parvum -infected cells, suggesting possible mechanisms of action.

### **CXCR6 is required for tissue resident memory T cell formation across peripheral non-lymphoid tissues**

Taylor Heim - New York University, Maria Steele, Tenny Mudianto

CD8 T cell control of viral infections in peripheral tissues is largely dependent upon their spatial positioning. Clearance of pathogens requires direct contact between CD8 T cells and infected cells. Chemokine receptors play an essential role in this process by facilitating T cell entry into tissues, directing T cell positioning within tissues and by influencing egress out of tissues. Tissue resident memory T cells (T<sub>RM</sub>), which fail to egress after pathogen clearance and scan cells for signs of infection, are potent sources of immunity to secondary encounters with pathogens. Here we show that expression of the chemokine receptor CXCR6 is a conserved feature of T<sub>RM</sub> after viral infection in many non-lymphoid tissues. Using CXCR6 KO TCR-tg T cells, we found that CXCR6 is necessary for proper formation of T<sub>RM</sub> in all non-lymphoid tissues examined. CXCR6 was necessary for early accumulation of effector T cells in several tissues such as the kidney and salivary gland but CXCR6 was dispensable for early accumulation in the skin. After vaccinia infection in the skin, CXCR6 expression is reinforced upon antigen re-encounter and promotes CD8 T cell accumulation in a migration-independent manner. Using single cell transcriptional analysis, we show that CXCR6 KO T<sub>RM</sub> appear to exhibit a survival disadvantage and dysregulated spatial positioning in skin. This work reveals a critical requirement for generating T<sub>RM</sub> in diverse anatomical locations and may benefit strategies for vaccine design, anti-tumor immunity and reducing T-cell mediated autoimmunity.

### **The Cystic Fibrosis Transmembrane Conductance Regulator regulates B cells and mucosal antibody responses**

Prosper Boyaka - The Ohio State University, Eunso Kim, Logan Mazik, Sungjun Yoo, Haley E. Steiner, Estelle Cormet-Boyaka, Prosper Boyaka

Cystic fibrosis (CF) is a genetic disorder that causes severe damage to the lungs and digestive system. CF affects the cells that produce mucus, sweat, and digestive enzymes and is caused by mutations in the Cystic Fibrosis Transmembrane

Conductance Regulator (CFTR). Expression and function of CFTR have been extensively studied on epithelial cells and several recent publications have shown that CFTR also regulates the functions of myeloid cells. We addressed whether CFTR is expressed by B cells and the role of CFTR in B cell functions. We found that CFTR is expressed on murine and human B cells. Compared to control wild-type (WT) mice, CFTR KO exhibited reduced frequency of B cells in lymphoid tissues and lower levels of serum immunoglobulin isotypes. Upon systemic immunization with an alum-based vaccine, CFTR KO mice developed lower antibody responses than control mice. Interestingly, vaccine supplementation with an inhibitor of the serine protease elastase (NEI) enhanced the titers of vaccine-specific serum antibody responses in CFTR KO mice and CFTR heterozygote (CFTR Het) mice. We have previously reported that NEI supplementation could promote mucosal immunity in mice immunized with the alum-based injected vaccine. In this regard, CFTR KO and CFTR Het mice immunized with NEI-supplemented alum-based injected developed higher IgA and IgG responses in saliva than control WT mice. These results provide new insights on the regulatory role of CFTR for B cell differentiation and function. They also suggest that vaccine supplementation with NEI could improve protection of individuals leaving with CF against respiratory pathogens.

### Cytokine and chemokines profiles in the nasopharynx of healthy individuals vary with age and sex

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The nasopharynx is an important immunological site where many bacteria and viruses can be found. The epithelium lining the nasopharynx and local immune cells can detect the presence of microorganisms and produce chemokines and cytokines. We aimed to establish nasopharyngeal immune marker profiles in the general population across all ages and between sexes. We will investigate this using nasopharyngeal swabs from the nationwide cross-sectional PIENTER-3 cohort (n=955 (n=475 female), age 0-89 years). We selected a panel of 35 immune markers and measured their presence in the nasopharyngeal swabs using multiplexed Legendplex assays. The acute phase proteins IL-6 and TNF- $\alpha$  appeared to be present at high levels in the nasopharynx of young children

and decreased between 0 and 25 years, after which the levels stayed constant. The same pattern was observed for CCL2, CCL20, and B-cell activator BAFF. The levels of anti-viral markers IFN- $\alpha$ , IFN- $\gamma$ , and IFN- $\lambda$ 1 decreased with age. In contrast, T cell attractants and activators CCL11, CCL17, and IL-33 levels increased between 0 and 25 years of age and stayed constant afterwards. Furthermore, the levels of neutrophil chemoattractants CXCL5, CXCL8, and the reactive oxygen species enzyme MPO appeared to be lower in females over 60 years compared to men of the same age. These data show that chemokine and cytokine levels change drastically between the age of 0 and 25, and that differences between sexes are present. In future research we want to investigate if we can correlate specific chemokine profiles with age, sex, and basic health conditions.

### Defective humoral immunity disrupts bile acid homeostasis which promotes inflammatory disease of the small bowel

Jason Kubinak - University of South Carolina, Ahmed Mohammed, Zahraa Mohammed, Mary Roland, Ioulia Chatzistamou, Amy Jolly, Lillian Schoettmer, Mireya Arroyo, Khadija Kakar, Yuan Tian, Andrew Patterson, Mitzi Nagarkatti, Prakash Nagarkatti

Mucosal antibodies maintain gut homeostasis by promoting spatial segregation between host tissues and luminal microbes. Whether and how mucosal antibody responses influence gut health through modulation of microbiota composition is unclear. Here, we use a CD19<sup>-/-</sup> mouse model of antibody-deficiency to demonstrate that a relationship exists between dysbiosis, defects in bile acid homeostasis, and gluten-sensitive enteropathy of the small intestine. The gluten-sensitive small intestine enteropathy that develops in CD19<sup>-/-</sup> mice is associated with alterations to luminal bile acid composition in the SI, marked by significant reductions in the abundance of conjugated bile acids. Manipulation of bile acid availability, adoptive transfer of functional B cells, and ablation of bacterial bile salt hydrolase activity all influence the severity of small intestine enteropathy in CD19<sup>-/-</sup> mice. Collectively, results from our experiments support a model whereby mucosal humoral immune responses limit inflammatory disease of the small bowel by regulating bacterial BA metabolism.

### Deficiency in cDC1 causes TH2-driven immunopathology upon recall infection in mice primed with respiratory syncytial virus as neonates

Katharina Lahl - Technical University of Denmark, Anna Hammerich Thysen, Isabel Ulmert, Agnès Garcias Lopéz, Cecilia Johansson

Respiratory Syncytial Virus (RSV) frequently causes respiratory disease in young children and those hospitalized due to RSV infection are relatively more likely to develop wheezing later in life, suggesting that early life RSV infection can have lifelong consequences on pulmonary immune homeostasis by initiating a type 2 immunity-favored environment. This can be modeled in mice, as RSV reinfection during adulthood following neonatal priming causes Th2-driven immunopathology in the Th2-prone mouse strain BALB/c. In contrast, type 1-prone C57BL/6 mice are relatively protected from immunopathology upon RSV reinfection, as they do not lose significant weight and show only a mild influx of type 2 cells. Type 2 immunity is thought to be initiated by type 2 classical dendritic cells (cDC2), while cDC1 were shown to be potent drivers of type 1 immunity. Following reinfection in adulthood, we found that mice lacking cDC1 showed weight loss, increased type 2 immunity at the expense of type 1 immunity, and slightly higher pathology in the lung despite their C57BL/6 background. Importantly, viral clearance upon reinfection was the same as in wildtype littermates, showing that pathology is not dependent on viral load and that at least some immune memory has been induced in all cases. Together, our data suggest that the flavor of early life immune imprinting to environmental triggers is influenced by a DC subset-specific induction of adaptive immunity and that cDC1 directly or indirectly regulate type 2 immunity in addition to their known ability to drive anti-viral immune responses.

### Degradation of epithelial barrier by IL-18-induced granzyme B during mucosal viral infection

Ying Shiang Lim - Washington University, Aisha Lee, Haina Shin

Disease associated with viral infection can be driven by inappropriate or overexuberant immune responses, and identification of molecules that drive pathology could have

important implications for development of therapeutics that avoid widespread immunosuppression. Using a mouse model of genital herpes simplex virus-2 infection, we show that IL-18 can act upon lymphocytes to enhance production and release of granzyme B, a prototypical cytotoxic granule. In vivo, we find that deletion of IL-18 receptor (IL-18R) specifically in NK cells leads to a reduction of extracellular granzyme B and is associated with decreased mucosal pathology. Examination of the spatial kinetics of granzyme B expression further reveals increased granzyme B+ cells in proximity with damaged epithelium relative to undamaged epithelium. Genetic ablation of granzyme B, results in reduced genital disease and epithelial damage, including destruction of hemidesmosomal proteins, without impairing vaginal viral control and immune cell recruitment. Finally, therapeutic inhibition of vaginal granzyme B markedly reduced mucosal pathology after HSV-2 infection. Taken together, our study reveals a pathogenic role for NK cells and granzyme B during vaginal HSV-2 infection, and identifies granzyme B as a potential therapeutic target for genital herpes.

### Dendritic cell-T-cell interactions are critical for enhanced IgA and IgG2b class switching in the Peyer's Patches of SFB-colonized mice

Brian Kelsall - National Institutes of Health

Dendritic cell-T-cell interactions are critical for enhanced IgA and IgG2b class switching in the Peyer's Patches of SFB-colonized mice Eun-Do Kim 1, \*, Byunghyun Kang 1, \*, Tomohiro Tomachi 2, #, Jianping He 1, Brian Kelsall 1 1 Mucosal Immunobiology Section, Laboratory of Molecular Immunology, NIAID, NIH, Bethesda, MD, USA 2 Department of Allergy and Clinical Immunology, Chiba University, Chiba, Japan Segmented filamentous bacteria (SFB, Candidatus savagella) are commensal bacteria that preferentially colonize the ileum, particularly over Peyer's patches (PPs). SFB colonization of germ-free mice induces IgA responses by unclear mechanisms. We found that SFB-colonized SPF mice also have higher baseline IgA levels and develop more potent IgA responses to oral immunization and reovirus infection than uncolonized mice. PPs are the major site for T-cell-dependent, and a minor site for T-cell-independent IgA B-cell differentiation. CCR6 + B-cells and dendritic cells (DCs) interact in the PP subepithelial dome region (SED) to initiate IgA B-cell isotype switching, followed by further B-cell

development in germinal centers. Using single cell mRNA sequencing of PP cells, IgA + , but also IgG1 + and IgG2b + B-cells were present in the germinal center B-cell population. SFB colonization increased IgA + and IgG2b + , but not IgG1 + PP B-cells, that was dependent on CCR6. In addition, a higher frequency of CCR6-expressing dome B-cells and T-cells was found in SFB+ than in SFB- PPs together with a significant increase of activated CD11c + SIRPα + BST2 - CD11b + DCs with high levels of H2-Ab1 and Cd86 by scRNAseq and flow cytometry. Finally, results from TCRα -/- and Zbtb46 Cre MHCII fl/fl mice indicate that increases in IgA + and IgG2b + B-cells in PPs of SFB+ mice were T-cell- and DC-dependent. Together these data support the hypothesis that SFB drives DC maturation, possibly from CD8 - CD11b - DCs present in the SED as suggested by others, and that DC-T cell interactions in PPs are essential for the enhanced IgA class switching in PPs of SFB colonized mice. \*E.D.K., and B.K. contributed equally to this work. # T.T. generated initial findings in SFP mice.

### Depletion of Yolk-sac-derived Macrophages Alters Enteric Neuron Density in Neonatal Mice

Ellen Schill - Washington University in St. Louis, Shreya Gaddipati, Vini John, Bibana Barrios, Sreeram Udayan, Alexandria Floyd, Keely McDonald, Rodney Newberry

Neuroimmune interactions between the enteric nervous system (ENS) and immune cells play a significant role in regulating intestinal homeostasis. The myenteric plexus of the ENS communicates with a population of tissue resident macrophages, to regulate gut motility and tissue repair. Intestinal tissue resident macrophages are initially all yolk-sac-derived but are mostly replaced by bone marrow-derived macrophages over the murine preweaning period. However, the macrophages that preferentially interact with the ENS are long-lived yolk-sac-derived cells. We wanted to understand the role of yolk-sac-derived macrophages on the early postnatal development of the ENS and intestinal homeostasis. CX3CR1-ERT2Cre<sup>+/+</sup>, diphtheria toxin receptor (DTR) flox/+ mice were injected with tamoxifen (controls were injected with sunflower oil) on day of life 1 (DOL 1) to induce DTR expression on CX3CR1+ cells. Mice were treated with diphtheria toxin on DOL 5, 7, and 9 before sacrifice on DOL 10 (Figure 1A). Tamoxifen-treated mice were smaller than control littermates (Figure 1B). The muscle layer was carefully

dissected away from the submucosal layer of the intestine for specific analysis of the myenteric plexus and associated macrophages by immunofluorescence. Despite depletion of DTR expressing macrophages, tamoxifen-treated mice had a significant increase in the density of colonic macrophages compared to controls (Figure 2D). Additionally, tamoxifen treated mice had a significant increase in colonic enteric neuron density (Figure 2H). These data suggest that depletion of yolk-sac-derived macrophages causes and influx of new (likely bone marrow-derived) macrophages and enteric neurons.

### Detection of anti-SARS-CoV-2 neutralising antibody at mucosal surfaces

Ore Francis - University of Bristol, Holly Baum, Alice Halliday, Amy Thomas, Elizabeth Oliver, Joyce Smith, Benjamin Hitchings, Kapil Gupta, Anna Long, Kathryn Shelley, Hayley Jones, Jennifer Oliver, Begonia Morales-Aza, Rachel Barr, Ruth Mitchell, Nicholas Timpson, Kate Northstone, Anu Goenka, Imre Berger, Andrew Davidson, Kathleen Gillespie, Mick Bailey, Adam Finn

Background Recently, pseudotype neutralisation assays have become a preferred tool to measure anti-SARS-CoV-2 neutralising antibody (nAb) in serum and characterise functional humoral responses of infection. However, neutralisation at the initial site of SARS-CoV-2 infection, the upper respiratory tract, is not necessarily reflected in serum-based assays. We have developed a saliva neutralisation assay to address this need. Methods Convalescent saliva samples were collected from donors and subject to analysis by enzyme-linked immunosorbent assays (ELISAs) against whole SARS-CoV-2 spike protein, specific for IgA and pan-IgG antibody subclasses. Matched aliquots of each sample were included in saliva neutralisation assays, where reducing volumes of saliva were each tested for their ability to neutralise vesicular stomatitis virus (VSV), pseudotyped with SARS-CoV-2 spike over ACE2 and TMPRSS2 expressing Vero-E6 cells. Results While saliva mediated neutralisation was observed in many samples, significant neutralisation was only observed in five out of twenty samples (3, 4, 10, 16 and 20) as exemplified by IC50 (ND50) values greater than a dilution factor of 20. Non-neutralising samples corresponding to neutralisation curves entirely above an infection proportion of 0.5 were observed in addition to weakly neutralising samples with an



IC50 value less than 13. IC50 values were used to compare neutralisation between samples and varied over two-logs (range 7-407). When appended to ELISA data, low antibody binding corresponded to lower neutralisation. Conclusion Saliva pseudotype neutralisation assays can be used to measure antibody neutralisation at mucosal surfaces. These methods may reveal relative antibody subclass contributions to mucosal anti-SARS-CoV-2 immune responses.

### Developing a chronic model of *Candida albicans* cerebral mycosis through gut colonization

Lynn Bimler - Baylor College of Medicine, Yifan Wu, Kelsey Mauk, Julian Naglik, Bernhard Hube, David B. Corry

Recent evidence suggests that Alzheimer's Disease (AD) is linked to fungal brain infections. We have previously established an acute model of cerebral mycosis by intravenously injecting the pathogenic yeast *Candida albicans*. The resulting infection induces mild transient memory deficits and fungal induced glial granulomas consisting of microglia and amyloid  $\beta$  deposits surrounding yeast aggregates. This structure essentially duplicates AD's characteristic senile plaque. AD involves numerous senile plaques and tauopathy that presumably accrue over many years potentially from chronic infection. This raises the possibility that *C. albicans* persists in a remote tissue, such as the intestines, from which it periodically mobilizes to chronically re-infect the brain. As both *C. albicans* colonization of the GI tract and low-level candidemia deriving from the GI tract have been documented in humans, we hypothesize that chronic *C. albicans* enteritis leads to low-level transmission of fungal cells into the bloodstream and persistent cerebral mycosis. To test this hypothesis and establish a more translationally relevant chronic model, we administered *C. albicans* to wildtype C57BL/6 mice via oral gavage. Live yeast were recoverable from the brain as soon as 2 days post gavage and for at least two months. Additionally, these colonies are polymicrobial, consisting of both yeast and bacteria, which is consistent with published analysis and our own cultures of AD brains. Through this study we will establish if this infection produces an AD phenotype. This research is groundbreaking for the AD field, producing an unprecedented model that could be used for AD therapeutic and mechanistic studies.

### Development of a spectral flow cytometry-based neutrophil-parasite killing assay

Julio Revilla - University of Virginia, Stacey L. Burgess, Brandon A. Thompson

Our group has shown that metabolic activity from commensal gut bacteria is linked to protection from infection with *Entamoeba histolytica*. *C. scindens* colonized mice exhibit elevated concentrations of serum deoxycholic acid (DCA) and bone marrow neutrophil progenitors. Following a challenge with *E. histolytica*, neutrophils in the gut increased significantly, suggesting a link between *C. scindens* colonization and neutrophil response. Supplementing mice with DCA recapitulated these results. We aim to test one hypothesis that an increase in serum DCA alters the ability of neutrophils to kill amoeba. To measure amoebic killing we developed a strategy for identifying and enumerating populations of live and dead amoeba, that were co-incubated with murine neutrophils, using spectral flow cytometry. Samples of live and heat-killed amoeba were stained with cell tracker dye or amoeba specific fluorescently tagged antibodies and a live/dead dye to establish the assay. Populations of live and heat-killed amoeba, along with their relative proportions, were identified in the software platform OMIQ using data gathered from the spectral cytometer. Neutrophils were isolated from murine bone marrow using a negative selection kit after evaluating several commercial kits for cell recovery. Neutrophils were then identified via spectral flow cytometry and neutrophil specific markers, their ability to kill amoeba was evaluated in a killing assay with and without IFN- $\gamma$  for neutrophil priming. Future experiments will include additional neutrophil and amoeba markers to allow for high dimensional analysis of parasite and granulocyte interactions. Neutrophils will also be isolated from *C. scindens* colonized mice, DCA treated mice and controls in order to compare killing ability of neutrophils. These studies will further our understanding of the downstream effects DCA has on neutrophil activity during infection.

### Development of Tolerance to Quaternary Ammonium Compounds in the Human Skin Microbiota

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The use of disinfectants and sanitizers are a common practice in homes, workplaces, industries and hospitals. Quaternary ammonium compounds (QACs) are positively charged polyatomic ions with broad-spectrum antimicrobial activity that are frequently used as the active ingredient in many antimicrobial products. Considering the abundant use of these products, QACs are perpetually in contact with the skin. While the human skin functions as a physical barrier between the external environment and the body proper, it is also colonized by a diverse microbiota that actively influence health and disease. To investigate the impact of QACs on the human skin microbiome, common skin bacterial species were exposed to purified benzalkonium chloride (BAC) and cetyltrimethylammonium bromide (CTAB) QACs with various alkyl chain lengths in short-term and long-term cultures. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined. We found that the standard microorganism used for antimicrobial efficacy testing, *Staphylococcus aureus*, was 10-100 times more sensitive to QAC inhibition than other opportunistic and commensal skin bacterial species. Repeated exposure to sublethal QAC concentrations significantly reduced bacterial susceptibility to QAC inhibition. These results suggest that prolonged exposure to sublethal doses of QACs can lead to the development of QAC tolerance that may render these QAC disinfectants and biocides ineffective at the directed use concentrations. These results provide insight into the potential consequences of widespread use of QACs on human health and help guide the selection and use of QAC-containing products.

### Dexamethasone induces the steroidogenic pathway regulator LRH-1 in intestinal mucosa

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Glucocorticoids are used as anti-inflammatory therapy in Ulcerative Colitis (UC), although some patients show refractory/dependent response. Transcription factor LRH-1 is critical to intestinal cortisol production (steroidogenesis), being reduced in UC patients. However, steroidogenic pathways in

UC patients with different glucocorticoid responses has not been studied. We therefore determined LRH-1, CYP11A1/CYP11B1 and glucocorticoid receptor (GR) expression in intestinal mucosa from UC patients, together with mechanisms involved in Dex-induced cortisol production. Colonoscopic biopsies from active UC patients (n=21) and healthy controls (n=10) were analyzed for LRH-1, CYP11A1, CYP11B1, GR isoform levels by IHC/qRT-PCR, and Dex effect on cytokine production by CBA. Dex effect on steroidogenic pathway components in biopsies, primary intestinal organoids, an intestinal epithelium-specific GR KO (GRiKO) dextran sodium sulfate (DSS)-colitis mice model and CCD841CoN cells was evaluated by ELISA, qPCR, IF, and ChIP assays. Results show Dex inducing cortisol production in control and responder patient's mucosa, and CCD841CoN cells. Moreover, LRH-1 transcript levels are lower in UC patient's vs controls; however, LRH-1 and GR $\beta$  protein content increase in lamina propria (LP) cells in dependent and refractory patient's vs controls. Interestingly, we found GR directly induces LRH-1 expression in organoids and CCD841CoN cells, binding to two glucocorticoid-responsive elements within the LRH-1 promoter. Additionally, LRH-1 expression is decreased in epithelium and increased in LP in the GRiKO-DSS-colitis mice model compared to GRflox/DSS mice. Despite high LRH-1 levels, GR-mediated intestinal steroidogenesis is altered in dependent and refractory UC patients, possibly related to mucosal GR $\beta$  content, linking their role in intestinal homeostasis and corticoid response.

### Diet at birth conditions successful development of type 2 immunity

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Gut immunity heavily influences susceptibility to infectious and non-communicable diseases. This highlights the need to establish early life factors that shape gut immunity development. Infant nutrition is known to play an important role in gut immune imprinting. Colostrum is the physiological food for the first 2-3 days of life and is extremely rich in biologically active components such as antibodies, growth factors, and oligosaccharides. While this composition suggests colostrum is designed to satisfy the needs of the developing newborn, there is a major gap of knowledge on its role in immune

development. To fill this gap of knowledge, we established a unique mouse model of colostrum deprivation where mice were cross fostered at birth by mothers that were at an advanced stage of lactation and not providing colostrum anymore. We analyzed gut immunity at weaning and found a major decrease in gut barrier, goblet cells, innate lymphoid group 2 and T helper 2 cells in the small intestine of mice deprived of colostrum as compared to physiologically breastfed mice. We then addressed the impact of abnormal gut development on susceptibility to infection to intestinal helminth parasite, *Heligmosomoides polygyrus*. At 3 weeks of age, mice deprived of colostrum at birth showed a dramatic increase in susceptibility to infection as shown by the amount of eggs in feces and worms in their intestine. In conclusion, our data demonstrate that colostrum is critical for type 2 immunity development and suggest that promotion of colostrum feeding is required to prevent parasitic infection in childhood.

### Dietary considerations in a murine model of Early Life Stress

Jamie Choe - University of North Texas Health Science Center, Harlan P. Jones

Early life stress (ELS) is known to have negative effects on long-term human health. A major challenge of studying the impact of stress on immune competency has been difficulty developing a reliable mouse model with reproducible stress effects. Animal models of ELS emulate the nature of childhood neglect through scheduled separation. Although variations of maternal separation in rodents have been published, the reported results are inconsistent. This may in part be attributed to variations in animal housing conditions between research institutions, such as diet. In the present study, we utilize a modified version of the maternal separation with early weaning (MSEW) paradigm with C57BL/6 mice and compare the effects of standard (PicoLab® 5058) and purified high carbohydrate (ClearH2O® DietGel®) diet on peripheral stress hormones and cytokine profiles of select primary and secondary lymphoid tissues. Pups were produced via in-house breeding procedures and subjected to our MSEW protocol. Euthanasia occurred at postnatal day (PD) 21 for tissue harvest and blood collection via cardiac puncture. Cytokines and stress hormones were detected using commercially available ELISA. This pilot study sheds light on the impact dietary variations have on immune outcomes in the context of stress. We

demonstrate diet is a critical component of our stress model and significantly impacts cytokine production within select lymphoid tissues at PD21. This work provides insight into the need for MSEW diet standardization to improve the reliability and reproducibility of murine models designed to study ELS.

### Dietary control of interspecies interactions in the human gut microbiota

Michael Patnode - UCSC, Bo Huey Chiang, Nicolette M. P. Hernandez-Kaempf, Giovanni Vega, Alexander Y. Newman

Which microbial species in the human gut form interdependent relationships with one another? Which dietary nutrients selectively promote beneficial species of interest, without also inadvertently targeting their non-beneficial interaction partners? Existing knowledge is insufficient to address these questions, in part because it is based on the numerical abundances, genome content, and gene expression of gut bacterial species. These types of data lack information about the physical proximity between cells, which may dictate whether reproducible interactions evolve in the turbulent physical environment of the gut. We sought to test whether human gut microbes adhere tightly to food particles, and whether the types of carbohydrates present in a food particle control which species cohabitate and interact on its surface. We generated a panel of artificial food particles with each type composed of microscopic paramagnetic beads coated with a fluorescent barcode and one of 30 different dietary or host glycan preparations. Analysis of 160 human-derived *Bacteroides* and *Parabacteroides* strains revealed diverse strain-specific and glycan-specific binding phenotypes, several of which were modulated by bacterial metabolism of plant-derived carbohydrates. This research establishes a novel method for dietary control of microbial interactions in the human gut ecosystem and sets the stage for identifying the bacterial genes that mediate selective adhesion to dietary particles. This approach may facilitate the design of diets that enhance the suppression of pathogenic bacteria by beneficial species.

### Dietary fibers increase net B vitamin output by the gut microbiome and support host immune homeostasis

Erica Grant - Luxembourg Institute of Health, Amy Parrish, Erica T. Grant, Marie Boudaud, Oliver Hunewald, Shinji Fukada, Mahesh S. Desai

Dietary fibers are often heralded as a tool to alter the microbial metabolic output and to sustain healthy immune function, however, the role of the fiber source and its potential immunomodulatory impact beyond the production of short-chain fatty acids is underexplored. To address this gap, we employed 5 distinct rodent diets with varying fiber content and source to decipher their effects on host immunity in mice with 3 distinct colonization profiles: specific-pathogen-free, gnotobiotic (containing a 14-member synthetic human gut microbiota), and germ-free. Broad-scale metabolomic analysis revealed a reduced concentration of microbially-produced B vitamins in the cecal contents of fiber-deprived mice. Correspondingly, RNA sequencing of the cecal contents indicated that a fiber-free diet shifted the ratio of bacterial B vitamin synthesis versus downstream utilization, resulting in a net reduction of host-available B vitamins. In parallel, broad immunophenotyping showed that effector immune populations and activated T cells accumulated in the colonic lamina propria of fiber-deprived mice in a microbiota-dependent manner. Supplementing the fiber-free diet with the prebiotic inulin, but not diverse crude fiber sources, recovered the microbial B vitamin output and host immune homeostasis observed in mice fed standard, fiber-rich diets. Our study calls attention to the unrealized biotechnological potential of fiber polysaccharides to boost availability of microbially-produced B vitamins and regulate local innate and adaptive immune populations, which has potential therapeutic consequences in the context of various gut-linked diseases.

## Dietary sugar alters colonocyte pyruvate metabolism and impairs the early proliferative response to damage

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The colonic epithelium requires continuous renewal by intestinal stem cells (ISCs) to maintain barrier integrity, especially after inflammatory damage. Epithelial renewal is

regulated in part by direct effects of dietary metabolites on ISCs and adjacent epithelial cells, yet how excess dietary sugar affects renewal of colonic epithelium is unknown. We utilized murine and human 3-dimensional colonoids to demonstrate direct effects of sugar on the metabolomic and transcriptomic signature of developing crypt-like structures. We validate these effects in vivo using a mouse-model fed high-sugar or high-fiber diets and measured the proliferative response to dextran sodium sulfate (DSS)-damage. We demonstrate that ISCs cultured under high sugar concentrations directly limited murine and human colonoid development, with a reduction in the expression of proliferative genes and an accumulation of glycolytic metabolites but not TCA cycle intermediates. These results suggest high sugar concentrations shift ISC metabolism and impair pyruvate entry into the TCA cycle. Restoring metabolic flux by inhibiting pyruvate dehydrogenase kinase rescued sugar-impaired colonoid development. In vivo lineage tracing experiments and transcriptomic analysis indicated that dietary sugar impeded the proliferative potential of ISCs. Metabolic analysis of colonic crypts revealed that a high sugar diet primed the epithelium for glycolysis without a commensurate increase in aerobic respiration. Finally, mice fed a high-sugar diet failed to repair DSS-induced colonic damage, resulting in lethal intestinal pathology. Our results indicate that short-term, excess dietary sugar can directly inhibit epithelial proliferation in response to damage and may inform diets that better support the treatment of acute intestinal injury.

## Differential expression of miRNAs in IBD intestinal mucosa and fibroblasts in search of biomarkers for associated colorectal cancer

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MicroRNAs (miRNAs) are small and non-coding RNAs, which induce or repress target gene expression. Specific miRNAs have been associated with inflammatory bowel diseases (IBD) and/or colorectal cancer (CRC). Besides, mucosal myofibroblasts are key stromal cells involved in IBD pathogenesis and in tumor microenvironment. We aimed to



study whether there is differential expression of miR-21-5p and miR-155-5p in the inflamed mucosa, and particularly in intestinal fibroblasts from IBD and CRC patients compared with healthy control intestinal mucosa, as potential early biomarkers for CRC. Total RNA was obtained from mucosal explants from IBD patients, polyp and colon biopsies from patients with CRC and healthy control patients. Intestinal fibroblasts were isolated from colon surgical pieces and primary cultures were established. cDNA from biopsies and/or fibroblasts was obtained and miR-21-5p and miR-155-5p expression was quantified by real-time qPCR with specific primers. Target genes for these miRNAs, PDCD4 and CBX7, were also evaluated, along with TNF- $\alpha$  and TGF- $\beta$  gene expression. We detected higher expression levels of miR-21-5p in inflamed tissue compared to non-inflamed mucosa, and in tumor biopsies from CRC patients, whereas expression of miR-155-5p was variable among groups. The miR-21 expression level correlated with its target gene expression, as PDCD4 was lower in inflamed tissue and fibroblasts compared to healthy samples. Mucosal inflammation and fibroblasts activity in IBD and CRC correlated with miR-21 increase. Further studies are underway, including fibroblasts RNA deep sequencing, to confirm that miR-21 and potentially other miRNAs could be helpful to predict IBD outcomes to early prevent CRC onset.

## Disarming antiviral immunity at the oral mucosal barrier

Juhi Bagaitkar - The Ohio State University and Nationwide Childrens Research Institute, Carlos Rodriguez-Hernandez, Richard J Lamont

The oral cavity is a prime site for initial viral infections and dissemination to other tissues. We found that Type III IFNs or IFN lambdas (IFN-Ls) are preferentially expressed by oral epithelial cells, and IFN-L-associated signaling confers robust, broad-spectrum, antiviral immunity at the oral mucosal barrier. Bacterial colonizers at barrier sites have the potential to modulate host susceptibility to viral infection. Consistent with this, we found that *Porphyromonas gingivalis* (Pg), which is associated with oral dysbiosis and periodontal disease, singularly and totally dampened all aspects of IFN signaling in response to viral agonists. Pg transcriptionally suppressed IFN production by downregulating several IFN regulator factors (IRFs-1,3,7,9). Downstream interferon-stimulated genes (ISGs) were also suppressed by the proteolytic degradation of STAT1

and consequent reduction of nuclear translocation of the ISGF3 complex, resulting in profound and systemic repression of multiple ISGs. Increased colonization with Pg in murine models and oral tissues of human periodontal disease patients showed significant suppression in inducible IFN-L responses and antiviral immunity. Mechanistically, multiple virulence factors and secreted proteases (gingipains) produced by Pg transcriptionally suppressed IFN promoters, and also cleaved IFN receptors making cells refractory to exogenous IFN. This multipronged virulence strategy employed by Pg led to a state of broad IFN paralysis by inhibiting constitutive as well as inducible arms of host antiviral immune responses. Thus, our data show, for the first time, a bacterial pathogen and resident of the oral microbiome can enhance susceptibility to viral infections at the oral mucosal barrier.

## Dissecting early epithelial-specific immune responses to an enteric bacterial pathogen

Moshe Biton - Weizmann Institute of Science, Sacha Lebon

The gut is lined with a specialized monolayer epithelium composed of different intestinal epithelial cell types specialized in sensing and responding to environmental substances. *Salmonella enterica* (*S. enterica*) is a common pathogenic foodborne infectious agent of the gut. Although the physiopathology of *Salmonella* systemic infection has been investigated in-depth, the first steps of natural infection, occurring in the gut, by which *S. enterica* invades and affects the epithelial cells are still unknown. We explored the epithelial cell-specific responses to *S. enterica* infection using single cell RNA-seq (scRNA-seq) and GFP-labeled pathogenic bacteria, coupled with computational approaches to identify an epithelial-specific signature of *Salmonella* infection. We showed that mice infected with *S. enterica* resulted in epithelium remodeling on the first day of infection, with depletion of intestinal stem cells (ISCs) and massive enterocyte differentiation. Our data indicate two subtypes of intestinal epithelial cells prone to *S. enterica* infection: Paneth cells and enterocytes. These cells increased their production of antimicrobial proteins, which in turn help to eradicate this pathogenic bacterium from the lumen. Surprisingly, close inspection of ISCs, residing next to Paneth cells revealed a novel stem cell protective mechanism against *S. enterica* infection. We identified that infected stem cells undergo programmed differentiation toward Paneth cells and

enterocytes to eliminate infected ISCs from the stem cell pool. Overall, we identified a novel epithelial-specific *S. enterica* infection signature and specific-epithelial cell responses to this infectious pathogen. This study illuminates a novel stem cell mechanism to cope with insults arriving from the lumen.

### Do early life commensal microbes prevent type 1 diabetes?

Jamal Green - Children Hospital of Philadelphia, Jean-Bernard Lubin, Sarah Maddux, Tereza Duranova, Julia Flores, Michael Silverman

Early-life microbes are critical for the development of the immune system and disruption of the microbiome in early life can lead to autoimmunity, yet the specific mechanisms that support healthy immune system development remain largely unknown. To rigorously study the role of early-life microbiota in autoimmune diabetes (T1D), we developed a novel consortium of 9 culturable bacteria that dominate the early life microbiome (PedsCom) and colonized germ-free, non-obese diabetic (NOD) mice. The PedsCom consortia represent over 90% of the bacteria in pre-weaning NOD mice. We find that early life colonization with the PedsCom consortia protects NOD mice from developing diabetes compared to germ-free mice. PedsCom colonization of NOD mice increases peripheral regulatory T cells in the intestinal and lymphatic systems suggesting a potential mechanism by which early-life commensal microbes may prevent system autoimmunity. Additionally, we demonstrated that specific early life microbes drive distinct adaptive mucosal and systemic immune responses. We aim to leverage PedsCom NOD mice to identify specific mechanism by which commensal bacteria educate the developing immune system and inform preventive therapies for T1D.

### Early life allergen-induced lung inflammation causes pericyte loss and vascular remodelling: a role for mast cell activation?

Regis Joulia - Imperial College, Franz Puttur, Lewis Entwistle, Helen Stoelting, Simone Walker, Laura Yates, Clare Lloyd

Allergic asthma is common during childhood and although the association with inflammation and lung dysfunction is well

known, cardiovascular disorders in later life are also a feature. Gas exchange is an essential feature of the respiratory function and is ensured by a highly complex network of blood vessels, however the mechanism by which allergic inflammation modulates the vascular system remains enigmatic. Typically, blood vessels are lined with an interrupted layer of endothelial cells (ECs) and surrounded by structural cells called pericytes, which are essential to the integrity and development of the vasculature. Using Precision Cut Lung Slices (PCLS) approach and high-resolution confocal microscopy, we showed that PDGFR-b + pericyte distribution is highly heterogenous with the highest cell number and coverage observed in the adventitia (areas including intermediate-to-large blood vessels and airways). During allergic inflammation, this distribution changed dramatically with significant vascular remodelling occurring in the adventitia, but also at distant site such as the parenchyma. Mechanistically, we observed that mast cell (MC) derived granules, enriched in proteases such as Mcpt1, were associated with the pericyte network and could destabilise pericyte/EC interaction. These granules were present across the inflamed lungs despite the restricted localisation of MCs in the adventitia, indicating a potential remote effect. In summary, our data outline a heterogenous pulmonary pericyte landscape during early life, with potentially important functional role in remodelling. Moreover, allergic inflammation leads to major changes in the lung vasculature suggesting short- and long-term detrimental effects on lung function. Funded by the BHF and the WT.

### Early Life Antibiotic Exposure Reduces Tolerance to Dietary Antigens

Keely McDonald - Washington University School of Medicine, Jenny K Gustafsson, Kathryn A. Knooop, Phillip I Tarr, Rodney D Newberry

Early life is a unique time when the microbiota and the intestinal immune system co-evolve for mutual beneficial co-habitation. Exposure to antibiotics in early life has life-long consequences, such as increased sensitivity to colitis, food allergy, and intestinal infections. The mechanisms and microbial players involved in shaping the tolerogenic intestinal immune responses are poorly understood. Microbial metabolites are known to control T cell differentiation and proliferation, but the metabolites that are enriched in early life

due to the unique diet and microbial community are largely unexplored. We used antibiotic exposure in early life to determine the impact on dietary allergen tolerance in adulthood. Only when antibiotics treatment coincided with opening of colonic goblet cell associated passages (GAPs, day of life 10-21) did we observe a loss of tolerance. However, when individual antibiotics were used in early life only b-lactams replicated the phenotype observed with a cocktail of broad spectrum antibiotics. These b-lactams induced a loss of lactate producing bacteria suggesting that lactate could be a microbial metabolite inducing tolerance in early life. Indeed, in vitro treatment of T cells with lactate increased their proliferation. This is the first report that a microbial metabolite enriched in early life is involved in intestinal immune tolerance.

### Early life immune responses modulated by maternal gut microbiome composition and diet

Erica Grant - Luxembourg Institute of Health, Erica T. Grant, Marie Boudaud, Mahesh S. Desai

The neonatal intestine experiences microbial colonization alongside the maturation of the host mucosal barrier and immune system, yet little is known about how diet-microbe interactions shape the early immune system. To understand the influence of the maternal microbiome on early life immune maturation, we colonized mice with a 13 or 14-member synthetic human microbiome (13SM or 14SM), with or without *Akkermansia muciniphila*, a health-associated bacterium with milk oligosaccharide and mucin-degrading capacities. We simultaneously bred 14SM mice on a fiber-free (FF) diet since dietary fiber also exerts a powerful impact on the microbiome, immune function, and *A. muciniphila* abundances. Pups of fiber-rich (FR)-fed dams exhibited an initial expansion of *A. muciniphila* in the colon, which dropped after pups began consuming solid food. However, *A. muciniphila* did not proliferate in the colons of mice born to dams fed a fiber-free (FF) diet until the pups were switched from maternal milk to a FF diet (20 postnatal days), after which the mucin-degrading bacteria rapidly expanded and remained abundant into adulthood. Prior to weaning, FF-fed mice also exhibited enrichment of transcripts corresponding to pathways for defense response against external antigens and exhibited elevated levels of CD8<sup>+</sup> T cells and group 3 innate lymphoid cells compared to pups of FR-fed mice. Furthermore, the abundance of *A. muciniphila* was inversely related to the

proportions of RORγt<sup>+</sup> T effector cells. Our results highlight the powerful influence of maternal dietary fiber intake in the postnatal establishment of mucolytic communities and the maturation of the intestinal mucosal barrier.

### Early Life Ingestion of Maternal Antibodies Suppresses CD4<sup>+</sup> T-Cell Activity Post-Weaning

Shannon Gordon - Fred Hutchinson Cancer Research Center, Meera K Shenoy, Meghan A Koch

Breastfeeding provides numerous immunomodulatory factors to neonates including non-digestible sugars to select for commensal microbiota and cytokines and antibodies to educate the developing immune system. Recent studies have even implicated breastfeeding in protection from allergic disease, potentially by modulating CD4<sup>+</sup> T-cell activity. Using mouse models, we previously demonstrated that pups reared by B cell-deficient dams experience elevated intestinal CD4<sup>+</sup> T-cell responses following weaning. We hypothesized that maternal antibodies ingested during breastfeeding are responsible for attenuating CD4<sup>+</sup> T-cell activity in the developing neonate. To investigate this mechanism, we optimized collection and processing of breastmilk from wild-type lactating dams one to two weeks post-partum. We used protein chromatography to isolate antibodies from matched serum and breastmilk and determined antibody titers via isotype-specific ELISA. To assess the direct effect of maternal antibodies, we reared wild-type pups on B cell-deficient dams and fed them antibodies isolated from breastmilk or serum, breastmilk depleted of antibodies, or PBS. Pups fed antibody-depleted breastmilk or PBS exhibited significantly higher intestinal CD4<sup>+</sup> T-cell responses compared to mice that were fed any antibodies. We previously showed that pups born to B cell deficient dams have elevated CD4<sup>+</sup> T-cell responses in the intestine. Here we demonstrate feeding maternal antibodies back to pups reverses this phenotype. This work definitively illustrates that maternal antibodies are responsible for suppression of offspring CD4<sup>+</sup> T-cell responses. Further investigations will define how maternal antibodies limit offspring immunity to potentially promote immune tolerance. If translatable to humans, antibody enrichment of infant formula could have great public health implications.

## Early Life Origin of Anti-commensal IgGs

Brigida Rusconi - Washington University School of Medicine, Adina Bard, Ryan McDonough, Colleen Rouggy-Nickless, Keely G McDonald, Sreeram Udayan, Rodney D Newberry, Phillip I Tarr

Maternal IgGs protect infants from enteric and systemic infections in early life. However, when, where, and how these protective maternal anti-commensal IgGs are generated remains enigmatic. In adult mice, circulating IgGs display more specificity pre-weaning gut bacteria than to their own adult bacterial community. IgG-secreting plasma cells specific for pre-weaning gut bacteria appear in the small intestine and colon shortly after weaning, where they remain into adulthood. Manipulating exposure to gut bacteria or plasma cell development in the pre-weaning period, but not later, reduces systemic IgG specificity towards early life gut bacteria. We also found that the offspring of dams perturbed in early life had diminished IgG specificity to their pre-weaning commensal bacteria, and were susceptible to an enteric infection model, including systemic dissemination. In contrast to classic concepts, protective maternal IgGs were specific for gut commensals that translocate during enteric infection in offspring of dams manipulated in early life, and not to the pathogen, indicating that protective maternal IgGs are generated in early life and are specific for gut commensals.

## Early life stress induced hypothalamic–pituitary–adrenal (HPA) axis dysfunction promotes chronicity of experimental colitis in mice

Rachel Muir - University of Alabama at Birmingham, Barbara J. Klocke, Jeremy B. Foote, Melissa S. Jennings, Patrick A. Molina, Cailin E. Kellum, Jennifer S. Pollock, Craig L. Maynard

Several studies have linked early life stress (ELS) with hypothalamic–pituitary–adrenal (HPA) axis dysfunction that ultimately increases susceptibility for adverse health outcomes later in life, including elevated risk of inflammatory diseases. The aim of this study was to determine whether ELS in mice affects susceptibility to, and/or severity of colitis induced subsequent to stress exposure. We used an established mouse model of ELS, maternal separation with early weaning (MSEW), where newborn mice are separated from their mothers for 4-8 hours daily from postnatal day (PD) 2 until

early weaning on PD 17. Beginning at PD 28, colitis was induced in MSEW mice and their normally-reared (NR) counterparts by transient blockade of the interleukin-10 receptor (IL-10R). Relative to their NR counterparts, MSEW mice exhibited systemic and colon-specific deficits of the HPA-axis endpoint molecule corticosterone, prior to induction of colitis. Following disease onset, NR mice were mostly in remission by 15 days after cessation of IL-10R blockade, whereas MSEW mice displayed sustained histological pathology with an emphasis on epithelial damage, in addition to consistently elevated colonic tumor necrosis factor (Tnf). Additionally, pharmacologic induction of HPA-axis dysfunction via early life exposure (PD 1-14) to the synthetic glucocorticoid, dexamethasone, emulates the results we observed in the MSEW model of ELS – both prior to and subsequent to colitis induction. Our results indicate that early life exposure to prolonged stress leads to colonic corticosterone deficits which may subsequently promote persistent colonic inflammation in susceptible hosts.

## Effector memory CD4+ T cells induce damaging innate inflammation and autoimmune pathology by engaging CD40 and TNFR on myeloid cells

Margaret McDaniel - University of Washington, Aakanksha Jain, Amanpreet Singh Chawla, Hannah E. Meibers, Irene Saha, Yajing Gao, Viral Jain, Krishna Roskin, Sing Sing Way, Chandrashekhar Pasare

Cytokine storm and sterile inflammation are common features of T cell-mediated autoimmune diseases and T cell-targeted cancer immunotherapies. Although blocking individual cytokines can mitigate some pathology, the upstream mechanisms governing overabundant innate inflammatory cytokine production remain unknown. Here, we have identified a critical signaling node that is engaged by effector memory T cells (T EM) to mobilize a broad proinflammatory program in the innate immune system. Cognate interactions between T EM and myeloid cells led to induction of an inflammatory transcriptional profile that was reminiscent, yet entirely independent, of classical pattern recognition receptor (PRR) activation. This PRR-independent “de novo” inflammation was driven by preexisting T EM engagement of both CD40 and tumor necrosis factor receptor (TNFR) on myeloid cells. Cytokine toxicity and autoimmune pathology could be completely rescued by ablating these pathways genetically or



pharmacologically in multiple models of T cell-driven inflammation, indicating that T EM instruction of the innate immune system is a primary driver of associated immunopathology. Thus, we have identified a previously unknown trigger of cytokine storm and autoimmune pathology that is amenable to therapeutic interventions.

### **The effects of obesity-associated dysbiotic gut microbiota on the intestinal and adipose tissue inflammation.**

Devesha Kulkarni - Washington University School of Medicine, Alexandria Floyd, Ryan McDonough, Elizabeth Joyce, Khushi Talati, Samuel Klein, Rodney Newberry

Obesity is a complex disorder resulting from multiple factors including genetics, diet and gut microbes. Further not all obese individuals develop metabolic disease. Individuals with metabolically unhealthy obesity (MUO) suffer from low-grade inflammation of the adipose tissue (AT) characterized by increased pro-inflammatory immune cells, which directly contributes insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. In contrast, individuals with metabolically healthy obesity (MHO) have a lower AT inflammation, improved insulin resistance, and a better liver function profile. Seminal studies indicate dysbiotic gut microbiota not only promote obesity, but also alters the immune system. However, whether gut microbes drive MUO-associated abnormalities remains unknown. To address this, we established a humanized microbiota-associated model colonizing wildtype mice consuming a normal chow diet with stool specimens collected from MUO, MHO, or lean human subjects with known degrees of AT inflammation, glucose intolerance and whole-body insulin sensitivity. Mice colonized with MUO and MHO microbiota had significantly higher weight gain at 5 weeks. However, only MUO mice showed metabolic abnormalities. Additionally, we observed that MUO mice showed the expansion of macrophages in the gut and AT compared to MHO or lean mice. Given the crucial roles of macrophages in metabolic diseases, it is plausible that macrophages contribute to the phenotype we observed and that this macrophage expansion is driven by gut microbes. This knowledge will help explain the heterogeneity among MUO and MHO individuals and will provide insights towards modulation of the gut microbiota as a therapy for obesity-related metabolic disease.

### **Effects of the human lung mucosa of the elderly population on the pathogenesis of Mycobacterium tuberculosis in alveolar epithelial cells**

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The ongoing pandemic of Tuberculosis (TB), caused by the infectious agent Mycobacterium tuberculosis (M.tb), still kills one person every 21 seconds. M.tb is transmitted into the alveolar space, where it gets in close contact with the alveolar mucosa, a.k.a. alveolar lining fluid (ALF). We have published that age-associated changes in ALF soluble components, such as increased levels of oxidized and dysfunctional innate proteins, accelerate M.tb growth within human macrophages. We are now exploring how human ALF influences M.tb infection of alveolar epithelial cells (ATs), non-professional phagocytes, and the major cell population in the alveolus. Based on our published studies with phagocytes, we hypothesized that M.tb exposure to elderly human ALF (E-ALF) accelerates M.tb replication within ATs due to impaired E-ALF innate soluble components; thus, defining ATs as a unique niche for M.tb growth and propagation in the elderly. Our results indicate that M.tb exposure to E-ALF significantly increases its intracellular growth in ATs, minimizing the production of inflammatory mediators. Interestingly, M.tb exposure to E-ALF also drove bacterial translocation to both endosomal and cytosolic compartments in ATs. Overall, rapid bacterial replication and increased growth within ATs were observed in M.tb exposed to E-ALF, and together with inadequate AT activation, could lead M.tb to potentially exploit the AT cytosol lumen as a favorable intracellular niche for replication. Our findings highlight how the status of lung mucosa as we age influences M.tb infection of ATs and define E-ALF as an unexplored contributing factor to the increased susceptibility of the elderly population to TB.

### **Endogenous retrovirus reactivation promotes allergic inflammation**

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Mammals co-evolved with a multitude of microorganisms that include the commensal microbiota and endogenous retroelements such as the endogenous retroviruses (ERVs) that comprise a substantial fraction of the mammalian genome. Recently, we showed that ERVs expression acts as endogenous adjuvants that prime the host for immune responses to the microbiota thereby controlling both tissue homeostasis and inflammation. However, how ERVs activation impact on the pathogenesis of inflammatory disorders such as allergic diseases remain unknown. Employing a murine model of house dust mite (HDM)-induced allergic inflammation we found that HDM intranasal sensitization significantly upregulated the expression of ERVs in several innate cell types in the lung. Antiretroviral treatment (a cocktail of two reverse transcriptase inhibitors) profoundly inhibited innate cells accumulation within the lung following HDM-allergic sensitization, including eosinophils, a hallmark for type-2 inflammation. Antiretroviral treatment also significant decreased the number of B cells, ILCs and CD4+ T cells during HDM sensitization as well as their ability to produce immunoglobulins and the type 2/17 cytokines such as IL-9, IL-33, IL-5, IL-13 and IL-17A. This phenomenon was associated with an intense reduction of perivascular and peribronchial infiltrate of inflammatory cells, and mucus production by the goblet cells in the lung epithelium. Of particular interest, we also found that genetic ablation of *Emv2* (a highly expressed endogenous retrovirus) was sufficient to impair both innate and adaptive Th2 immunity during HDM sensitization. Together this work reveals an unexpected role for endogenous retroelements in the control of allergic inflammation in the lung.

## Endotracheal Aspirate Concentrations of MCP-1, Eotaxin-1, and IL-12p70 Are Associated with Risk of SARS-CoV-2 Induced Pulmonary Fibrosis

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Pulmonary fibrosis (PF) is a major long-term complication of severe, acute SARS-CoV-2 infection. The mechanisms underlying SARS-CoV-2 induced PF remain largely

unidentified. We set out to identify lung-specific biomarkers that associate with the development of PF to elucidate mechanisms of disease and identify patients at high risk of this outcome. We collected and analyzed 68 endotracheal aspirates (ETAs) from mechanically ventilated SARS-CoV-2 positive patients from Seattle area hospitals. ETAs were collected at two visits, V1 and V2, at 1 and 4 days post-intubation. A board-certified chest radiologist blinded to the data adjudicated PF by reviewing chest imaging. Proteins involved in extracellular matrix remodeling and inflammation were measured by immunoassay and analyzed using Mann-Whitney U tests. We identified PF in 27 patients. We found that higher concentrations of Monocyte Chemoattractant Protein-1 (MCP-1), Eotaxin-1, and Interleukin-12p70 (IL-12p70) were associated with PF at V2 ( $p < 0.05$ ). In patients with PF, ETA concentrations of MCP-1 and Eotaxin-1 increased between V1 and V2. IL-12p70 concentrations remained stable between V1 and V2 in patients with PF but trended downwards in patients without fibrosis between visits. Biomarkers of acute inflammation that have been associated with COVID-19 severity, such as Interleukin-6 (IL-6), Interferon-gamma (IFN- $\gamma$ ), and Tumor Necrosis Factor alpha (TNF- $\alpha$ ), were not significantly associated with PF at any timepoint in ETAs. These data indicate that changes in the lung concentrations of MCP-1, Eotaxin-1, and IL-12p70 between days 1 and 4 post-intubation, not a general pro-inflammatory response, may play a role in the pathogenesis of PF from SARS-CoV-2 infection.

## Environmental influences on gut microbiota, immunity, and inflammation

Michal Kuczma - Georgia State University, Timothy Denning, Edyta Szurek, Leszek Ignatowicz

Inflammatory bowel disease (IBD) is the result of chronic inflammation of the digestive tract. Genetics, lifestyle, environment, food, and dysregulation of the immune system reacting to gut-resident microbial antigens and diet, are the most prominent factors contributing to IBD. The immune system evolved to ensure that microbes are kept in check through tolerogenic mechanisms present in the gut and periphery. Gut-resident bacteria dynamically regulate the composition and function of innate and adaptive immune cells, including CD4+ cells. Nevertheless, the break in the tolerance to endogenous microbiota causes activation of CD4+ cells leading to IBD. Recent data indicate that the SPF conditions of

conventionally-housed (CNV) mice have led to the development of specific microbial niches within the gut, which may impact the translational potential of pre-clinical colitis models of human IBD. In our studies, we exposed CNV, triple-reporter (Foxp3 hCD2 Nur77 GFP IL10 Thy1.1) B6 mice to a key environmental determinant, namely soil, which resulted in dramatic changes in the intestinal microbial communities and innate and adaptive immune cells of this environmentally-exposed (ENV) mice. Prominently, the innate immune cells expanded in ENV mice at the expense of the adaptive immune cells, which in turn created a suppressive/tolerogenic/anergic environment with increased numbers of Tregs and IL-10-expressing cells, and this effect required active microbial colonization. Importantly, ENV mice displayed altered severity of colitis. Concurrently, our studies will reveal how exposure to environmental factors protects from or reduce the severity of IBD.

### Epithelial MHC class II directs microbiota-specific intestinal immune homeostasis

Emily Eshleman - Cincinnati Children's Hospital Medical Center, Tzu-Yu Shao, Vivienne Woo, Taylor Rice, Jordan Whitt, Laura Engleman, Sing Sing Way, Theresa Alenghat

The intestinal microbiota is essential for instructing the host immune system, however, dysregulated immune responses to resident commensal microbes can promote pathologic inflammation. Despite this relationship, the mechanisms regulating tissue-intrinsic, microbiota-specific T cell responses remain poorly understood. Here, we find that non-hematopoietic intestinal epithelial cells (IECs) represent dominant cells expressing major histocompatibility complex (MHC) II at the host-microbiota interface. Interestingly, epithelial MHCII and commensal-specific CD4<sup>+</sup> T cells were simultaneously induced by post-natal microbiota colonization, prompting the hypothesis that epithelial MHCII regulates intestinal microbiota-specific CD4<sup>+</sup> T cells. While classical MHCII-expressing antigen presenting cells promote antigen-specific T cell expansion, loss of IEC-intrinsic MHCII surprisingly resulted in elevated commensal-specific CD4<sup>+</sup> T cells in the intestine. Further, epithelial MHCII actively limited accumulation of microbial-antigen specific CD4<sup>+</sup> T cells in adult mice. Expansion of commensal-specific Th17 cells was restricted by epithelial MHCII, and remarkably mice lacking epithelial MHCII were highly susceptible to microbiota-

triggered intestinal inflammation. Collectively, these data suggest that altered epithelial MHCII-T cell regulation in the intestine may impair tolerance to resident microbes and predispose to chronic inflammation. Future investigation into the relationship between the microbiota, IECs, and local immune cells could aid in the development of novel therapeutics to prevent or limit inflammatory conditions.

### Epithelially expressed Interferon $\epsilon$ supports regulatory T cells and promotes intestinal homeostasis

Eveline de Geus - Hudson Institute of Medical Research, Jennifer S. Volaric, Nicole A. de Weerd, Niamh E. Mangan, Edward M. Giles, Paul J. Hertzog

Type I interferon (IFN) signalling is crucial for maintaining intestinal homeostasis and disruption of intestinal immune balance can lead to inflammatory bowel diseases (IBD). The type I IFN family includes multiple IFN $\alpha$ , IFN $\beta$  and IFN $\epsilon$ , and it is unclear what role the individual type I IFN play. We previously showed IFN $\epsilon$  is highly expressed by epithelial cells of the female reproductive tract, where it is involved in protection against pathogens. Expression has also been shown in jejunum and rectum of rhesus macaques. We hypothesise IFN $\epsilon$  is important for maintaining intestinal homeostasis. We show IFN $\epsilon$  is expressed in human and mouse intestinal epithelium and expression is lost in inflamed conditions. Furthermore, our results show IFN $\epsilon$  limits intestinal inflammation in the DSS colitis model, as IFN $\epsilon$ -/- mice had more severe disease when compared to wildtype mice. Regulatory T cell (Treg) frequencies were decreased in DSS-treated IFN $\epsilon$ -/- mice suggesting a role for IFN $\epsilon$  in maintaining the intestinal Treg compartment. Like IFN $\beta$ , IFN $\epsilon$  can bind to IFNAR1 in the absence of IFNAR2, resulting in a distinct non-canonical gene signature. To characterise a role for non-canonical signalling in experimental colitis, IFNAR2-/- mice were treated with IFN $\epsilon$ -neutralising Ab before induction of colitis. Interestingly, non-canonical IFN $\epsilon$  signalling was pathogenic, as neutralising IFN $\epsilon$  resulted in ameliorated symptoms. These findings show that IFN $\epsilon$  is a new factor involved in the pathogenesis of IBD. Furthermore, we show non-canonical IFNAR signalling is pathologically relevant in intestinal inflammation. This makes IFN $\epsilon$  a promising therapeutic target for the treatment of IBD.

## Establishing A Mouse Model Of Coeliac Disease-Associated Lymphomagenesis

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**Background and objective:** The frequent first step in coeliac-disease (CeD)-associated lymphomagenesis is an intraepithelial clonal lymphoproliferation called type II refractory CeD (RCDII). RCDII arises from a small subset of iCD3+ innate-like intraepithelial lymphocytes (IELs), which carry somatic gain-of-function (GOF) mutations in JAK1 and/or STAT3 that license their clonal expansion in the IL-15-rich coeliac intestine. Our present objective was to establish a preclinical mouse model to recapitulate this physiopathogenic scheme allowing us to test therapeutic strategies. **Methods :** Murine iCD3+ innate-like cells were generated from FACS-purified murine common lymphoid cell progenitors by co-culture on OP9-DL1 stromal cells in the presence of IL-7, SCF, Flt3l and IL-15. The differentiation status was followed weekly by FACS and qPCR. Progenitors were transduced with wild-type or S tat3 GOF (p.D661V), differentiated on OP9-DL1, and injected into immunodeficient mice overexpressing IL-15 (Rag-/-gc-/-IL15tg) for 8 weeks. **Results :** iCD3+ innate cells appeared after two weeks of culture only in presence of IL-15 and displayed phenotypic and transcriptomic features comparable with human iCD3+ innate-like IELs, with the potential to colonize preferentially the intestinal epithelium upon injection into Rag-/-gc-/-IL15tg hosts. However, mice receiving iCD3+ innate-like cells harboring S tat3 GOF did not induce an RCDII phenotype upon 8 weeks. **Conclusions and perspectives :** We have successfully generated a murine model with iCD3+ innate-like IELs that can be modified with wild-type or Stat3 GOF. Reconstitution will now be performed for an extended period, and also be done with additional mutations such as Jak1.

## Evaluating immunogenicity of a next-generation intranasal outer membrane vesicle pertussis vaccine using ex vivo nasopharynx mucosal immune tissue of humans

Qibo Zhang - University of Liverpool, Sean Hammond, Rene Raeven, Madhan Krishnan, Emma Whitehall, Ravi Sharma, Paul S McNamara

**Background:** The resurgence of whooping cough is mainly attributed to the induction of relatively short-lived immunity by acellular pertussis vaccines and their incompetence to prevent transmission. Improved vaccines should therefore preferably induce systemic and mucosal IgA/IgG and T-helper (Th) 1 and 17 responses that provide better protection. **Preclinical research** on the development of a next-generation pertussis vaccine based on outer membrane vesicles (omvPV) demonstrated that intranasal immunization with this vaccine in mice blocked colonization in the nasopharynx through induction of mucosal Th17 and IgA responses. **Aims and methods:** We characterized the induction of mucosal adaptive immunity following ex vivo omvPV stimulation of human nasopharynx-associated lymphoid tissues (NALT). This includes, T cell immunity (including Th1 and Th17) and antibody (IgG/IgA) responses, using ELISA/multiplex immunoassay (MIA) and flow cytometry. **Results:** Following stimulation by the candidate omvPV vaccine, we have shown a marked T and B cell proliferative response, also demonstrated both Th1 (IFN $\gamma$ -producing) and Th17 (IL17A-producing) responses in adenotonsillar cells. We further demonstrated significant antigen-specific IgG antibody responses to several pertussis antigens contained in the omvPV vaccine. **Conclusion:** This induction of local immunity in the nasopharynx, which is not induced by current immunization strategies, may prevent nasal colonisation/carriage in humans, thus reducing B. pertussis transmission in addition to the prevention of disease and infection of the lower respiratory tract.

## Evaluation of isotype specific salivary antibody assays for detecting recent SARS-CoV-2 infection in children and adults

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**Background** Large-scale epidemiological studies using mucosal samples for immunological surveillance are uncommon. We aimed to develop, evaluate, and field-test salivary enzyme linked immunosorbent assays (ELISAs) for



detecting recent SARS-CoV-2 infection. Methods ELISAs to detect IgA and IgG antibodies to whole SARS-CoV-2 spike protein, its receptor binding domain region and nucleocapsid protein in saliva were developed. Using 230 saliva samples collected pre-pandemic and 90 from SARS-CoV-2 PCR confirmed cases, thresholds for positivity were set and test accuracy was evaluated using receiver operator characteristic curves. Antibody responses for paired sera and saliva were evaluated in the context of demographic characteristics. Assays were field-tested in household outbreaks (n=22). Results For discriminating between known negative and positive saliva samples, anti-spike IgG was the best performing assay (ROC AUC: 95%, CI95%: 92.8-97.3%), followed by anti-spike IgA (ROC AUC: 89.9% CI95%: 86.5-93.2%). Individuals tended to mount either spike IgA or IgG antibody responses following infection. Serum IgA antibody was not predictive of salivary IgA antibody, while serum IgG was predictive of salivary IgG, consistent with salivary antibody reflecting a systemic IgG response and a mucosal IgA response. In household outbreaks, anti-spike antibody was a reliable indicator of recent infection - all PCR confirmed cases salivary-converted while fewer individuals converted to N-protein. Some naïve (PCR negative), unvaccinated children show evidence of exposure with salivary IgA response. Conclusion Saliva is a non-invasive sample that can be used to monitor trends in mucosal immune responses reliably. Consequently, assays are currently being deployed at scale to track SARS-CoV-2 infection in school settings in Bristol, UK.

### Examining the function of lung CD8+ tissue resident memory T cells in humans.

Cameron Mattingly - Emory University, Kristiana Lancetti, Jennifer Elliot, Jeronay Thomas, Jenna Lobby, Sarah Michalets, Jacob Kohlmeier

Due to their position in the lung tissue, CD8 + tissue resident memory T cells (T<sub>RM</sub>) act as sentinels of the respiratory tract that rapidly respond and mediate protection against respiratory viruses. In mice, T<sub>RM</sub> have been shown to mediate protection at barrier sites around the body by producing cytokines, chemokines, and performing cell lysis. In the lungs specifically, our lab has shown that influenza-specific CD8 + T<sub>RM</sub> rapidly produce IFN $\gamma$ , but airway T<sub>RM</sub> are poorly cytolytic in mice. In humans, less is known about the effector functions of antigen specific CD8 + T<sub>RM</sub> in the lungs and

thus this study seeks to fill that gap in knowledge. Using cells from healthy human lungs, we first identified and quantified the frequency of antigen specific cells in our lung donors by performing intracellular cytokine staining (IFN $\gamma$  + ) and activation induced marker assays (CD137 + CD25 + ). Then, by performing a series of in vitro peptide stimulation and cytokine neutralization experiments, we investigate which cytokines are produced by lung CD8 + T<sub>RM</sub> and how those cytokines impact local innate cells. Initial results show that, when stimulated with their cognate antigen, lung CD8 + T<sub>RM</sub> produce cytokines, such as IFN $\gamma$ , that serve to activate innate immune cells. Results of this study suggests that human CD8 + T<sub>RM</sub> in the lungs act as secondary messengers and these results will ultimately help us how understand how CD8 + T<sub>RM</sub> fit into the overall immune response to respiratory viruses.

### Exploring liver-associated microbes: Who are they and how did they get there?

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Nonalcoholic steatohepatitis (NASH) is characterized by inflammation that can lead to liver damage, cirrhosis, and hepatocellular carcinoma. Recent studies revealed a positive association between bacterial burden in the liver and metabolic disease, suggesting that bacterial translocation from the gut, and/or their metabolites, potentially contribute to liver inflammation. How and which bacteria colonize the liver and exacerbate NASH progression is unknown. Using the GAN model of NASH, we aimed to test the hypothesis that GAN mice would have increased bacterial translocation to the liver. At sacrifice, GAN and control (CON) livers were perfused, homogenized, and cultured for 24-48hrs at 37°C. Of CON mice, 6/28 and 8/28 livers had culturable aerobic and anaerobic bacteria, respectively (75% concordance). In contrast, 17/26 and 18/26 GAN livers had culturable aerobic and anaerobic bacteria, respectively (94.4% concordance). By Fisher's exact test, GAN mice had significantly increased liver-associated bacteria aerobically (p=0.0021) and anaerobically (p=0.0059) compared to CON mice. Bacteria were then isolated and identified by 16S PCR amplification and Sanger sequencing from the genera Enterobacter, Enterococcus, Escherichia, Klebsiella, Proteus, Pseudomonas,

Staphylococcus, and Streptococcus. We then tested the ability of these bacteria to induce intestinal permeability, therefore permitting their escape from the gut. Caco2 monolayers were cultured with bacteria supernatants and assessed for changes in permeability by TEER and FITC-dextran assays. This resulted in bacterial-specific changes in permeability, with the biggest changes by Escherichia and Enterococcus; this was not due to cell death, as determined by a LDH assay. Based on these data, we conclude that the GAN diet promotes bacterial translocation to the liver, and specific bacteria can impact intestinal permeability as modeled by the Caco2 assays. Future studies will investigate the impact of live bacteria in vitro and in vivo, to determine the specific effects on liver inflammation.

### Expression and novel function of FcαRI (CD89) in human vaginal mucosa

Emilie Mausser - Boston University School of Medicine, Jeffery Pudney, Kadryn Kadasia, Deborah Anderson

In the human female reproductive tract (FRT), most interactions with sexually transmitted pathogens occur at the vaginal epithelium, where both IgA and IgG are key mediators of antigen-specific humoral responses. If antibodies are to move through the vaginal epithelium from circulation, FcRn is abundantly present for the transport of IgG, but pIgR or other receptors for trafficking IgA are noticeably absent. We have found that FcαRI (CD89) is highly expressed in basal layers of human vaginal epithelium. While its presence and function on myeloid cells is well characterized, CD89 has not previously been described in FRT epithelial cells. The existence of CD89 was confirmed using several antibody clones for immunohistochemistry and western blotting in both human vaginal and ectocervical tissues. The MatTek EpiVaginal tissue model was found to express CD89 with similar distribution patterns to human surgical samples. Application of IgA to the basal surface of this tissue model showed strong co-localization of IgA and CD89, as well as transport of IgA across the epithelium. Blocking the EC1 domain on CD89 reduced this co-localization and IgA transport across the epithelium by about 40%. The CD89 blocker was specific for monomeric IgA and aggregated IgA complexes but did not affect the transport of IgG or other proteins. In conclusion, we describe for the first time, expression of CD89 by human vaginal epithelial cells, and its possible role in the transport of IgA from the circulation

through the vaginal epithelium to the lumen where it can protect against pathogens.

### The Expression and Regulation of Oncostatin M and its Receptor in Intestinal Inflammation

Roodline cineus - Charite Medical University, Yanjiang Luo, Aya Abdel Rahman, Diana Boesel, Saskia Hainbuch, Doga Bingoel, IBDome Consortium, Chiara Romagnani, Stefan Wirtz, Ahmed N. Hegazy

Oncostatin M (OSM), a pro-inflammatory cytokine, has recently been identified in mouse and human intestinal inflammation. Dysregulations of the OSM-OSMR pathway in inflammatory bowel disease (IBD) lead to enhanced leukocyte recruitment and activation, tissue retention, and tissue remodeling through the modulation of the stromal cell function. The impact of OSM on other resident intestinal cells remains unexplored. We, therefore, assessed the influence of OSM on intestinal epithelial cells (IECs). We utilized a combination of in vitro and in vivo techniques to evaluate the role of OSM and OSMR in epithelial cell biology. In vitro investigations using a high-throughput approach showed that IL-22 and IFN-γ were central cytokines inducing OSMR in epithelial cells through the activation of STAT1. Moreover, in vivo blocking of IFN-γ and IL-22 showed a substantial reduction of OSMR on epithelial cells during colitis and a decrease in inflammatory gene signature. Furthermore, bulk RNA sequencing of inflamed mice treated with the anti-OSM blocking antibody showed a strong modulation of intestinal inflammation. Our results have shown that both IL-22 and IFN-γ are expressed during colitis and subsequently promote the induction of OSMR in IECs. Furthermore, blocking of OSM influences different resident cell types during inflammation. In addition, OSMR signaling is relevant in IECs as targeting it reduces intestinal inflammation. Therefore, we hypothesize that OSM might drive distinct transcriptional responses in various gut-resident cell populations. Thus, differential targeting of the OSM receptor might be a potential therapeutic approach in IBD.

### Extraintestinal roles of intestinal vitamin D receptor in protecting against dysbiosis and tumorigenesis in breast

Jun Sun – University of Illinois-Chicago, Jilei Zhang, Yinglin Xia, Yong-guo Zhang

Microbiota has critical role to regulate the function and health in intestine and extraintestinal organs. A fundamental question whether there is an intestinal-microbiome-breast axis during the development of breast cancer. If yes, what are the roles of host factors. Vitamin D receptor (VDR) involves host factors and human microbiome. Vdr gene variation shapes human microbiome and VDR deficiency leads to dysbiosis. We hypothesized that intestinal VDR protects mice against tumorigenesis in breast. We used an 7,12-dimethylbenzanthracene (DMBA)-induced breast cancer model in intestinal epithelial VDR knockout (VDR  $\Delta$ IEC) mice. We reported that VDR  $\Delta$ IEC mice with dysbiosis are more susceptible to breast cancer induced by DMBA. Intestinal and breast microbiota analysis showed that lacking VDR leads to bacterial profile shift from normal to susceptible carcinogenesis. We found enhanced bacterial staining within breast tumors. At the molecular and cellular levels, we identified the mechanisms by which intestinal epithelial VDR deficiency led to increased gut permeability, disrupted tight junctions, microbial translocation, and enhanced inflammation, thus increasing the tumor size and number in breast. Furthermore, treatment with beneficial bacterial metabolite butyrate or probiotics reduced the breast tumors, enhanced the tight junctions, and inhibited inflammation in the VDR  $\Delta$ IEC mice. Gut microbiome contribute to the pathogenesis of diseases, not only in the intestine, but also in the breast. Our study provides new insights into the mechanism by which intestinal VDR dysfunction and gut dysbiosis led to high risk of extraintestinal tumorigenesis. Gut-tumor-microbiome interactions indicate a new target in prevention and treatment of breast cancer.

## Faecal microbiota transplantation to Tackle Antimicrobial Resistance in Chronic Liver Disease - improves intestinal barrier function and modulates mucosal IL-17 immunity.

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Background : The World Health Organization states that Antimicrobial Resistance (AMR) is "the biggest threat to global health", causing 1.27Million deaths in 2019 globally, predicted to rise to 10million/year, by 2050. Patients with chronic liver

disease (CLD) are particularly susceptible to developing infections and AMR resulting in hospitalisation, organ failure and potential death. Susceptibility to infection results from gut-barrier-damage (GBD), microbiota dysbiosis, and translocation of bacteria and their products across the gut-epithelial-barrier. This translocation induces innate immune dysfunction. We hypothesised that modifying the gut microbiota with faecal microbiota transplant (FMT) may alter intestinal barrier function and mucosal immunity in CLD-patients. Methods: We conducted a prospective, randomised, single-blinded, feasibility trial evaluating FMT (n=15) against placebo (n=6) [NCT02862249]. Patients were administered FMT/placebo into the jejunum within 7 days of baseline. We assessed efficacy in modulating the patient's own microbiome and inflammatory status: stool was collected at baseline and 7, 30 and 90 post-FMT/placebo. Assessing cytokine production and barrier integrity markers (electrochemiluminescence/ELISA) and metabolite profile (1 H-NMR). Results: Administering FMT to CLD patients significantly reduced stool carriage of E.faecalis and AMR genes. Inflammation/GBD increases AMR-gene carriage; FMT reduced gut inflammation, whilst enhancing butyrate production (p =0.014) and restoration of gut barrier function. Particularly, reducing luminal IL-17A at day7 (mean - 68%, p =0.0037) in contrast with placebo, which increased 30days post-treatment (mean +36%). High IL-17A levels induce epithelial-cell tight-junction permeability. Conclusions: These data support FMT as playing an important role in enteric pathogen reduction, altering the gut-microbiota to promote inflammatory restoration of the gut barrier, and reducing AMR.

## The fate of Th17 cells in periodontitis

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Periodontitis is a Th17-mediated disease leading to local inflammation of the periodontal ligament and alveolar bone; it is also a risk factor for a plethora of systemic diseases. At sites of inflammation Th17 cells are notoriously unstable, adopting features of other T-helper lineages and losing IL17 expression. Here we aimed to investigate the stability of Th17 cells during periodontitis. Utilizing the ligature-induced model of periodontitis and IL17a Cre -R26R eYFP mice, we show that cytokine production from Th17 cells was stable during periodontitis, with the bulk of YFP + -Th17 cells continuing to

produce only IL17 during peak disease. However, in the lymph node draining the gingiva, a subset of Th17 cells exhibited distinct plasticity, adopting a T follicular helper (Tfh) cell fate. Converted YFP + Tfh cells no longer produced IL17 and instead expressed Bcl6, the central regulator of Tfh cells. Immunofluorescence revealed that in periodontitis a subset of YFP + Th17 cells in the gingival-draining lymph node were located within the germinal centre, suggesting their interaction with germinal centre B cells. Conversion of Th17 to a Tfh phenotype during pathological inflammation was not seen during other Th17-mediated pathologies including Antigen-induced arthritis (joint), *Citrobacter rodentium* infection (gastrointestinal tract) or Imiquimod-induced psoriasis (skin); indicating that in periodontitis the gingiva-draining lymph node is a unique environment. Exploring the factors driving this conversion we demonstrate an important role for IL-6, but not TGF $\beta$ . We are now utilizing Bcl6f/f-IL17 eYFP mice to explore the impact Th17-Tfh cells have on disease pathology. Combined our data will define previously unappreciated Th17 plasticity during IL17-driven disease.

### Fatty acid overproduction by gut commensals exacerbates metabolic abnormalities

Tadashi Takeuchi - RIKEN, Keishi Kameyama, Eiji Miyauchi, Yumiko Nakanishi, Takashi Kanaya, Hiroshi Ohno

Although recent studies have revealed the important roles of gut microbiota in metabolic abnormalities such as obesity, the mechanisms by which these microbes aggravate the disease progression have not been fully elucidated. Several studies have documented that carbohydrate and amino acid metabolites derived by gut microbiota affect the host metabolism, highlighting the impact of diet-microbe interactions in these diseases. Since dietary fat is also involved in obesity and diabetes, we speculated that gut microbiota may also modulate the effects of dietary fat on the host metabolism. We here report that a commensal species highly colonized in both diabetic humans and mice produces abundant fatty acids to exacerbate metabolic abnormalities. *Fusimonas intestini* (FI), a novel strain of Lachnospiraceae isolated from db mice, substantially aggravated body mass gain and insulin resistance in gnotobiotic mice fed a high-fat diet. Metabolome analyses revealed that FI particularly increased a trans-unsaturated fatty acid in both mice and bacterial culture, which appeared to be converted from dietary fat. High fat intake

altered the expression of fatty acid metabolism regulator FadR in FI, and monoclonization with a FadR-overexpressing *E. coli* partly recapitulated the metabolic phenotypes. Finally, we confirmed that FI and the microbe-derived fatty acids impaired intestinal epithelial integrity in vivo and in vitro, and that these fatty acids indeed aggravated insulin resistance in db mice. Together, our study shows a novel mechanistic insight into the host-microbe relationship, where the overproduction of fatty acid by the gut commensals exacerbates metabolic abnormalities.

### Fermented Food-Derived Bacterial Metabolites Participate In A Transkingdom Metabolic Network To Regulate Intestinal Immunity

Sean Spencer - TBA, Evelyn Lemus, Elisa Caffrey, Matthew Olm, Hannah Wastyk, William Van Treuren, Erica Sonnenberg, Justin Sonnenburg

Fermented foods are ancient and ubiquitous—likely consumed in nearly all known cultures over the last 10,000 years. Increased consumption of fermented foods (e.g., yogurt, kombucha, kimchi, sauerkraut) was shown in a nutritional intervention clinical trial to increase intestinal microbiota diversity and reduce serum inflammatory markers (Wastyk et al, Cell 2021). We defined the chemical space of fermented foods consumed by trial participants using a bacterial metabolite-focused mass spectrophotometry (LC-MS) pipeline (Han et al, Nature 2021), in combination with short chain fatty acid (SCFA) quantification to find that lactate and acetate are abundant in fermented foods (~80-200mM). We colonized germ free mice with trial participant stool and administered fermented vegetable brine to test if we could recapitulate reduced inflammation observed in the clinical trial. Indeed, we observed an immunoregulatory shift in the small intestine lamina propria of mice given fermented food, with increased Foxp3+ T-regulatory cells and IgA+ Plasma cells. By separating the microbial and chemical fractions of fermented vegetable brine, we found that bacterial metabolites, without live bacteria, conferred a similar immunoregulatory shift. Finally, we found that lactate alone, recapitulated the immune effects of whole fermented food. Lactate is produced and used by both host and bacterial metabolism, with consumption of fermented foods leading to a transkingdom metabolic network in small intestinal tissue, in which bacterial derived lactate dominantly influences small intestinal immunity via cell type-



specific immunometabolic alterations. This study highlights the potential for bacterial metabolite-based therapies targeting the small intestinal microbiota-immune metabolic axis to regulate inflammation.

### **Flagella and indole produced by commensal bacteria protect the intestinal barrier to prevent food allergy**

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Lifestyle-induced changes to the diversity of the commensal microbiota have been causally linked to the increasing prevalence of food allergies and other non-communicable chronic diseases. We have shown that bacteria from the Clostridia class prevent an allergic response to food by eliciting an IL-22 dependent barrier protective response that limits allergen access to the systemic circulation. We have now examined the mechanisms by which commensal Clostridia induce this allergy-protective effect. We identified taxa in a consortium of Clostridia that possess flagella and produce indole, which are ligands for TLR5 and AhR, respectively. Lysates and flagella isolated from this consortium induced IL-22 in mouse intestinal explants. IL-22 was not induced in explants from mice in which TLR5 or MyD88 was knocked out globally or conditionally in CD11c + cells. Treatment with the commensal flagellar isolate also reduced detection of intragastrically administered FITC dextran in the serum of antibiotic-treated mice. Similarly, indole exposure induced IL-22 in intestinal explants and reduced intestinal permeability to FITC dextran. Importantly, AhR signaling in ROR $\gamma$ t + cells was necessary for IL-22 induction by flagella. These results suggest that flagella and indole act synergistically to prevent an allergic response. Finally, we have isolated and characterized two Clostridial taxa which bear flagella and produce indole. We hypothesize that germ-free mice colonized with these two taxa will exhibit improved IL-22 dependent barrier function and be protected against an allergic response. Our work reveals novel features of Clostridia key to their allergy-protective capability which may be further exploited to develop therapeutics.

### **Functional metabolomics of the asthma airway identifies L-tryptophan as a biomarker and mediator of neutrophilic asthma**

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Asthma is the most common chronic airways disease worldwide and the severe subtype of asthma is responsible for the majority of hospitalizations and healthcare costs. Asthma is a heterogenous disease and can be characterized both by classical allergic Type 2/eosinophilic infiltrates in the airways as well as a neutrophilic phenotype with a dominant mixture of Type 1 and Type 17 cytokines in airway tissue, sputum, and bronchoalveolar lavage. Severe neutrophilic asthma (NA) currently has no effective treatment as these patients are refractory to inhaled glucocorticosteroids and ineligible for monoclonal antibody therapies. Using a global metabolomics screen of bronchoalveolar lavage fluid across 2 independent cohorts (n=260) we have identified the asthma airway metabolome and a specific NA signature uniquely characterized by high levels of airway L-tryptophan (Tryp). Blood-derived neutrophils treated with Tryp demonstrate increased barrier chemotaxis in vitro suggesting a plausible functional role for Tryp in NA. In vivo inhaled Tryp administered during a house dust mite model of asthma led to increased airway neutrophilia, steroid-resistant airway-hyperresponsiveness, goblet cell metaplasia, and thus more severe disease. These findings re-capitulate many of the hallmark features of human NA. Treatment with epacadostat reversed many of these features offering a potential therapeutic candidate. This study identifies the localized airway metabolome of asthma, reveals a conserved dysregulation of metabolites in NA defined by high Tryp, and identifies a key functional role for Tryp in the promotion of both airway neutrophilia and steroid-resistant disease severity. Tryp may represent both a novel biomarker and therapeutic target in this difficult to treat subtype of asthma.

### **GATA4 regionalizes intestinal metabolism and bacterial colonization to prevent immunopathology**

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The mechanisms how tissues regionalize metabolism and immunity to maintain homeostasis are unknown. Here we show that the intestinal epithelial transcription factor GATA4 promotes regionalization of metabolism, immunity, and the microbiome between the proximal and distal small intestine. GATA4 prevented adherent bacterial colonization and inflammation in the proximal small intestine by regulating retinol metabolism and luminal IgA. In the absence of epithelial GATA4, the regionalization between the jejunum and ileum was lost. This altered the colonization of both commensals, segmented filamentous bacteria (SFB), and pathogens, *Citrobacter rodentium* from the distal to proximal small intestine to promote pathogenic inflammation. This altered regionalization of *C. rodentium* led to severe mortality of GATA4 deficient hosts which was dependent on TNF $\alpha$  induced immunopathology driven by SFB. Furthermore, we show in active celiac patients with villous atrophy, low GATA4 expression was associated with regional metabolic defects, mucosal *Actinobacillus*, and increased IL-17 immunity. This study reveals a critical role of GATA4 regulated intestinal regionalization in homeostasis and disease.

## Gut microbiota modulates lung immunity and improves ability to cope with influenza virus infection

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Probiotics play a critical role in modulation of immune responses locally and systemically. Recent studies proposed cross-talk between gut microbiota and lung immunity, referred to as gut-lung axis. However, their concrete evidences and mechanisms have not been elucidated. To determine whether probiotics influence gut-lung axis, we firstly screened *Lactobacillus* sp. which augmented type I interferon (IFN) signal using IFN  $\alpha/\beta$  reporter cell line. We picked up *Lactobacillus* (L.) *paracasei* strain (MI29) from feces of health volunteers which enhanced predominant levels IFN $\alpha/b$  in vitro. Oral administration of selected L. *paracasei* to wild-type B6 mice for two weeks resulted in increased expressions of IFN-stimulated genes (e.g., *Oas1*, *Cxcl10*, *Ifit1*) and pro-inflammatory cytokine (e.g., *Il1b*, *Il6*, *Il18*) in the lung. We detected significantly increased numbers of CD11c + PDCA-1 + plasmacytoid dendritic cells and Ly6C hi monocytes in the lung of L. *paracasei* -treated mice compared with in PBS-

treated mice. Importantly, pre-treatment with L. *paracasei* for two weeks showed less weight loss and viral loads in the lung after sub-lethal dose of influenza virus infection. Furthermore, gene ontology pathways enriched in the transcriptome of lung tissues revealed upregulation of genes related to activation of defense immune responses. These findings suggest that our newly isolated L. *paracasei* strain help host defense immunity to prevent symptoms of infections caused by influenza virus.

## Gut microorganisms induce Peyer's patch-dependent maternal IgA production in milk

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Promoting breastfeeding is of great importance to public health and offers multiple benefits for both mothers and newborns. Maternal antibodies, transferred via breastfeeding, protect the gastrointestinal tracts of neonates from several infections caused by pathogens. However, the molecular mechanism through which maternal antibodies, especially IgA, are produced in mothers during lactation is not completely understood; hence, the immunological and microbiological approach to increasing breastfeeding quality for newborn health has not yet been discovered. The aim of our research was to identify the source and the immune modulator that promote maternal IgA production in milk. Our goal is to develop an ultimate immune strategy that contributes to public health by increasing the amount of maternal IgA in milk. We demonstrated that an interorgan network between the mammary glands and the small intestine in mothers is essential for maternal antibody transfer to the newborns via breastfeeding. Specifically, Peyer's patches (PP), which are a primary immune organ in the gastrointestinal tract, play a key role in producing maternal IgA in milk. Moreover, we succeeded in identifying that *Bacteroides acidifaciens* and *Prevotella buccalis*, both of which cohabit in the gastrointestinal tract of mothers, facilitate the induction of immune responses in PP to promote maternal IgA transfer to the newborns. These results provide significant insights into the development of probiotics, as well as vaccine carriers of

maternal immunization, that can be used to transfer sufficient amounts of maternal antibodies with meaningful specificity that contributes to public health.

### Heat stress affects the function and structure of cecal tonsils in broiler chickens

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Global warming causes environmental heat stress (HS), which adversely affects the growth and health of various animals. Among them, birds are highly susceptible to HS because they lack the ability to dissipate body heat due to the absence of sweat glands. Therefore, it is supposed that HS may also influence on the mucosal immune function, which affects their health. Using broiler chickens exposed to chronic HS condition (34.5 °C) for 2 weeks, we here demonstrated the influence of HS on the intestinal immune system, especially in the cecal tonsils. Specifically, HS induced the atrophy of the cecal tonsils. The numbers of mature B cells and T cells including both CD4 + and CD8 + cells depressed significantly under the HS conditions. Consistently, the expression levels of CXCL13 and CCL19, which promote B and T cell recruitment into the cecal tonsils were both reduced significantly in the cecal tonsils in the HS conditions. Importantly, HS caused hypoplasia of follicular regions of cecal tonsils, thus preventing B cell proliferation and differentiation into IgA + plasmablasts. In contrast, the number of fully differentiated plasma cells (PCs) was maintained adequately in intestinal lamina propria even under the HS conditions. Importantly, the levels of IgM, IgA and IgY in the gut of heat-stressed chickens were sustained sufficiently at same level as those of thermoneutral chickens. Our findings suggest that plasma cells (not mature B cells) may be a potential target for maintaining a functional intestinal immune system, such as antibody production in the gut, under the HS conditions.

### Helicobacter pylori induces cag-PAI dependent expression of immune checkpoints in gastric cancer cells

Lucia Figueroa-Protti - University of Costa Rica, Silvia Molina-Castro, Javier Mora, Warner Alpizar-Alpizar

Helicobacter pylori colonization of the gastric mucosa, in combination with environmental factors ( i.e. diet and lifestyle), trigger a sustained inflammatory reaction resulting in chronic gastritis that, in a subset of patients, may progress through a stepwise process that results in invasive adenocarcinoma. One of the most important H. pylori virulence factors that is associated with this clinical outcome is cytotoxin-associated gene A (CagA), which is located at the cag pathogenicity island (cag-PAI). Tumor microenvironment is characterized by an increase in the amount and diversity of inhibitory receptors, so-called immune checkpoints (ICs), which avert T cells from getting properly activated and, thus, dampen an effective anti-tumor immune response. The paramount role of H. pylori in the development of chronic inflammation and gastric carcinogenesis, prompted us to investigate the mRNA expression levels of ICs ligands in gastric cancer cell lines when co-cultured with H. pylori . We found that PD-L1, TNFRSF14, PVR, and ICOSLG were significantly upregulated at mRNA levels compared to unchallenged cells. Interestingly, this induction was absent when cells were exposed to a cag-PAI negative H. pylori strain. These results suggest that cag-PAI positive H. pylori strains can directly induce the expression of ICs ligands in gastric epithelial cells very early in gastric carcinogenesis, which may eventually lead to the creation of a suppressive immune microenvironment thus facilitating cancer development and progression. Studies on a mouse model of H. pylori -induced gastritis are ongoing to support these findings. Key words: immune checkpoints, Helicobacter pylori , gastric mucosa, gastric adenocarcinoma, cag-PAI.

### Helminth-Induced IFN $\gamma$ Remodels the Intestinal Epithelium to Compromise Host Resistance

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Repair of the intestinal epithelial barrier is a dynamic process involving the intestinal stem cell (ISC) niche and local immune responses. The intestinal helminth Heligomosomoides polygyrus bakeri ( Hpb ) disrupts the epithelial monolayer resulting in a robust IFN $\gamma$ -mediated type 1 immune response during the tissue-invasive phase of infection. While we have previously shown that IFN $\gamma$  production promotes disease tolerance to Hpb infection, it remains unclear how a type 1 immune response is orchestrated and its relevance to host

resistance. Here we hypothesize that an early type 1 immune response to Hpb promotes disease tolerance at the expense of compromising host resistance to infection. Using a combination of cell transfer, bone marrow chimera and gnotobiotic approaches, we identify a tissue-resident CD8 T cell population that secretes IFN $\gamma$  following breach of the epithelial wall by Hpb larvae. Notably, activation of intestinal CD8 $^{+}$  T cells occurs in an antigen-independent manner but requires exposure to the commensal microbiota. These events coincide with a loss of proliferating homeostatic Lgr5 $^{+}$  ISC responsible for epithelial repair. Deletion of IFN $\gamma$  receptor signaling results in accelerated regeneration of the Lgr5 $^{+}$  ISC compartment, elevated numbers of mucus-producing goblet cells and reduced parasite burden. Together, our results indicate that Hpb exploits a gut microbiota-dependent type 1 immune response to alter the epithelial "weep and sweep" response and facilitate chronic infection.

## HIV interactions with colorectal macrophages in early infection

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New HIV infections remain a significant global health issue despite antiretroviral therapy and pre-exposure prophylactics helping to reduce transmission rates. In Australia, as in most of the developed world, most new infections occur via anal intercourse. Despite this, little is known of the early events that follow HIV entry into the colorectal mucosa, particularly the role that macrophages play in early infection. This constitutes a fundamental gap in our knowledge of early HIV targets and understanding HIV-macrophage interactions may provide a basis for macrophage-specific HIV studies as well as microbicide design. Utilising extensive collaborations with colorectal surgeons, human colorectal tissue was digested, and liberated cells subjected to high-parameter flow cytometry. Four distinct subsets of macrophages were found in the colorectum, confirming what was recently defined in human small intestine. Moreover, we have shown that these macrophage subsets have varied expression of key HIV binding receptors. These subsets were isolated utilising FACS sorting to investigate their ex vivo HIV interactions. Interestingly, these macrophage subsets take up significantly differing amounts of HIV 2-hours post-exposure, indicating that early HIV interactions are subset specific. The subsets also

demonstrated differing ability to transfer HIV to CD4 T cells, though all can significantly enhance the infection of CD4 T cells. By further investigating the uptake mechanism and productive infection in these macrophage subsets, we can better understand how these subsets are contributing to early infection in the colorectum, further characterising HIV interactions at this critical transmission site.

## Hookworm-derived pro-tolerogenic compounds to prevent allergy onset in neonates

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Nearly a billion-people suffer from asthma, resulting in considerable morbidity and poor quality of life. With the highest prevalence in the world, the cost of allergic diseases in Australia is over \$28 billion per year. Specific bacterial species and low microbial diversity have been associated with allergy. There are no preventative modalities to date, and treatment do not address the aetiology of the disease. The human immune system has co-evolved together with parasites, and helminths in particular, inhabiting the gut. The "old friend" hypothesis suggests that gastrointestinal parasites contribute to immune education by providing essential stimuli in early childhood that allow for the development of tolerance. A defect in immune regulation, and regulatory (Treg) cells in particular, is one of the major factors that contribute to asthma onset. We have identified a hookworm anti-inflammatory protein (AIP)-2 that promotes Treg development in vivo. We have shown that the protein targets key regulatory players in the gut-draining lymph nodes that involves a specific subtype of dendritic cells, conventional dendritic cells type (cDC)-1. AIP-2 modifies mesenteric-cDC1 function in that it enhances the proportion of Tregs trafficking to mucosal sites. When administered to neonate animals, the protein induces a state of tolerance that persists into adulthood and prevents the onset of asthma. Preliminary evidence suggests that this mechanism is underpinned by gut microbiome-immune crosstalk and a long lasting "imprint" on mesenteric cDC-1 cells.

## Host-microbe interactions during colorectal cancer development using transgenic mouse models



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Colorectal cancer (CRC) is one of the most prevalent cancers in Western society, and increasing evidence indicates a strong contribution of environmental factors and the microbiota to CRC development. Several bacteria are identified as CRC-driving bugs, or 'oncobacteria', including *Fusobacterium nucleatum* and adhesive invasive *E.coli* (AIEC) strains. In healthy conditions, goblet cells are essential to establish a protective mucus layer which prevents excessive bacterial-epithelial interactions, chronic inflammation and cancer development. To study host-microbiota interactions in conditions of mucosal barrier defects, we generated several unique transgenic mouse models. First, we generated mice with intestinal epithelial specific expression of the EMT-regulator Zeb2 (Zeb2 IEC-Tg mice), which develop spontaneous microbiota dependent invasive CRC (Slowicka et al., Nature Cancer). Using gnotobiotic Zeb2 IEC-Tg mice, we are now studying CRC-promoting functions of *F. nucleatum* and different AIEC strains. In addition, we generated a new goblet-cell specific Cre line (Muc2Cre), which enables cell specific gene targeting. Using diphtheria-toxin based depletion models, we can transiently or constitutively delete intestinal goblet cells and the mucus layer, and study epithelial-bacterial interactions in mucus deprived conditions. In addition, we generated a goblet cell reporter line (Muc2YFP mice), and performed single-cell RNA sequencing analysis on sorted goblet cells in various physiological conditions (Trichuris muris, *C. rodentium* infection, DSS colitis and AOM/DSS CRC). Using this approach, we describe dynamics in different goblet cell subsets during inflammation, infection and cancer. Together, using unique transgenic mouse models and germ-free mouse technology, we study the molecular mechanisms underlying complex host-microbe interactions during the development of colorectal cancer.

## Human intestinal CD103+SIRPα+ conventional dendritic cells from patients with Crohn's disease prime IL-17+ T-cells.

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Although human intestinal conventional dendritic cells (cDC) maintain the balance between immunity and tolerance, their function in the human gut in Crohn's disease (CD) is scarce. Hence, tissue resections from patients with ileocolonic CD and controls (healthy tissue from patients with proximal colonic cancer) were obtained, being subsequently enzymatically digested. Total intestinal cDC were identified within singlet viable leukocytes as CD14-CD64-HLA-DR+CD11c+. Three cDC subsets were sorted based on the expression of CD103 and SIRPα (CD103-SIRPα+; CD103+SIRPα+; CD103+SIRPα-) while total macrophages were sorted as CD14+CD64+HLA-DR+. cDC subsets and macrophages were further cultured with allogeneic CD4+ naïve T-cells. All 3 intestinal cDC subsets from controls stimulated naïve T-cells as opposed to intestinal macrophages. Indeed, cDC stimulated T-cells produced IL-10 and co-expressed FoxP3, being this capacity increased in both CD103+ subsets (CD103+SIRPα- and CD103+SIRPα+). Moreover, all three ileal cDC subsets stimulated T-cells more efficiently than their paired colonic counterparts, although the acquired T-cell profile did not differ among tissues. None of the cDC subsets, on the contrary, induced the expression of IFNγ or IL-17 on the stimulated T-cells. Referred to CD patients, all three colonic cDC subsets were more stimulatory than their counterparts from the control tissue, while CD103+SIRPα+ cDC from the inflamed ileum primed the generation of IL-17+ responding T-cells suggesting that their regulatory functions can be reshaped by the pro-inflammatory tissue microenvironment in CD.

## Human skin tissue-resident virus-specific memory T cells after shingles

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Herpes zoster (HZ) skin rash is due to varicella zoster virus (VZV) reactivation. We hypothesized that virus-specific T cells localize to HZ lesions and that tissue-resident memory T cells (T<sub>RM</sub>) persist in the skin after viral clearance. Rash site and contralateral skin biopsies and blood from 17

immunocompetent subjects with HZ were collected 45 days after rash onset and at serial timepoints over the following year. VZV DNA at the rash site and blood VZV-specific T and B cell responses declined over time. T cells in HZ skin showed classical T RM CD69 and CD103 phenotypes, with decreasing cytotoxicity (TIA-1) and increasing regulatory (FoxP3) marker trends over time. VZV-specific T cells preferentially localized to VZV DNA-positive skin and remained resident in healed HZ skin after viral clearance for up to 500 days. Specific CD8 T RM were more frequently detected than CD4 T RM. Unbiased proteome-wide assays revealed VZV ORF9 as a population-prevalent skin CD8 T RM antigen across diverse HLA alleles. VZV-specific skin CD8 T RM target specificity was consistent over time within-subject. Virus-specific T RM persistence was validated at the clonotype level by TCR $\beta$  CDR3 sequencing and tetramer staining. Quantitative CDR3 analyses showed that up to 25% of skin T cells were VZV-specific. In summary, HZ attracts and retains abundant, clonotypically stable, virus-specific T cells in the skin.

### **The human small intestine contains two subsets of regulatory Foxp3<sup>+</sup> CD4<sup>+</sup> T cells with very different lifespan and functional properties.**

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Gut resident regulatory Foxp3<sup>+</sup> CD4 T (Tregs) cells have been shown to play an important role in suppressing immune responses against harmless dietary antigens and members of the microbiota in mice. However, information about the phenotype and function of Tregs in the human gut is limited. Here, we characterize Foxp3<sup>+</sup> CD4 Tregs in human small intestine (SI). SI Foxp3<sup>+</sup> CD4 T cells were CD45RA<sup>-</sup>CTLA4<sup>+</sup>CD127<sup>-</sup> and suppressed proliferation of autologous T cells in vitro. In normal SI, Tregs constituted only on average 2.4% of all CD4<sup>+</sup> T cells, and of these approximately 60% expressed the transcription factor Helios. In inflamed SI from active celiac disease patients, both Treg subsets were similarly increased 5-10-fold. However, Helios<sup>-</sup> Tregs produced IL-17, IFN- $\gamma$  and IL-10 upon stimulation, whereas Helios<sup>+</sup> Tregs produced very low levels of these cytokines. In human SI

transplants, donor Helios<sup>+</sup> Tregs rapidly disappeared, whereas Helios<sup>-</sup> Tregs persisted for at least 1 year after transplantation. Taken together, these findings show that human gut contains two phenotypically and functionally distinct Treg subsets, which resemble the rapidly renewed dietary antigen-specific RoRyt<sup>-</sup> Tregs and the resident microbiota-specific Tregs resident RoRyt<sup>+</sup> Tregs in mice.

### **Human urothelial organoid: A model to study host-uropathogen interaction**

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The urinary tract is constantly exposed to microorganisms but can usually resist infection. Protection from microbial colonisation is achieved by resident immune cells, and physical barriers. The lower urinary tract is lined by the urothelium, consisting of several layers of stratified epithelial cells forming the first line of defence. As the urothelium needs to act as a constant permeability barrier to protect the underlying tissues from toxic urinary compounds, the urinary tract immune responses need to be rapid but contained to maintain barrier integrity and structure. UTIs are caused by a wide range of pathogens. Although UPEC has been identified as the most prevalent agent, *E. faecalis* is the frequent cause of UTI in hospitalised patients. The aim of this study is to develop and characterise an improved urine-tolerant human urothelial organoid model and to elucidate the mechanisms by which *E. faecalis* and UPEC interact with this model. The expression of UP-1A, -1I, -1III, and CK-13, -20 was detected by immunofluorescence staining. The model was further examined for the expression of tight and adherens junctions. The paracellular permeability and barrier integrity were assessed by FITC-dextran and TEER, pre- and post-infection. Taken together, these data suggest that the bladder organoids display very similar structural and physiological features compared with human bladder urothelium. The host-pathogen interaction was investigated by confocal-microscopy indicating the infection mechanisms can be species/strain-dependent. The innate immune response was assessed by measuring expressed cytokines/chemokines. Our work supports the use of this model to elucidate UTI host-pathogen interactions in vitro.

### IBD Gene Rnf186 Regulates Intestinal Homeostasis by Targeting Paneth Cells

Yu-Wen Chen - Academia Sinica, Hung-Yu Chiang, Jr-Wen Shui

Paneth cells, at the crypt base, provide key niche factors to support nearby stem cells and regulate crypt cell remodeling. Clinically, dysfunction of Paneth cells is associated with inflammatory bowel disease (IBD) and is linked to extra-intestinal manifestations (EIM) in patients. RING finger protein 186 (RNF186), an E3 ubiquitin-protein ligase, is reported as a risk gene for IBD and is known to regulate basal autophagy in intestinal epithelial cells by genetic deletion studies. However, the cellular and molecular targets of RNF186 in the gut have not been revealed. As we found Rnf186 mRNA is highly expressed in sorted Paneth cells, we therefore hypothesize that RNF186 might regulate autophagy-active Paneth cells and the nearby stem-cell niche, which are crucial to maintaining intestine homeostasis. Using Paneth cell-specific Rnf186 conditional knock-out mice ( Defa6-Cre + Rnf186 f/f ) and primary ileum organoids, we found CD24 + Lysozyme-producing Paneth cells are significantly reduced in the absence of RNF186, leading to autophagy blockade, increased ER stress, and apoptosis. Unexpectedly, at the age of 4-month old, Defa6-Cre + Rnf186 f/f mice developed spontaneous fatty liver, which is likely caused by Paneth-cell dysfunction and disrupted intestinal homeostasis. In organoids and fresh crypts, anti-microbial MMP7 and  $\alpha$ -defensins were aberrantly upregulated in the absence of Paneth-cell RNF186, which likely contribute to microbiota dysbiosis and loss of tight junctions. Collectively, our results reveal a crucial role for RNF186 in Paneth cells in maintaining gut homeostasis and preventing non-alcoholic steatohepatitis, a pronounced EIM in IBD patients, via a gut-to-liver axis.

### Identification of HIV Transmitting Mononuclear Phagocytes in Human Anogenital Tissues

Jake Rhodes - Westmead Institute for Medical Research, Rachel Botting, Kirstie Bertam, Hafsa Rana, Erica Vine, Heeva Baharlou, Anthony Cunningham, Andrew Harman

When an invading pathogen penetrates the physical barriers of mucosal surfaces the first cellular arm of the immune system it interacts with are mononuclear phagocytes (MNP). In tissue

these are comprised of Langerhans cells (LC) dendritic cells (DC) and macrophages. Despite this, very little is known about the exact role MNPs play in the early events of sexual transmission of HIV. Our lab has access to all the human anogenital and colorectal tissues that HIV may encounter: labia, vagina, cervix, glans penis, foreskin, penile urethra, perineum, anus, rectum and colon. Using our labs highly optimised tissue processing/isolation protocols alongside high parameter flow cytometry, we were able to define all tissue MNP subsets, including two previously undefined epithelial subsets; CD11c + DC and CD33 low MNP. We then went on to fully define the pathogen binding lectin receptor profiles at the transcriptional and cell surface expression levels of all identified subsets. Following this we characterised how each of these cells interact with HIV and identified three key HIV target cells; epithelial CD11c + DC, sub-epithelial langerin + cDC2 and sub-epithelial CD14 + CD11c + monocyte derived dendritic cells. We showed these cells were more efficient at HIV uptake, supported higher levels of productive infection and transferred the virus to CD4 T cells more efficiently compared to other tissue MNPs. Furthermore, these cells were shown to be significantly enriched in anogenital tissues and thus are likely to be preferential HIV target cells that may play a key role in HIV transmission. Identifying the initial HIV target cells will be essential for guiding the development of a vaccine and better PrEP regimens, to aid in putting an end to the global HIV pandemic.

### Identifying colorectal cancer-promoting microbiotas and their mechanisms of action

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An altered microbiome composition has been associated with a variety of diseases, including inflammatory bowel disease (IBD) and colorectal cancer (CRC). However, the link between the gut microbiome, inflammation, and tumorigenesis remains incompletely characterized, as many gut microbiota strains have not been evaluated for their ability to influence tumorigenesis. To better elucidate causal links between gut microbes and disease state, we colonized germ-free (GF) CRC-susceptible mice ( Apc Min/+ ;IL10 -/- ) with human donor microbiota from 5 CRC, 4 IBD, and 7 healthy donors (HDs). We found the number of tumors in the colon of Apc Min/+ ;IL10 -/- mice varied significantly by donor microbiota and was

correlated with intestinal inflammation, as measured by elevation of fecal lipocalin 2. Intestinal microbial diversity remained stable across time, suggesting tumorigenesis was mediated through the complex microbiome present at the time of colonization. We hypothesized that a small subset of strains in the tumorigenic microbiotas was driving tumorigenesis via DNA damage. We screened 80 individual strains from tumorigenic and non-tumorigenic human gut cultured communities for potential genotoxic effects (i.e., DNA double-strand breaks) using flow cytometry. We found several unique bacterial strains in the tumorigenic microbiotas consistently induced DNA damage (histone H2AX phosphorylation) when co-cultured with a human colorectal adenocarcinoma cell line, and we are testing their tumorigenic capacity in vivo. Across our in vivo and in vitro screens, we identified tumorigenic microbial communities and strains not previously associated with tumorigenesis. These data suggest that the tumorigenic potential of the gut microbiota may be influenced by previously unexplored bacteria.

### **IgA B cell receptor signaling regulates Peyer's patches germinal center competition by protecting from Fas counterselection**

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Immunoglobulin A (IgA) is the major antibody isotype in the intestine: IgA deficiency reduces tissue response to oral vaccine, increases enteric pathogen susceptibility, and alters microbiome composition. IgA can bind multiple unrelated bacterial taxa, but mechanistic insights into the generation of this cross-reactivity is lacking. IgA B cells arise during the germinal center (GC) reaction in Peyer's patches: however, the role of IgA B cell receptor (BCR) in shaping the GC response and controlling humoral response at the intestinal interface remains unknown. Here we showed during the GC reaction, B cells lacking IgA are outcompeted by IgA+ B cells in Peyer's patches and were unable to contribute to memory B cell and intestinal-homing plasma cell compartments. IgA BCR did not influence cell proliferation, and Bcl-2 overexpression did not rescue IgA-deficient GC B cells in mixed bone marrow chimeras. In contrast, IgA-deficient GC B cells underwent increased apoptosis in the light zone. Supporting these

findings, we observed that IgA BCR drove faster and stronger intracellular Ca<sup>2+</sup> signaling and increased BCR-dependent phosphorylation events. Mechanistically, IgA BCR signaling conferred resistance to Fas ligand (FasL)-dependent cell death in vitro, and genetic inhibition of Fas-FasL pathway restored IgA-deficient B cell fitness during GC reaction in vivo. Thus, our results showed that IgA-expressing GC B cells are protected from FasL counterselection in the light zone via enhanced BCR intracellular signaling. This process may allow low affinity B cell clones to participate to the GC reaction and assure a comprehensive antibody response to poorly immunogenic commensal species.

### **IL-13 Receptor $\alpha 1$ regulates colonic homeostasis and inflammation**

Yaara Gordon - TAU, Shmulik Avlas, Avishay Dolitzky, Hadar Reichman, Michal Itan, Prof. Ariel Munitz

Rationale: Colonic inflammation is a key characteristic and therapeutic target in IBD. Interleukin (IL)-13 is a hallmark cytokine of "type 2 immunity" that is associated with tissue repair. IL-13 signals through IL-13 Receptor  $\alpha 1$  (IL-13Ra1), which is expressed by hematopoietic and non-hematopoietic cells. The IL-13-IL-13Ra1 signaling axis has a key role in allergic inflammation, nonetheless, whether IL-13 and/or IL-13Ra1 have a role in colonic inflammation, is unclear. Methods: DSS was administered to wild type (WT) and Il13ra1<sup>-/-</sup> mice for five days. Thereafter, DSS was replaced with regular drinking water. Mice were monitored for their weight and disease activity index (DAI), and their colons were taken for histopathology. Colonic RNA was obtained for RNA sequencing. Results: DSS treatment induced significant weight loss in WT and Il13ra1<sup>-/-</sup> mice. By day 11, Il13ra1<sup>-/-</sup> mice lost more weight than WT mice (% Weight loss in Il13ra1<sup>-/-</sup> vs WT). In addition, DSS-treated Il13ra1<sup>-/-</sup> mice displayed significantly decreased survival rates when compared to DSS-treated WT mice (68% vs 95%). Histopathological analysis revealed that Il13ra1<sup>-/-</sup> mice exhibited increased infiltration of immune cells, increased epithelial cell erosion and decreased cell proliferation in comparison with WT mice. Global RNA sequencing analysis of control and DSS-treated mice revealed that under baseline conditions Il13ra1<sup>-/-</sup> mice display reduced expression of 326 genes responsible for innate immune responses, cell death and cell cycle process including Ang4, Ccl8 etc. Conclusions: These data highlight Il13ra1 is a key



counter regulator of colonic homeostasis with important protective roles in settings of colonic inflammation.

### IL-1RAcP: A Central Mediator in Mucosal Candida Immunity

James Griffiths - King's College London, Julian Naglik

Fungal pathogens represent a severe disease burden and kill ~1.5 million individuals per year. *Candida albicans* is one of the most widespread human fungal pathogens and causes superficial mucosal and life-threatening systemic infections. Candida disease (candidiasis) typically arises from the mucosa when commensal yeast switch to invasive hyphae and produce the fungal toxin candidalysin, which damages host cells, drives immune responses and promotes infection. Candidalysin is central to inducing epithelial IL-1 family signalling (IL-1 $\alpha$ , IL-1 $\beta$  and IL-36 $\gamma$ ) which results in potent Th17 immunity and neutrophil recruitment. These responses are largely host-protective in oral and gut candidiasis; however, drive excessive inflammation and disease in vaginal candidiasis. Additionally, aberrant IL-1 family signalling is strongly implicated in asthma, COPD, IBD and psoriasis. Whilst the induction of IL-1 family members during candidiasis is clear, their function mediating immunity at different mucosal sites requires investigation. In this study, we use an in vivo model lacking IL-1RAcP, the adaptor protein required for IL-1, IL-33 and IL-36 signalling, in mucosal models of candidiasis. We show that during oral candidiasis IL-1RAcP-deficient mice are unable to drive key early cytokines and chemokines resulting in severely reduced IL-17 and IL-23 signalling and impaired Th17 immunity. This leads to poorly controlled *Candida* growth, invasive candidiasis, and high susceptibility to an otherwise non-lethal mucosal *Candida* challenge. Here, we identify IL-1RAcP as a central mediator of oral mucosal immunity promoting crucial Th17 immunity, fungal clearance and ensuring barrier protection. Modulating IL-1 family members to promote mucosal immunity and barrier protection would likely have huge therapeutic potential.

### IL-22 initiates an IL-18-dependent epithelial response circuit to enforce intestinal host defense

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High prevalence of ileal adherent-invasive *Escherichia coli* (AIEC) is frequently associated with Crohn's disease. IL-22, a key barrier keeper in the gut and a promising target for IBD therapy, is indispensable for host defense and new evidence show IL-22 induces epithelial IL-18 during intestinal infection. However, the definitive targets for IL-22-IL-18 axis in epithelial barrier have not been revealed. Here, we explore the response circuit of IL-22 and IL-18 during intestinal AIEC infection and show that these two cytokines exert integrated regulatory functions in Paneth cells and epithelial Lgr5 + stem cells during early host defense. IL-22 coordinates with IL-18 in early host defense against Crohn's AIEC, via induction of Lgr5 + stem cell-mediated epithelial regeneration and Paneth cell-directed Lysozyme production. IL-22 induces phospho-Stat3 binding to the IL-18 promoter in gut epithelial cells and additively promotes crypt immunity with IL-18. In organoid culture, while IL-22 primarily increases organoid size and inhibits stem cells, IL-18 robustly promotes organoid budding and induces Lgr5 + stem cells in a Stat3-independent but Akt-TCF4 dependent manner. At the early stage of infection, administration of IL-18 into mice corrects compromised T-cell IFN $\gamma$  production and restored Lysozyme + Paneth cells in IL-22 -/- mice, however, IL-22 injection failed to do so in IL-18 -/- mice. Together, IL-22-Stat3 signaling triggers an IL-18 response circuit for host defense at the barrier: an IL-18-Stat3 signaling to boost Paneth cells for anti-microbial response, an IL-18-Akt-TCF4 signaling to promote Lgr5 + stem cells for tissue repair, and an IL-18/IL-12 signaling to promote IFN $\gamma$  + T cells for AIEC clearance.

### IL2-antibody complex boosts maternal Treg cells to protect against fetal loss in pregnant mice

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Regulatory T cells are essential for pregnancy tolerance, and Treg cell insufficiency is implicated in female infertility, recurrent miscarriage, and preeclampsia. We utilised an abortion-prone mouse model to evaluate efficacy of IL-2 complexed with JES6-1 anti-IL-2 antibody (IL-2/JES6-1) to

boost uterine Treg cells and improve reproductive success. CBA/J female mice mated with DBA/2 males to generate abortion-prone pregnancies were administered IL-2/JES6-1 three times at 24h intervals on days 0.5-2.5 post coitum. Evaluation of Treg cells on day 3.5 pc showed IL-2/JES6-1 but not IL-2/IgG or PBS control (all 16-20 dams per group) elicited an >3-fold increase in the proportion of CD4<sup>+</sup> T cells expressing Foxp3, and an increase in the ratio of Foxp3<sup>+</sup> Tregs to Foxp3<sup>-</sup> Tconv cells, in the uterus and draining lymph nodes. Both thymic-derived and peripheral Treg cells showed an attenuated phenotype with elevated expression of Ki67 and suppressive function markers CTLA4, CD25, and Foxp3. IL-2/JES1-6 treatment reduced fetal loss from 31% to 10%, a rate comparable to non-abortion prone CBA/J female x Balb/c male matings (all P<0.05, ANOVA). There was no adverse impact of IL-2/JES1-6 treatment on perinatal parameters at day 18.5 pc, although a small decline in fetal weight was attributable to larger litter size. These experiments show that boosting uterine Treg cells through targeting IL-2 signaling is effective in promoting uterine receptivity to embryo implantation and mitigating immune-mediated fetal loss. The results are relevant to design of clinical interventions to target Treg cells in order to manage immune-mediated infertility and pregnancy disorders in women.

## Immune markers in saliva are associated with SARS-CoV-2 infection and humoral immunity

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Severity of COVID-19 associates with specific immune markers detected in blood. Since less is known about immune markers at the mucosa, the site of entry and initial immune responses to the virus, we studied immune markers in saliva by SARS-CoV-2 infection status. SARS-CoV-2 PCR-confirmed cases and their household members were sampled within 3 days after confirmation of the index case (T1), at 2-3 weeks (T2), at 4-6 weeks (T3) and at 8-11 months (T4). At T1-T3 individuals were tested for SARS-CoV-2 by PCR and saliva samples were analyzed for levels of 13 chemokines and 11 cytokines. SARS-CoV-2-specific antibodies were measured in saliva (T1-3) and serum (T1-4). Symptoms associated with viral load, were more frequent in adults (18+) compared to

minors (0-17) and mostly resolved by T2. Infection with SARS-CoV-2 was associated with altered levels of 10 chemokines described to recruit neutrophils and NK cells for early immune defense, and 7 cytokines, including different IFN types. Levels of CXCL5 were reduced in infected minors, CXCL11 elevated in adults and CCL2 in female adults. INF- $\lambda$ 2/3 was inhibited in infected male minors, but not in other participants. In saliva, the development of Spike-specific IgA preceded specific IgG. Levels of specific antibodies associated negatively with levels of inflammatory markers in saliva. In conclusion, we identified changes in the levels of immune markers in saliva upon SARS-CoV-2 infection, that was age and sex dependent. These data warrant further study to assess whether these markers are associated with disease severity or local protection against SARS-CoV-2 infection.

## Immune response of mRNA COVID-19 vaccines in elderly adults and patients with lymphoid malignancies.

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COVID-19 affects the population unequally with a higher impact on aged and immunosuppressed people. Hence, we aimed to characterize the immune response triggered by mRNA vaccines in three different cohorts: 25 healthy adults (under 70); 28 institutionalized elderlies (over 70 years); and 48 hematologic patients untreated or treated with B-cells antiproliferative drugs (lenalidomide, ibrutinib and rituximab). Peripheral blood mononuclear cells (PBMCs) were obtained pre-vaccination and 3 months after immunization. Cellular memory towards SARS-CoV-2 was determined by ELISPOT while a 40-plex spectral cytometry panel was applied to characterize the immune system. A consistent IFN $\gamma$  response was performed on post-vaccine COVID-stimulated conditions confirming immunization and pro-inflammatory T-cell

phenotype after vaccination in all groups except in hematologic patients treated with lenalidomide and ibrutinib. t-SNE analysis of the spectral cytometry results revealed that while healthy and elderly adults changed their immune profile following vaccination, hematologic patients grouped apart and were not affected by vaccination (Figure 1). Indeed, Post/Pre vaccination ratio for every immune subset was also analyzed referred to healthy adults revealing that untreated hematologic patients had a significant increase in monocytes, NK, CD4 + and CD4 + CD8 + T-cells and T regs coupled with a decrease on ILCs. On the contrary, elderly adults had an increase in NK, T $\gamma\delta$ , and CD8 + T-cells. In addition, the NK subset was reduced on rituximab patients. In summary, here we have shown that oncohematologic patients display a reduced immune response to mRNA COVID vaccines, rendering patients treated with lenalidomide and ibrutinib incapable of generating cellular memory.

### Immunogenetic determinants of HSV-2 infection and disease

Jessica Graham - Fred Hutchinson Cancer Research Center, Jessica Swarts, Michael Mooney, Jennifer Lund

Herpes simplex virus-2 (HSV-2) is one of the most prevalent sexually transmitted infections, and can result in life-long, chronic disease. Disease severity, frequency of reactivation, and shedding rates vary between individuals, though little is known about how host genes regulate tissue-specific immune responses. We have previously used the Collaborative Cross (CC) mouse model system, which incorporates the extent of genetic variation found in the human genome, to better model the diversity of outcomes found in human viral infections, so we next probed the CC to identify host genetic regions that regulate viral shedding and disease following HSV-2 infection, as well as tissue-specific immune responses. We performed a screen of mice from different CC strains to assess viral titers and disease following vaginal HSV-2 infection, and then used this data to perform quantitative trait loci (QTL) mapping to identify chromosomal regions linked to vaginal viral shedding rates and levels, as well as virus-associated clinical disease. In parallel experiments, we assessed lymphoid, nervous system, and mucosal immune cell frequencies at innate, adaptive, and memory response timepoints. We observed a distinctive suppressive signature on vaginal Tregs compared to lymph node Tregs, at various times following HSV-2 infection.

Additionally, these suppressive responses varied in mice with either higher viral titers, or more tissue inflammation, highlighting the interplay between host immune response and viral infection kinetics. Understanding host factors that contribute to HSV shedding, clinical disease, and immune responses may provide critical insights for developing new preventive strategies or interventions to HSV-2 infection.

### Immunomodulation therapy offers new molecular strategies to treat bacterial infections

Ines Ambite - Lund University, Daniel S.C. Butler, Murphy Lam Yim Wan, Thi Hien Tran, Catharina Svanborg

Innovative solutions are needed for the treatment of bacterial infections. The immune system and its efficient defence strategies can be mined for effective molecular solutions for use as alternative to antibiotics. Immunomodulation is now shown to be a realistic option for treating acute bacterial infections in the urinary tract, reducing tissue pathology and boosting the protective antimicrobial defense, accelerating bacterial clearance from infected kidneys and bladders. By targeting molecular nodes controlled by these genes, effective disease-specific, immunomodulation strategies have been developed for acute pyelonephritis (APN) or acute cystitis. IRF7-specific siRNA interference therapy was successfully used to treat acute pyelonephritis in the murine UTI model, preventing destructive hyper-inflammation in the kidneys and restoring innate immune homeostasis. Acute cystitis is caused by excessive IL-1 activation and treatment with an Interleukin-1 receptor antagonist (IL-1RA) in the murine acute cystitis model prevented destructive hyper-inflammation and pain. Both approaches accelerated bacterial clearance from infected tissues with similar efficacy as antibiotics. Clinical relevance of IL-1RA treatment was recently confirmed in patients with bladder pain syndrome, who experienced reduced symptoms and improved quality of life. These clinical findings offer "proof-of-concept" and support for continued development of immunomodulation therapy for acute UTI and its sequelae.

### Impact of Pattern Recognition Receptors During Intestinal Inflammation

Stephanie Tribble - The University of Arizona, Dakota Reinartz, Sydney Verdugo, Justin Wilson

The incidence of Inflammatory Bowel Disease (IBD) has increased ~85% since 1990. IBD- associated inflammation can result from environmental factors, genetic susceptibility and an intolerance to commensal organisms, which are recognized by pattern recognition receptors (PRRs). Several PRRs are associated with type 1 inflammation, microbiota recognition, and barrier repair during experimental models of IBD. However, the role of PRRs in the small intestine (SI) has not been fully explored, and it is unclear if they impact immune-regulatory type 2 responses during IBD. Type 1 and type 2 inflammation can dictate the induction vs. resolution of intestinal inflammation. A better understanding of the molecular links between microbial sensing and inflammatory resolution may be key to limiting IBD. Type 2 immune responses in the SI can be activated by Tuft cells (TCs) and type 2 innate lymphoid cells (ILC2s). TCs secrete IL-25 in response to the metabolite succinate, leading to IL-4 and IL-13 production by ILC2s. This promotes TC and Goblet cell differentiation, thus resulting in a feedforward circuit for optimal type 2 immune activation. Here, we show PRR-deficient small intestine organoids (SIO) treated with IL-4 have diminished TC and goblet cell gene expression profiles compared control wild type (WT) SIOs. Succinate exposure resulted in reduced TCs and ILC2-associated IL13 in PRR-null mice compared to WT controls. No differences were detected in IL-4 and IL-13 receptor expression between WT and PRR-null SI-Os and mice, suggesting intact IL-4/IL-13 signaling. These data implicate an important role for PRRs during type 2 inflammation in the SI.

### The impact of *Helicobacter hepaticus* infection on host innate immunity

Anna Heawood - University of Glasgow, Holly C. Webster, Tezz Quon, Annika Frede, Kevin J. Maloy

Infection of mice with the intestinal bacterium *Helicobacter hepaticus* (Hh) has been used to model human inflammatory bowel disease (IBD) and provided important insights into pathogenetic mechanisms. However, mice with intact regulatory immune functions do not develop intestinal pathology following Hh colonization, as the bacteria appear to induce dominant tolerance that allows persistent colonization without pathology. We hypothesise that chronic Hh infection may even confer host benefits, by enhancing resistance to other challenges and/or promoting barrier responses.

Combining in vitro systems and in vivo infection models, we are investigating the effects of Hh on the intestinal epithelium and on myeloid cells. We found that Hh colonization reduced innate inflammatory responses during chemically induced colitis, including decreasing neutrophil infiltration with a shift towards anti-inflammatory macrophages. In vitro, macrophages responded to Hh with upregulation of co-stimulatory and antigen presentation molecules, as well as production of inflammatory cytokines. However, when we tested the macrophage response to Hh under microaerophilic conditions required for Hh survival, we observed a downregulation of macrophage inflammatory responses. We are investigating the epithelial response to Hh using a primary intestinal epithelial cell (IEC) organoid system to assess effects on proliferation, differentiation, and barrier functions. This work aims to contribute to our better understanding of the mechanisms through which the host is influenced by its resident immunomodulatory microbiota.

### Impact of pre-existing immunity on the development of de novo virus-specific TRM following live attenuated influenza vaccination

Jenna Lobby - Emory University, Shamika Danzy, Anice Lowen, Jacob Kohlmeier

Live attenuated influenza vaccine (LAIV) elicits both humoral and cellular immune memory in children, but its efficacy is limited in adults. We hypothesize that pre-existing immunity from past infections and/or immunizations prevents the attenuated vaccine from establishing an immune response. To determine if we can overcome this limitation by increasing the antigenic distance of the vaccine strain from previous circulating seasonal strains, we generated a series of drifted LAIVs with successive mutations in the HA protein, allowing for increasing levels of escape from pre-existing antibody. We also inserted a CD8 + T cell epitope from the Sendai virus nucleoprotein (SeV-NP) as a readout for generation of a de novo T RM response following immunization. Surprisingly, we were unable to identify SeV-NP + CD8 + T RM following LAIV immunization in PR8-immune mice, even with LAIV strains that can fully escape pre-existing antibody. As these data suggested a role for cell-mediated immunity in limiting LAIV efficacy, we investigated several scenarios to assess the impact of pre-existing LAIV-specific TRM in the upper and lower respiratory tract. Ultimately, we found that deletion of the



immunodominant influenza NP 366-374 epitope was sufficient to escape pre-existing CD8 + T RM and to establish de novo CD8 + T RM . Combined, these studies demonstrate that both pre-existing humoral and cellular immunity can limit the effectiveness of LAIV, thereby informing future design of vaccine vectors against respiratory pathogens.

## Impaired Th17 cell immunity to intestinal bacteria in inflammatory bowel disease and *C. difficile* patients.

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**Introduction :** Bacterial flagellin is a key antigen in inflammatory bowel disease (IBD) pathogenesis. IBD is characterized by intestinal dysbiosis, with patients at increased risk for *Clostridioides difficile* infection (CDI), an urgent antimicrobial resistant threat. Studies of antibody responses to flagellin and *C. difficile* toxins have not been clinically informative. We hypothesised CD4 + T cell responses would be of greater clinical use and may uncover unique therapeutic targets. **Objective :** We aimed to quantify and characterize flagellin-specific and *C. difficile* toxin B (TcdB)-specific CD4 + T cells in Crohn's disease (CD) and ulcerative colitis (UC) patients, non-IBD CDI patients and healthy controls. **Methods and Results :** Peripheral blood CD4 + T cell responses to bacterial antigens were detected using a flow cytometric activation induced marker assay and could discriminate patients from healthy controls. Flagellin-specific responses were increased in IBD patients and contained significantly fewer Th17 cells compared to healthy controls. Similarly, toxin-specific Th17 cell proportions were significantly reduced in both IBD and CDI patients compared to healthy controls. 16S rDNA sequencing of IBD stool samples revealed differentially abundant bacterial species that correlated with T cell immunity. Successful fecal microbiota transplant treatment of CDI was associated with reconstitution of the Th17 cell compartment. **Conclusions :** A common feature of healthy immune responses to commensal and pathogenic bacterial antigens is a predominance of Th17 cells. These data help explain the failure of IL-17 inhibitors in IBD clinical trials and highlight a need for further work into the role of Th17 cells in intestinal disease.

## Increased intestinal bacterial mucin foraging facilitates clearance of a parasitic worm

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Host-secreted intestinal mucus plays an important role in the expulsion of intestinal nematode parasites. While mucin secretion is strongly regulated by the metabolic activities of commensal gut microbes, it is not clear how changes in bacterial mucin foraging impact the course of worm infection. In this study, we utilized a gnotobiotic mouse model with a well-characterized, 14-member synthetic human gut microbiota along with dietary fiber deprivation to stimulate elevated microbial mucin foraging. During infection with a robust murine intestinal nematode ( *Trichuris muris* ), we detect abrupt changes in the structure of this defined bacterial consortia and an elevation in mucolytic enzyme activity, which alters mucin glycosylation patterns. Furthermore, among fiber-deprived mice, but not mice fed a standard chow, the host immune response shifts from susceptible (chronic, Th1 type) to resistant (acute, Th2 type), which promotes worm clearance. These results document an unexplored diet-driven mechanism to skew the metabolic activity of the microbiome toward mucin glycoproteins and confer resistance to worm infection. Our study reveals a link in the microbiome–parasite–host immune axis, which has a compelling potential in the treatment of parasitic worm infections.

## In-depth characterization of the immune response of infected and non-infected bystander alveolar macrophages during *Legionella pneumophila* lung infection

Ann-Brit Klatt - Charité - Universitätsmedizin Berlin, Facundo Fiocca Vernengo, Ivo Röwekamp, Sandra Caesar, Gitta Anne Hein, Mir-Farzin Mashreghi, Bastian Opitz

Various important pathogens including *Legionella pneumophila* replicate in tissue-resident macrophages. While the interaction of *Legionella* with cell culture models such as bone-marrow derived macrophages has been thoroughly studied, little is

known about how tissue-resident alveolar macrophages, the primary host cell of *Legionella*, react in response to infection in vivo. In this study, we aim to characterize the immune response of infected and non-infected bystander alveolar macrophages during early stages of lung infection in vivo. By infecting mice intranasally with a GFP-expressing strain of *Legionella*, we are able to isolate and discriminate infected and non-infected cells by FACS-sort. As a control, we additionally isolated leucocytes from PBS-infected mice. Subsequent scRNAseq analysis indicates that the expression of both inflammatory- and metabolism-associated genes differs considerably between infected and non-infected alveolar macrophages. Ongoing studies aim to further explore the interaction of alveolar macrophages with *Legionella* on both transcriptional and posttranslational levels by evaluating the proteome and metabolism of in vivo and ex vivo infected and non-infected bystander alveolar macrophages in more depth.

### The Individual Short Chain Fatty Acids Acetate, Propionate and Butyrate Differently Regulate Intestinal Epithelial Cell Gene Profile, Function and Organoid Development

Silvia Melgar - APC Microbiome Ireland, University College Cork, Aine Fanning, Keith O'Donoghue, Ana Ramon Vazquez, Mary Ahern, Ken Nally, Fergus Shanahan

Short chain fatty acids (SCFAs), especially butyrate, influence inflammatory and barrier function and act as energy source for intestinal epithelial cells (IECs). Herein, we examined the individual regulation of acetate, propionate and butyrate on IEC-functions and organoid differentiation. Human colon epithelial (C2Bbe1) and reporter (HEK) cells were treated with individual SCFAs (1-100mM) and assayed for viability (CelltiterBlue), proliferation (BrdU), gene expression (qRT-PCR), and cytokines (ELISA). Murine intestinal organoids were differentiated in media supplemented with individual SCFAs for 7-days followed by gene expression and NOTCH-PCR array assessment. Acetate and propionate (100mM) were slightly toxic to C2Bbe1s provoking minor alterations in proliferation, cell cycle and epithelial-differentiation genes, while butyrate (5-10mM) increased proliferation and apoptosis. Pre-treatment with propionate and butyrate attenuated IL-1b-induced IL-8 secretion, while acetate enhanced it. Butyrate and propionate dose dependently downregulated HEK-derived-IFN $\gamma$ , while upregulating HEK-derived-TGF $\beta$  (1-10mM). Organoids

differentiated in acetate, propionate or butyrate reduced Paneth cells' gene profile, while propionate reduced Enteroendocrine/Goblet cell's gene profile and acetate reduced Goblet cells gene expression. IPA analysis revealed that acetate and butyrate similarly regulated NOTCH-associated genes at day-3, while propionate and butyrate regulated a similar gene profile at day-7 of organoid development. Collectively, our data identified distinct regulatory potential of individual SCFAs on IECs' function. Butyrate and propionate presented similar anti-inflammatory properties, but they have opposing effects on organoid differentiation, with propionate eliciting the highest effect. Our data provide new insights on the regulation of IEC-function and development by individual SCFAs, which is relevant for the discovery of new SCFAs producing bacteria strains.

### Inflammatory Cytotoxic Natural Killer B-cells appear in Colon During SIV infection.

Edward Barker - Rush University Medical Center, Andrew Cogswell, Sungro Jo, Natasha Ferguson, Kajal Gupta

Here we report the appearance of Natural Killer B cells (NKBs) within the colon and draining lymph nodes during SIV infection of susceptible monkeys. Using RNAseq and flow cytometry, we show that NKBs are unique cells with features and functions of NK and B cells. NKBs express receptors and ligands found on B-cells involved in 1) antigen presentation, 2) activities associated with class switching, affinity maturation, and B-cell memory formation in secondary lymphoid follicles, and 3) antigen recognition. The predominant Ig expressed on NKBs is IgA with lambda light chain, although surface IgM and IgG can be expressed. There is a dominant expression of lambda over kappa light chain. NKBs express NK cell activation receptors and Fas ligand. NKBs express the cytolytic molecules perforin and granzymes. NKBs lyse cells in a lytic assay. NKBs also produce inflammatory cytokines interferon- $\gamma$ , tumor necrosis factor- $\alpha$ , and interleukin (IL)-18, which were not made by NK cells and CD8+ T-cells from the same colon. Finally, we noted the increased capacity of NKBs to proliferate compared to NK cells and CD8+ T-cells from the SIV-infected colon. Increased proliferation and inflammatory cytokine production are related to the relatively high IL-15 receptor (R) beta expression, IL-7R, IL-18R, and 41BB. These properties of NKBs, absent from or reduced in NK cells and CD8+ T-cells in the SIV-infected gut, may lead to loss of CD4, increased

microbial translocation, and enhanced inflammation observed during SIV infection of the gut remains to be determined.

## Inflammatory monocytes restrict enteropathogenic *Yersinia* within intestinal pyogranulomas

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Granulomas are organized collections of immune cells that sequester chronic pathogens. Enteropathogenic *Yersinia* cause acute gastroenteritis and induce the formation of granulomatous lesions termed pyogranulomas in lymphoid tissues. Here, we uncover the rapid formation of pyogranulomas within the intestinal mucosa of mice following oral *Yersinia pseudotuberculosis* (Yp) infection. These intestinal pyogranulomas contain high numbers of viable bacteria surrounded by neutrophils and monocytes, in contrast to adjacent non-granulomatous tissue, demonstrating that Yp infection of the intestinal mucosa and the resulting immune response are spatially restricted. Bacterial burdens within these structures were equivalent to that of Peyer's patches, indicating that intestinal pyogranulomas are a previously unrecognized site for bacterial control during early Yp infection. Notably, *Ccr2* -deficient mice lacking circulating monocytes fail to form defined pyogranulomas, are unable to restrict bacteria in both the intestine and deeper tissues, and succumb rapidly to Yp infection, demonstrating that monocytes are necessary for acute control of oral *Yersinia*. Notably, mutant Yp unable to inhibit phagocytosis do not induce intestinal pyogranuloma formation. Furthermore, *Ccr2* -/- mice are able to control mutant Yp and survive infection, suggesting that pyogranulomas form in response to blockade of innate immune cell function, and that monocytes are essential in overcoming this blockade. Overall, this work defines a previously undescribed site of *Yersinia* intestinal invasion and reveals host and pathogen drivers of granuloma formation in the intestinal mucosa.

## Inhibition of Goblet Cell Associated Antigen Passages Results in an Intestinal Humoral Immune Response

Alexandria Floyd - Washington University in Saint Louis School of Medicine, Bibiana E. Barrios, Ryan T. McDonough, Khushi Talati, Keely G. McDonald, Rodney D. Newberry

In the steady state goblet cell associated antigen passages (GAPs) in the small intestine are open allowing for gut-immune interactions in many different contexts. In the face of enteric infections, such as *Salmonella typhimurium*, GAPs are inhibited in the small intestine due to the increased IL1 $\beta$  signaling in goblet cells (GCs). Single cell RNA sequencing on mice lacking GCs revealed an expansion of B lymphocytes and plasma cells. Bulk RNA sequencing performed on small intestine of mice where GAPs were inhibited in the absence of infection showed an upregulation of cell markers for B cell recruitment and differentiation into plasma cells as well as for Tfh cells including CD20, CXCL13, Prdm1, and PDL-1. These findings were confirmed using ELISPOTs and flow cytometry in mice where GAPs were inhibited. This could also be seen in mice lacking GCs altogether. We additionally observed an increase in isolated lymphoid follicles (ILFs) and an increase in Peyer's Patch size in mice where goblet cells were deleted or GAPs were inhibited. These findings suggest that inhibition of GAPs is a physiologic response to infection to promote adaptive immunity.

## Interactions of differentially charged nanoparticles with bacterias, mucus, and host tissues

Bumjun Kim - Princeton University, Nan Gao, Robert Prud'homme

Inflammatory bowel diseases (IBDs) including Crohn's disease (CD) and ulcerative colitis (UC) are characterized by an inappropriate immune response in a genetically susceptible host. Despite a variety of therapeutic options including anti-inflammatory, immunosuppressive, and biologics, up to 20% of UC patients and more than 50% of CD patients eventually require surgery. One of the major roadblocks for effective IBD intervention is the lack of a system for robust and targeted delivery of drugs specifically to the site of inflamed intestinal tissues. Two categories of nanoparticles (NPs) have demonstrated the efficacy of targeting inflamed intestines. Muco-penetrating particles (MPPs), which possess dense poly(ethylene glycol) (PEG) brushes, can diffuse through mucus layers, and are deeply retained in mucosa. Muco-adhesive particles (MAPs), on the other hand, have charged

surface and anchor to the adherent mucus on luminal surface. However, previous investigations on interactions between NPs and host tissues were macroscopic and/or non-quantitative. Moreover, the interaction of NPs with luminal contents, such as the microbiota and mucus, was overlooked. Herein, we systemically investigated the distribution of NPs in inflamed colons as a function of NP surface chemistry. Negatively charged MAPs, (N\_MAPs), neutral MPPs and positively charged MAPs (P\_MAPs) were synthesized with FDA approved excipients and rectally administered in a chemically-induced murine colitis model. To enable the microscopic visualization and quantification of differentially charged NP distribution throughout the colons, we developed a whole colon scanning method (Figure 1). Our work highlights the quantitative analysis of the interactions among NPs, microbiota, and inflamed mucosa.

### Interleukin-33 signaling exacerbates experimental infectious colitis

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Various bacterial pathogens can enter the gastrointestinal tract, thereby disturbing the gut microbiota composition and causing infectious diarrhea and colitis. A finely tuned balance between pro- and anti-inflammatory cytokines is necessary to eradicate the microbial threat and to avoid infection-related complications. Recently, we identified the alarmin interleukin (IL)-33 as a critical negative regulator of the immune response to the enteric pathogen *Citrobacter rodentium*. We observed that deficiency of the IL-33 signaling pathway attenuates bacterial-induced colitis. Conversely, boosting this pathway strongly aggravates the inflammatory response and makes the mice prone to systemic infection. Mechanistically, IL-33 mediates its detrimental effect by enhancing gut permeability and by limiting the induction of protective T helper 17 cells at the site of infection, thus impairing host defense mechanisms against the enteric pathogen. Furthermore, next generation sequencing of the gut microbiota composition reveals an IL-33-mediated dysbiosis associated with alterations of short-chain fatty acid levels in the feces of IL-33 treated mice. Thus, a deeper understanding of cytokine-mediated dysbiosis and the resulting consequences for the hosts' gut immunity are

indispensable for the accurate grasp and treatment of intestinal infectious diseases.

### Interplay between NAIPs and prostaglandins in colonic infection, inflammation and cancer

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Maintenance of intestinal integrity is dependent on crosstalk between epithelial, stromal and immune cells and the gut microbiota. Nod-like receptor (NLR) apoptosis inhibitory proteins (NAIPs) activate the NLRC4 inflammasome upon recognition of gram-negative bacteria, leading to pyroptosis/apoptosis, intestinal epithelial cell expulsion and release of IL-1 $\beta$ , IL-18 and prostaglandin E 2 (PGE 2). NAIPs also appear to have homeostatic roles within the intestinal epithelium, as our group has previously shown that NAIPs suppress colonic tumorigenesis but enhance colonic inflammation. Eicosanoids, such as PGE 2, have a well-established but complex role in gut maintenance, colorectal cancer and colitis. We aimed to further understand how epithelial NAIPs impact the immune compartment and understand the role of PGE 2 in this axis. Using co-culture models of colonic organoids and mouse splenocytes as well as ex vivo analysis of the intra-epithelial lymphocyte (IEL) compartment. We have identified altered basal levels of prostaglandins (PGF2 $\alpha$ ) and IL-15/IL-15R complex and concomitant alterations in IEL subsets as well as activation-induced IFN $\gamma$  production in CD4 T cells when NAIPs are lacking in the intestinal compartment. Our ongoing studies aim to further evaluate the mechanisms and impact on mucosal immunity in response to inflammatory challenge.

### Intestinal regulatory T cells control microbiota-specific T cell plasticity and memory potential.

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Plasticity is an inherent characteristic of CD4 + T cells, allowing for effective responses to a variety of pathogens and environments. However, microbiota-specific T cell plasticity can be deleterious and is associated with certain inflammatory



diseases such as IBD. Re-activation of microbiota-specific T cells can occur in the presence of heterologous gut infection; however, whether they exhibit plasticity or contribute to pathology is unknown. We hypothesize that microbiota-specific T cells are plastic and aid in pathogen clearance during infection but may contribute to inflammatory diseases and pathology long-term. To address this hypothesis, we utilized Segmented Filamentous Bacteria (SFB) TCR transgenic mice and MHC II tetramers to directly track SFB-specific T cell fate and function during intestinal infection with an invasive parasitic pathogen, *Toxoplasma gondii*. SFB-specific T cells became plastic in the presence of *T. gondii* infection through transient upregulation of Tbet and IFN $\gamma$  and reduction of IL-17. Interestingly, regulatory T cell (Treg) loss, either *T. gondii* induced or directly depleted, was necessary and sufficient to drive plasticity. Transcriptional analysis revealed that plastic T cells upregulated memory programs and associated metabolic pathways. Indeed, further functional analyses showed that plasticity licensed microbiota-specific T cells to become long-lived tissue resident memory T cells within the gut. Lastly, T cell plasticity led to more severe pathology in a transfer ileitis model. Taken together, these studies suggest a mechanism by which microbiota-specific T cells form long-term memory through Treg loss or dysfunction, and that they may potentially contribute to more severe host pathology over time.

## Intestinal resident memory T cells (Trm) and circulating ex-Trm in health and inflammatory bowel disease

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**Background.** Tissue-resident memory T-cells (Trm) persist in tissues and can contribute to inflammation. Trm can re-enter the circulation (ex-Trm) and give rise to new effector and Trm populations. Human skin-derived ex-Trm co-express the residency marker CD103 and cutaneous leukocyte antigen (CLA), a skin-tropism marker. The existence of ex-Trm derived from the gut would have implications for inflammatory bowel disease (IBD). **Methods.** PBMCs and colonic cells were obtained from healthy volunteers and patients with active IBD (Crohn's disease or ulcerative colitis), and analysed by multi-colour flow cytometry and single cell RNA sequencing. **Results.** Over 80% of colonic  $\alpha\beta$ T-cells were CD69+ Trm in health and IBD. Few CD4+ Trm co-expressed CD103, but CD8+ Trm comprised CD103+ and CD103- subsets, with

CD69+CD103- cells significantly reduced in IBD. CD8+ Trm from inflamed colonic tissue had high expression of genes associated with activation, cytotoxicity and ROS generation. Putative gut-derived ex-Trm were identified amongst TCR $\alpha\beta$ +CD45RA- blood cells as a  $\beta 7^{++}$ CD103+ population, indicative of cells expressing both  $\alpha 4\beta 7$  and CD103( $\alpha E$ ) $\beta 7$  integrins. Gut-derived and skin-derived (CLA+CD103+) ex-Trm shared a characteristic phenotype. Gut ex-Trm were significantly reduced in patients with Crohn's disease but not ulcerative colitis. **Conclusion.** The distribution of CD8+ Trm subsets is altered in IBD, and intestinal inflammation is associated with Trm expression of genes associated with pro-inflammatory pathways. A putative gut-derived ex-Trm population identified in blood is reduced in Crohn's disease; recruitment of ex-Trm could explain the patchy nature of Crohn's disease inflammation. The maintenance of residence and generation of ex-Trm may provide new therapeutic targets.

## Intranasal vaccination via lipid-conjugated immunogens promotes antigen persistence and transmucosal uptake to drive mucosal and systemic immunity

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To combat the global HIV epidemic as well as emerging threats such as SARS-CoV-2, immunization strategies are needed that elicit protection at mucosal portals of pathogen entry. Immunization directly through the airway surfaces is effective in driving mucosal immunity, but poor vaccine uptake across the mucus and epithelial lining is a major limitation. The major blood protein albumin is constitutively transcytosed bidirectionally across the airway epithelium via interactions with the neonatal Fc receptor (FcRn). Exploiting this biology, here we demonstrate a strategy of 'albumin hitchhiking' to promote mucosal immunity using an intranasal (i.n.) vaccine consisting of protein immunogens modified with an amphiphilic albumin-binding polymer-lipid tail (forming amph-proteins). Amph-proteins persisted in the nasal mucosa and exhibited increased uptake into the tissue in an FcRn-dependent manner, leading to significantly enhanced germinal center (GC) responses in the NALT. I.n. immunization with amph-

conjugated HIV Env gp120 or SARS-CoV-2 RBD proteins elicited robust antigen-specific IgG and IgA titers (100-1000-fold higher than unmodified protein) in the serum, upper and lower respiratory mucosa, and distal genitourinary mucosae of mice. Amph-RBD immunization induced high levels of SARS-CoV-2 neutralizing antibodies in serum, nasal washes, and bronchoalveolar lavage. Intranasal amph-protein immunization in rhesus macaques elicited ~10-fold higher antigen-specific IgG and IgA responses in the serum and nasal mucosa compared to unmodified protein, supporting the translational potential of this approach. These results suggest that employing amphiphile-protein vaccines to deliver antigen across the mucosal epithelium presents a promising and simple strategy to promote mucosal immunity against HIV, SARS-CoV-2, and other infectious diseases.

## Intravenous Administration of Tumor-Derived Extracellular Vesicles Augments Anti-Tumor Immunity with Attenuating Regulatory T Cell Characteristics

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**Background** Although tumor-derived extracellular vesicles (TEVs) have been utilized as a diagnostic tool for cancer, therapeutic application of TEVs is still in challenging. We validated the output of administration of TEVs in vivo with focusing on the phenotype of regulatory T cells (Treg). **Methods** The experimental lung metastasis model mice of colorectal cancer were generated by intravenous injection of Colon-26, Balb/c colon adenocarcinoma cells into Balb/c mice. TEVs derived from Colon-26 (Colon-26-EVs) and ASB-XIV, Balb/c lung squamous cell carcinoma (ASB- EVs) were isolated from culture supernatants by ultracentrifugation. Mice were analyzed following intravenous injection of TEV equivalent to 10 µg protein or PBS every other days for 2 weeks. **Results** Histopathology revealed that Colon-26-EVs reduced lung tumors compared to the PBS group ( $P<0.001$ ). Whereas the proportion of Treg/CD4<sup>+</sup> in lung was comparable between Colon-26-EV group and healthy mice, it was up-regulated in PBS group compared to that in healthy mice ( $P<0.001$ ). The expression of Treg functional marker such as PD-1 and GITR, and T cell activation marker such as CD69 in lung Treg were significantly up-regulated in PBS group compared to that in healthy mice (PD-1;  $P<0.001$ , GITR;

$P<0.0001$ , CD69;  $P<0.0001$ , respectively) but were comparable between Colon-26-EVs and healthy mice. Interestingly, ASB-EVs also did not up-regulate these expressions of Treg. Dye-labeled TEVs clarified that uptake of TEVs by T cells, Treg and B cells was negligible compared to that by CD11b<sup>+</sup> subsets. **Conclusion** TEVs might contain the elements that suppress tumor growth with attenuating characteristics of Treg in vivo.

## Invasive isolates of *Neisseria meningitidis* show strong complement evasion and difference in binding to differentiated primary nasal epithelial cells

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Since 2000 various countries experienced outbreaks of invasive meningococcal disease (IMD) caused by *Neisseria meningitidis* serogroup C (MenC) and W (MenW). Since IMD cases were often caused by clonal complex (CC)11 bacteria, the genetic makeup likely contributes to causing IMD. We investigated the response of air-liquid-differentiated primary nasal epithelial cells (PNEC) to a capsule-deficient isolate cultured from an asymptomatic carrier, and a CC11 and non-CC11 strain of MenC and MenW. The capacity to evade complement-mediated killing was tested for 85 isolates of varying CCs, serogroup and clinical presentation backgrounds. PNEC-binding differed between isolates, and at 2h was the highest (8-30 fold) for the capsule-deficient isolate. This adherence also differed between PNEC donors and was partially maintained at 24h. Infection resulted in 35-60% reduced trans-epithelial electrical resistance. Cytokine and chemokine responses of PNEC differed slightly between the isolates. Compared to isolates from carriers, invasive isolates showed >100-fold higher resistance to complement-mediated killing as assessed by the gold-standard serum bactericidal assay using sera from unvaccinated individuals. Complement-escape was observed for amongst others CC11 and clustered with core-genome profiles, but not with clinical presentation or outcome. All IMD isolates were lysed when serum from vaccinated individuals was used. These data show that epithelial responses to meningococci are influenced by the genetic background of the donor and particular isolate,

especially with respect to PNEC-binding, as proxy for colonization-potential. Profound capacity of invasive isolates to evade complement-mediated killing was observed. These data increase our understanding of the pathogenicity of *N. meningitidis* isolates.

### Investigating Apoptotic Cell Death Dependent-Antigen Sampling in the *Helicobacter pylori*-infected Stomach using the GOFLOWChip

Michelle Cherne - Montana State University, Barkan Sidar, Zahra Mahdiah, Jake Fredrikson, Humberto S. Sanchez, T. Andrew Sebrell, Connie B. Chang, Mark A. Jutila, James N. Wilking, Seth T. Walk, Diane Bimczok

Investigating Apoptotic Cell Death Dependent-Antigen Sampling in the *Helicobacter pylori* -infected Stomach using the GOFLOWChip Michelle D. Cherne 1 , Barkan Sidar 2 , Zahra Mahdiah 2 , Jake Fredrikson 2 Humberto S. Sanchez 2 , Andy Sebrell 1 , Connie B. Chang 2 , Mark A. Jutila 1 , James N. Wilking 2 , Seth T. Walk 1 , Diane Bimczok 1 Department of Microbiology and Cell Biology (1) and Chemical and Biological Engineering Department (2), Montana State University *Helicobacter pylori* infects nearly half the world's population. Infections mostly remain asymptomatic, but the chronic gastric inflammation induced by *H. pylori* may lead to gastric cancer. The causes of the variable host response to this pathogen remains unclear, but antigen uptake by mononuclear phagocytes (MNP) is thought to play an important role. How MNP take up antigen from the stomach lumen is still unknown. Some hypothesize MNP directly interact with the epithelium, reaching dendrites through to phagocytose antigen, while others suggest uptake through gaps in the epithelium created during the turnover of short-lived gastric epithelial cells. Initially, we observed an increase in apoptotic cell death of *H. pylori*- injected human gastric organoids (HGOs) compared to uninfected HGOs. Considering this, we investigated the role of apoptotic cell turnover to MNP sampling of *H. pylori*- infected gastric epithelium using a novel tissue chip platform, the gastrointestinal organoid flow chip (GOFLOWChip). *H. pylori*- infected HGOs were embedded in Matrigel microbeads to allow for cellular migration, and co-cultured with monocyte derived-dendritic cells (DCs) in the GOFLOWChip. A fluorescent apoptosis reporter was added to observe and quantitate interactions of the DCs with apoptotic gastric epithelium in real-time for 20 hours. While DCs

migrated rapidly towards HGOs and across the epithelium, DCs were not observed to sample bacteria from the organoid lumen but were attracted to HGOs with higher amounts of apoptotic cells, phagocytosing bacteria released from organoids. Our data suggests that antigen leakage through epithelial gaps may serve as an important mechanism for MNP sampling in the stomach.

### Investigating astrovirus goblet cell tropism reveals the importance of a host tryptophan catabolizing enzyme in the gut

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Astroviruses are small, enteric pathogens that cause a wide range of disease spanning from asymptomatic infections to severe diarrhea in birds and mammals. Despite being so widespread, they are one of the least characterized enteric RNA viruses. To better understand the tissue and cellular tropism of astrovirus, we previously identified a subset of small intestinal goblet cells as the primary target of murine astrovirus-1 using single-cell RNA sequencing analysis. Interestingly, this subset of goblet cells targeted by astrovirus express high levels of indoleamine 2,3-dioxygenase-1 (Ido1), the main host tryptophan catabolizing enzyme in the gut, and Ido1 expression in the gastrointestinal tract aligns with the biogeography of infection. Together this suggests that Ido1 may be an important host factor in virus replication. To investigate this further, we infected Ido knockout (KO) mice and observed a lower level of infection compared to wildtype animals that was not associated with fewer target cells but rather lower Muc2 expression. We also observed lower levels of infection in neonatal mice infected on postnatal day 3 as compared to day 7, which corresponded with increased expression of Ido1 in goblet cells during neonatal development. Finally, using human astrovirus-1 we similarly detected lower levels of infection in IDO1 KO Caco-2 cells compared to wildtype cells. Overall, this work led to the identification of a critical host factor important for the tropism of human and murine astroviruses. Our current work is focused on determining the role of Ido1 in the astrovirus replication cycle and more broadly in epithelial cell biology.

### Investigating commensal-specific B cells in the gastrointestinal tract

Sheenam Verma - Benaroya Research Institute, Oliver Harrison

Induction of adaptive immune responses to commensal microbes is critical for intestinal homeostasis, and perturbation of these responses is associated with multiple chronic inflammatory disorders. B cells play a key role in intestinal homeostasis, in part through local production of secretory Immunoglobulin A (IgA). However, the mechanisms underlying induction and regulation of commensal-specific B cell responses remain poorly understood, due to a lack of tools to identify commensal-specific B cells *in vivo*. To address this, we generated a novel B cell tetramer to investigate the induction of mucosal B cell responses elicited by Segmented Filamentous Bacteria (SFB). Using this tool, we identified SFB-specific B cell activation and differentiation in gut-associated lymphoid tissue (GALT). SFB-specific B cell response was found to be strongly T-cell as well as T<sub>H</sub>17-dependent. Further, in addition to induction of IgA<sup>+</sup> B cells, a high frequency of IgG<sup>+</sup> SFB-specific B cells indicate that gut microbiota also induces systemic immune responses that extend beyond the local tissue environment. In ongoing work, we are investigating the role and function of GC-derived SFB-specific B cells in intestinal homeostasis and inflammation.

### Investigating IL-36R signaling during intestinal infection

Tayla Olsen - University of Washington, Oliver J. Harrison

Mammalian barrier tissues, including the skin and gut, are colonized with commensal microbes that are critical for the education and function of the immune system. In contrast to our understanding of immunity to pathogens, how the host mounts immune responses to commensal microbes is poorly understood. Recent work from our laboratory, and others, demonstrated that commensal-specific T cells in the skin contribute to host defense against microbes generally, but also actively aid in wound repair, a differentiation state we term poised Type-2 immunity. In the skin, the IL-1 family member alarmin, IL-18, licenses these poised T cells to produce the type 2 cytokine IL-13. Preliminary data from the lab shows that the IL-1 family member, IL-36, may similarly license gut commensal reactive T cells in the intestines. IL-36 has been

shown to be important for tissue remodeling after intestinal damage in other contexts. I aim to expand upon these early findings by investigating the role of IL-36R signaling in commensal reactive T cells during bacterial or helminth infection to determine their contribution to pathogen clearance.

### Investigating the Distinct Tissue Repair and Immunosuppressive Roles of Regulatory T cells in HSV-2 Infected Mice

Irene Cruz Talavera - Fred Hutchinson Cancer Research Center, Brianna Traxinger, Tanvi Arkatkar, Jennifer Lund

Herpes Simplex Virus Type-2 (HSV-2) causes genital herpes, affecting around 500 million people worldwide. Infected individuals present with varying frequency and severity of viral reactivation, yet little is known about the contribution of regulatory T cells (Tregs) to the diverse mucosal responses and clinical symptoms. Tregs are necessary for CD4<sup>+</sup> T cell priming in the draining lymph node (dLN), though their role in shaping tissue-resident memory T cell (T<sub>RM</sub>) responses during HSV-2 reactivation is unclear. Our preliminary data indicate that activated Tregs accumulate in mouse vaginal tissue (VT) after infection and persist above baseline out to at least 90 days post-infection (p.i.), thus implicating Tregs in memory recall responses to re-infection. We hypothesized that Tregs restrain vaginal T<sub>RM</sub> recall responses to HSV-2 challenge. Additionally, we hypothesized that post-challenge, vaginal Tregs promote tissue healing in the VT in response to inflammatory alarmins and through the expression of epidermal growth factor ligands, such as amphiregulin (Areg). Female FoxP3 DTR mice were infected intravaginally with attenuated HSV-2, then systemically depleted of Tregs via intraperitoneal injection of diphtheria toxin and challenged with WT HSV-2 on day 30 p.i.. We found that Treg-depleted mice have increased local inflammation, increased frequency of CD44<sup>+</sup> and Ki67<sup>+</sup> CD8<sup>+</sup> and CD4<sup>+</sup> T cells, and increased granzyme-B<sup>+</sup> CD8<sup>+</sup> T cells in VT by day 3 post-challenge. By day 7 p.i., Treg-depleted mice have significantly higher pathology scores in H&E-stained VT. Ongoing work includes characterization of Areg expression in vaginal Tregs and further dissection of the T<sub>RM</sub> recall responses in Treg-depleted mice.



## Investigating the Role of AIM2 in Head and Neck Squamous Cell Carcinoma

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Head and neck squamous cell carcinoma (HNSCC) is the 6<sup>th</sup> most common cancer worldwide. HNSCC development is linked to chronic inflammation, while established HNSCC tumors often exhibit an immune suppressive microenvironment. However, both occur through mechanisms that are not fully understood. The cytosolic double-stranded DNA (dsDNA) sensor Absent in Melanoma 2 (AIM2) recognizes microbial- and host-derived dsDNA. Upon binding to dsDNA, AIM2 forms an inflammasome, which results in IL-1 $\beta$  and IL-18 release. AIM2 also restricts intestinal tumorigenesis through suppression of the PI3K pathway. Because PI3K and IL-1 $\beta$  promote HNSCC, we hypothesized that AIM2 regulates HNSCC. Here, we use an experimental model of HNSCC, involving treatment of wild type (WT) and Aim2<sup>-/-</sup> mice with the carcinogen 4NQO in drinking water for 20 weeks. Compared to WT mice, 4NQO-treated Aim2<sup>-/-</sup> mice exhibited larger tumor sizes and increased tissue dysplasia, but no obvious differences in PI3K or inflammasome activation. Instead, 4NQO-treated Aim2<sup>-/-</sup> mice presented elevated Ifng expression and enhanced expression of transcripts related to the MHC protein complex, cell killing and T cell activation following RNA sequencing of total tongue RNA. Aim2<sup>-/-</sup> mice given regular water following 4NQO displayed enhanced IL10, suggesting a shift towards immune suppression during recovery. Aim2<sup>-/-</sup> naïve CD4 T cells differentiated under Th1 conditions in vitro displayed greater expression and production of both IFN $\gamma$  and IL-10 compared to WT controls. These findings suggest AIM2 limits the progression of oral tumor development, which may occur through intrinsic suppression of IFN $\gamma$  and IL-10 production from CD4 T cells.

## Investigating the Role of the Transcription Factor, PPAR- $\gamma$ , in the Development and Long-term Persistence of Group 3 Innate Lymphoid Cells within the Neonatal Lung

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Lung-resident type 3 innate lymphoid cells (ILC3s) are critical for lung mucosal defense against bacterial pneumonia in newborns. During perinatal development, ILC3s localize to and proliferate within the newborn lung, however the signals that guide their development are incompletely understood. In mice, dysbiosis caused by early-life antibiotic exposure decreased numbers of pulmonary ILC3s and increased susceptibility to pneumonia infection. With bacterial pneumonia killing over one million infants around the world each year and high rates of early-life antibiotic exposure in neonates, understanding pulmonary ILC development and how it may be altered is clinically important. Simultaneous transcriptome and epigenome profiling of pulmonary and intestinal ILC3s implicated peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ), as a potential link between the tissue environment and the development and long-term persistence of pulmonary, but not intestinal, ILC3s. PPAR- $\gamma$  is a critical regulator of lipid homeostasis and plays an essential role in the development and functional maturation of alveolar macrophages, another tissue-resident innate immune cell in the lung. We hypothesize that PPAR- $\gamma$  integrates cues from the lung microenvironment to instruct the differentiation and functional fitness of pulmonary ILC3. We crossed RAR-related orphan receptor gamma (Rorc)<sup>cre</sup> mice with Ppar- $\gamma$  flox mice to generate Rorc $\Delta$ Ppar $\gamma$  mice. In preliminary studies, Rorc $\Delta$ Ppar $\gamma$  mice had altered frequencies of pulmonary ILC3. Continuing studies will test the necessity of Ppar- $\gamma$  in the development, long-term persistence, and functional maturation of pulmonary ILC3, as well as host defense against respiratory pathogens. Improved understanding of pathways directing pulmonary ILC3 development could inform novel therapeutic strategies for newborn immune support.

## Krüppel-like factor 4 is critical for CD4 T cell memory responses to oral vaccination

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Oral vaccination can prevent infectious diseases, particularly those that are spread via host-pathogen interactions at the gastrointestinal mucosa. For a successful oral vaccine (OV) response, the induction of immunological memory at the site of infection is critical, and Tissue resident memory cells (TRMs) are crucial players in the maintenance of long-term immunity

against mucosal pathogens. Here, we wanted to determine the factors that drive CD4 T-cell memory responses to OVs in the intestinal mucosa. We used the E.coli double mutant heat-labile toxin (dmLT) as a vaccine, to analyze the gene-expression patterns that are required for a successful OV response. Using dmLT-specific MHCII tetramers, we isolated intestinal vaccine-specific memory CD4 T-cells at various timepoints post-vaccination and identified genes associated with long-term survival in the intestine through RNAseq. The transcriptome of intestinal LT-specific CD4 T cells greatly resembled that of CD8 TRMS. Further, the transcription factor Krüppel-like factor 4 (KLF4) was enriched in CD4 TRMS in the siLP. Mice lacking KLF4 within CD4 T cells (CD4 Cre KLF4 fl/fl) had fewer vaccine-specific intestinal CD4 T cells, impaired intestinal IL-17 responses and were unable to restrict invasive infection by an LT-peptide expressing *Listeria*. Collectively, these findings reveal a role for KLF4 expression in the persistence and accumulation of intestinal CD4 TRM memory cells and establish KLF4 as a key transcription factor that controls CD4 T-cell memory responses to oral antigens.

## Lactobacillus acidophilus as an orally delivered mucosal vaccine platform

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Mucosal vaccines are currently limited to attenuated live or modified pathogens which confers risk of reversion to virulence, inability to be used in immune compromised individuals, and unpredictable immune response in altered mucosal environments. Subunit vaccines are attractive as next generation mucosal vaccines but often fail to induce robust immune responses. We have developed the Gram-positive lactic acid probiotic bacterium *Lactobacillus acidophilus* (LA) as an oral subunit vaccine platform. LA offers many advantages including gastric acid and bile resistance, oral administration, and heat stability. In addition, it expresses endogenous molecular patterns that activate innate immune receptors including Toll-like receptor (TLR) 2, Nucleotide-binding oligomerization domain-containing protein 2 (NOD2), and dendritic cell (DC)-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin (DC-SIGN). Here we present numerous LA subunit vaccines expressing viral epitopes

against various mucosal pathogens including HIV-1, rotavirus, SARS-CoV-2, and feline enteric coronavirus (FECV). Additionally, we have surface-displayed the immune stimulating adjuvants TLR5 ligand *Salmonella typhimurium* FliC and the microfold cell targeting protein and TLR4 adjuvant E. coli FimH. Adjuvant expression by LA results in differential induction of innate immune responses and increases LA trafficking to the mesenteric lymph nodes. Oral delivery of LA vaccine strains to mice and cats (FECV) induces anti-viral antibodies and B cells at local and distant mucosal sites, and there is a decreased duration of rotavirus shedding following challenge in LA-rotavirus vaccinated mice. LA expressing viral epitopes and adjuvants has the potential to be a powerful next generation oral vaccine platform against mucosal pathogens.

## LKB1 in intestinal epithelial cells regulates bile acid metabolism by modulating FGF15/19 production

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Background & Aim Liver kinase B1 (LKB1) is a master upstream protein kinase involved in nutrient sensing and glucose and lipid metabolism in many tissues; however, its metabolic role in intestinal epithelial cells (IEC) remains unclear. In this study, we investigated the regulatory role of LKB1 on bile acid (BA) homeostasis. Methods We generated mice with IEC-specific deletion of LKB1 (LKB1 D IEC) and analyzed the characteristics of IEC development and BA level. In vitro assays with small interfering RNA, liquid chromatography (LC) / mass spectrometry (MS/MS), metagenomics, and RNA-sequencing were used to elucidate the regulatory mechanisms underlying perturbed BA homeostasis. Results LKB1 deletion resulted in abnormal differentiation of secretory cell lineages. Unexpectedly, BA pool size increased substantially in LKB1 D IEC mice. A significant reduction of the farnesoid X receptor (FXR) target genes, including fibroblast growth factor 15/19 (FGF15/19), known to inhibit BA synthesis, was found in the small intestine (SI) ileum of LKB1 D IEC mice. We observed that LKB1 depletion reduced FGF15/19 protein level in human IECs in vitro. Additionally, a lower abundance of bile salt hydrolase-producing bacteria and elevated levels of FXR antagonist (i.e., T-bMCA) were observed in the SI of LKB1 D IEC mice. Moreover, LKB1 D IEC mice showed impaired conversion of

retinol to retinoic acids in the SI ileum. Subsequently, vitamin A treatment failed to induce FGF15 production. Thus, LKB1 D IEC mice fed with a high-fat diet showed improved glucose tolerance and increased energy expenditure. Conclusions LKB1 in IECs manages BA homeostasis by controlling FGF15/19 production.

### Local SARS-CoV-2 peptide-specific Immune Responses in Convalescent and Uninfected Human Lung Tissue Models

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Multi-specific and long-lasting T cell immunity have been recognized as indicators for long term protection against pathogens including the novel coronavirus SARS-CoV-2, the causative agent of the COVID-19 pandemic. Functional significance of peripheral memory T cells in individuals recovering from COVID-19 (COVID-19 + ) are beginning to be appreciated; but little is known about lung resident memory T cells (lung TRM) in SARS-CoV-2 infection. Here, we utilize a perfused three dimensional (3D) human lung tissue model and identify pre-existing local T cell immunity against SARS-CoV-2 proteins in lung tissues. We report ex vivo maintenance of functional multi-specific IFN- $\gamma$  secreting lung TRM in COVID-19 + and their induction in lung tissues of vaccinated COVID-19 + . Importantly, we identify SARS-CoV-2 peptide-responding B cells and IgA + plasma cells in lung tissues of COVID-19 + in ex vivo 3D-tissue models. Our study highlights the importance of balanced and local anti-viral immune response in the lung with persistent induction of TRM and IgA + plasma cells for future protection against SARS-CoV-2 infection. Further, our data suggest that inclusion of multiple viral antigens in vaccine approaches may broaden the functional profile of memory T cells to combat the severity of coronavirus infection.

**Longitudinal analysis in a Hnf4a $\Delta$ IEC mouse colitis model reveals that preclinical flaring is associated with increased Akkermansia muciniphila levels in the gut microbiota**

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Development of inflammatory bowel diseases (IBD) has been linked to altered homeostatic relationships between host and microbiota. Early "preclinical" stages of IBD are relatively understudied, especially with respect to host-microbiota interactions. Hepatocyte nuclear factor 4 alpha (HNF4A), a key transcription factor regulating intestinal epithelial cell (IEC) gene expression, is linked to human IBD. IEC-specific knockout of HNF4A in mice ( Hnf4a  $\Delta$ IEC ) leads to spontaneous colonic inflammation after 6-12 months. We previously discovered that HNF4A is a novel mediator of host-microbiota interaction in IECs, which is suppressed by microbiota. Here we investigated how early stages of spontaneous intestinal inflammation develop in Hnf4a  $\Delta$ IEC mice, and whether microbiota drive disease development in this model. We found that most Hnf4a  $\Delta$ IEC mice exhibit flares of fecal lipocalin 2 (LCN2) and episodic loose stools as early as 5 weeks of age, with a subset developing consistently elevated fecal LCN2 and mild histopathological features of colitis by 12 months. We demonstrated that these phenotypes were microbiota-dependent using both antibiotic treatment experiments and germ-free derivation of Hnf4a  $\Delta$ IEC mice. The microbiota composition of a subset of Hnf4a  $\Delta$ IEC mice diverged from wild-type and heterozygous controls between 6-9 weeks of age. Hnf4a  $\Delta$ IEC genotype as well as LCN2 flaring onset correlated with increased relative abundance of Akkermansia muciniphila during this time. An A. muciniphila strain isolated from an aged, inflamed Hnf4a  $\Delta$ IEC mouse was found to be genetically distinct from 52 other A. muciniphila human and mouse isolates. Future studies in this model may yield insights into host-microbiota interactions during preclinical stages of IBD.

### Long-term alteration of airway epithelial cells after early-life RSV infection mediated by IL-1R activation

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The development of asthma and lung function deficit in childhood has been associated with bronchial responsiveness

in infants. Respiratory Syncytial Virus (RSV) infection is the most prominent cause of bronchiolitis and childhood hospitalization in infants under six months old. In this study, we have demonstrated that early-life RSV infection (EL-RSV) alters the expression of epithelial cell cytokines IL-33, TSLP, and CCL2 in alveolar epithelial cells type 2 (AT2) isolated from neonate mice infected at 7 days old (EL-RSV infection). AT2 harvested at 4 weeks post-EL-RSV infection presented upregulation of TSLP and IL-33. The mechanism for these alterations was associated with increased active mark H3K4 trimethylated in the promoters of the epithelial-derived cytokines, as assessed by ChIP analysis. To understand if airway epithelial cells of EL-RSV infected mice were hyperreactive toward inflammatory stimuli, we challenged mice at 4 weeks post-EL-RSV infection with inhaled allergen *Alternaria alternata* for five consecutive days. In addition, we evaluated the production of epithelial cell cytokines in the lung and bronchioalveolar lavages. We observed that EL-RSV infected mice upregulated the expression and production of IL-33 and TSLP in the airways after *A. alternata* administration. However, these changes were reversed when neonate mice were treated with IL-1ra (anakinra) during the EL-RSV infection. These results suggest that EL-RSV infection generated long-term alteration on AT2 cells due to IL-1b signaling mechanisms. This modification may play a critical role in the predisposition to asthma after EL-RSV infection.

### Long-term consequences of maternal breast milk antibodies on mucosal immunity and homeostasis

Bingjie Wang - Fred Hutch, Meghan Koch

We have previously shown that maternal antibody deficient mice exhibit dysregulated intestinal homeostasis. These mice harbor increased numbers of live bacteria in the mesenteric lymph nodes and exhibit perturbations in mucosal immunity, including elevated T follicular helper (Tfh) cell and germinal center (GC) B cell responses in the gut-associated lymphoid tissues at the time of weaning. Importantly, microbes are needed to drive these responses as germ-free offspring lacking maternal antibodies do not exhibit altered intestinal immunity. Tfh and GC B cell responses can lead to the production of high affinity, T-dependent antibodies, which can persist up to the lifetime of the host. Antibodies are key regulators of host-microbiome interactions. To explore the long-term consequences of maternal antibodies on intestinal

immunity, we performed 16s rRNA sequencing of intestinal microbes to look at alterations in intestinal composition, RT-PCR of the bacterial 16s rRNA gene to look at abundance of wall-associated microbes, infection studies with *Salmonella typhimurium* and *Citrobacter rodentium* to look at susceptibility to small and large intestinal pathogens, and fecal IgA ELISAs to look at intestinal antibody levels. We found no differences between mice receiving or lacking maternal antibodies across all of the tested parameters. In summary, our data suggest that maternal antibodies do not alter intestinal microbial composition and abundance, susceptibility to intestinal pathogens, and fecal IgA levels. Future directions include exploring whether the elevated Tfh/GC B cell response is acting in a compensatory fashion to restore homeostasis in the maternal antibody deficient group.

### Long-term persistence of *Candida albicans* in the murine gastrointestinal tract

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*Candida albicans* is a dimorphic fungus that infects mucosal surfaces of humans. Although often considered a commensal organism, *C. albicans* has the potential to cause severe disease in immunocompromised individuals and has been associated with multiple chronic diseases, including allergic airway disease, inflammatory bowel disease, and Alzheimer's Disease. Despite its pathogenic potential, and the fact that *C. albicans* can be effectively cleared from other non-gastrointestinal (GI) mucosal sites, *C. albicans* both colonizes and persists within the GI tract of a large portion of the human population. Understanding how *C. albicans* interacts with the host immune system and intestinal tissue is important for informing how intestinal colonization with *C. albicans* plays a role in systemic infection and chronic disease. However, the mechanisms by which *C. albicans* avoids elimination from the gut are unclear. To investigate these mechanisms, we established a chronic murine model of *C. albicans* intestinal infection which we are using to study the host-fungal interactions within the GI tract which affect fungal colonization and persistence. Based on our studies in this model, we hypothesize that persistent infection with *C. albicans* is accomplished through the expression of multiple virulence factors required for gut epithelial invasion. Understanding how *C. albicans* persists within the GI tract will critically elucidate



the widespread presence of this fungus in humans and its participation in multiple chronic diseases.

### Loss of Junctional Adhesion Molecule-A in the intestinal epithelium results in increased severity in a murine model of food allergy

Catherine Ptaschinski - University of Michigan, Anny-Claude Luissint, Charles A. Parkos, Nicholas W. Lukacs

Junctional Adhesion Molecule-A (JAM-A) is a tight junction transmembrane protein that plays a major role in the maintenance of barrier function in epithelia and endothelia. In the small intestine, JAM-A has been shown to be important in epithelial barrier function, and mice that lack JAM-A (JAM-A <sup>-/-</sup>) have increased intestinal permeability. We hypothesized that these JAM-A <sup>-/-</sup> mice would have more severe food-induced anaphylaxis in a model of food allergy due to the enhanced ability of antigens to pass through the epithelial barrier and activate the immune cells in the lamina propria. We sensitized mice systemically with ovalbumin (OVA) adsorbed to alum, followed two weeks later by oral gavage with OVA three times per week. We found that JAM-A <sup>-/-</sup> mice had severely enhanced symptoms of anaphylaxis, including a significant drop in body temperature compared to wild-type controls. These animals also had an increased number of mast cells in the small intestine as analyzed by histology and enhanced mast cell activation as determined by serum levels of mMCP-1. In order to specifically confirm the role of JAM-A in the intestinal epithelium, we used *Jama* f/f -Villin Cre mice, in which *Jama* is deleted only in these epithelial cells. We again found increased severity of anaphylaxis, including a decrease in body temperature and increased numbers of activated mast cells. Together, these data provide evidence that impaired barrier function in the small intestine enhances the severity of food allergic reactions.

### Loss of Paneth cells promotes epithelial synucleinopathy in Parkinson's disease

SzuJu Chen - NTUH, Hung-Yu Chiang, Yi-Ting Weng, Chin-Hsien Lin, Jr-Wen Shui

Parkinson's disease (PD) is the second most common neurodegenerative disorder characterized by progressive

motor dysfunction relating to intraneuronal  $\alpha$ -synuclein aggregation and dopaminergic neuronal degeneration at central nervous system. Pathological study suggested that synucleinopathy in PD may start at gastrointestinal (GI) tract and later spread to brain via cell-to-cell transmission, yet, the mechanism initiating GI synucleinopathy is currently unknown. Changes in gut microenvironment including gut dysbiosis and impaired anti-microbial immunity, trigger dopaminergic neuronal degeneration and related motor dysfunction. As such, patients with inflammatory bowel disease have higher PD risk and share with PD common genetic risk factors, such as dysfunction of Paneth cell, the main epithelial cell that senses microbes and secretes anti-microbial agents to maintain epithelial barrier homeostasis. Herein, we propose that loss of Paneth cells may promote epithelial synucleinopathy during early MPTP-induced PD pathogenesis. We observed that, in Paneth cell-ablated mice at the early stage of PD induction (day-3) prior to motor dysfunction (day-5), the amount of  $\alpha$ -synuclein accumulation was significantly increased in ileal crypt cells, but not enteric Tubb3 + neurons in the lamina propria. Intriguingly, there was more monodansylcadaverine (MDC + ) autophagosomes in Tubb3 + neurons and TMEM119 + microglial cells, but not in crypt cells, suggesting epithelial  $\alpha$ -synuclein accumulation might initiate autophagy blockade in neurons that would promote synucleinopathy at the later stage. Taken together, our findings reveal that epithelial barrier dysfunction might promote an early epithelium-neuron transmission of synucleinopathy and autophagy blockade during PD initiation.

### LT $\beta$ R signaling promotes anti-CD137-mediated hepatotoxicity by inducing bystander CD11c+CD8+ effector T cells

Anna Korchagina - The University of Texas Health Science Center at San Antonio, Anna Korchagina, Ekaterina Koroleva, Peter Dube, Alexei Tumanov

Cancer immunotherapy causes immune-related adverse events (irAEs) in multiple organs including mucosal surfaces. Costimulatory molecule CD137 (4-1BB) represents an attractive target for cancer therapy. Unfortunately,  $\alpha$ CD137 agonistic antibody (ab) treatment also leads to antigen-independent expansion of CD8 + T cells in the liver and lungs causing tissue damage. The goal of this work was to define the mechanism of CD8 + T cell expansion and tissue damage

following  $\alpha$ CD137 ab treatment. We found that CD11c + and KLRG1 + cells were major subsets of infiltrated CD8 + T cells. CD11c + CD8 + effector T cells express high levels of genes associated with cytotoxicity: *Ifng*, *Gzma*, *Gzmb* and *Prf1*. Differentiation of CD11c + CD8 + T cells was accompanied by accumulation of dendritic cells (DCs) towards T cell zone in the spleen. We further found that genetic ablation of LT $\beta$ R-LT $\beta$  signaling, a critical pathway for the maintenance of lymphoid tissue architecture and DC development, prevented the accumulation of CD11c + CD8 + T cells. Pharmacological blockade with LT $\beta$ R-Fc, a soluble decoy fusion protein, prevented CD11c + CD8 + T cell accumulation and abrogated hepatotoxicity. Importantly, LT $\beta$ R-Fc treatment did not impair anti-tumor potency of  $\alpha$ CD137 ab. We propose that LT $\beta$ R signaling on fibroblastic reticular cells (FRC) promotes migration of DCs to T cell zone in the spleen via CCL19/CCL21 pathway, which in turn promote co-stimulation of CD8 + T cells in an antigen-independent manner. In summary, our work identified LT $\beta$ R as a critical regulator of bystander CD11c + CD8 + T cells, which promote hepatotoxicity following  $\alpha$ CD137 antibody treatment. Funding: William and Ella Owens Medical Research Foundation, The Peter Bradley Carlson Trust.

## Lung injury enhances resistance to influenza virus infection

Sang-Uk Seo - The Catholic University of Korea, Jae-Hyeon Jeong, Hyun-Jeong Ko, Mi-Na Kweon

Lung injury results in the release of self DNA and induces the production of type I interferon (IFN-I). Mice treated with bleomycin (BLM), which induces lung injury, were more resistant to influenza virus infection and exhibited higher levels of IFN-I transcription during the early infection period than PBS-treated control mice. By contrast, BLM-treated IFN-I receptor 1 (IFNAR1) knockout mice failed to show this attenuated phenotype, indicating that ALI-induced IFN-I is a key to antiviral response. Depletion of pDCs reduced the effect of BLM against influenza virus infection, suggesting that pDCs are the major source of IFN-I and are crucial for defense against viral infection after BLM-induced lung injury. Overall, our study showed that BLM-mediated lung injury potentiated IFN-I-dependent pulmonary viral resistance in mice.

## Lymphotoxin promotes IFN $\gamma$ -driven intestinal pathology in infectious colitis

Anna Korchagina - The University of Texas Health Science Center at San Antonio, Xi Jing, Sergey A. Shein, Ekaterina Koroleva, Alexei V. Tumanov

Enteric infections are a common cause of microbial dysbiosis which can exacerbate inflammatory bowel disease. *Campylobacter jejuni* (C. jejuni) is a foodborne pathogen and is the main causative agent of human gastroenteritis. Our recent study identified the critical role of IFN $\gamma$ -producing ILCs and T cells in driving C. jejuni -induced intestinal pathology, yet the mechanisms leading to activation of pathogenic IFN $\gamma$ -producing cells remain largely unknown. In this study, we identified lymphotoxin beta receptor (LT $\beta$ R), a member of TNFR cytokine receptor superfamily, as a critical regulator of C. jejuni -induced colitis. LT $\beta$ R -/- mice displayed reduced intestinal pathology caused by C. jejuni and decreased levels of IFN $\gamma$  and IL-12 in the colon. Based on these findings we hypothesized that LT $\beta$ R signaling promotes C. jejuni -induced intestinal inflammation by promoting IFN $\gamma$  production by ILCs and T cells. Mice with genetic inactivation of LT $\beta$ R in dendritic cells showed reduced intestinal pathology with decreased levels of IFN $\gamma$  and IL-12 in the colon. Flow cytometry analysis revealed reduced numbers of IFN $\gamma$  producing ILC1s in the colon in LT $\beta$ R -/- mice. Furthermore, LT $\beta$ R-deficient bone-marrow derived dendritic cells produced less IL-12 after C. jejuni stimulation in vitro. LT $\beta$ R interacts with its two ligands, lymphotoxin (LT) or LIGHT which are primarily produced by lymphoid cells. LT $\beta$  -/- mice but not LIGHT -/- mice exhibited reduced intestinal pathology and reduced levels of IFN $\gamma$  and IL-12 in the colon. Thus, our results reveal a previously unappreciated role of LT $\beta$ R signaling in controlling IFN $\gamma$ -driven responses by ILCs and T cells, thereby promoting C. jejuni -induced intestinal pathology. Funding: This research was supported by grant from NIH (AI135574).

## Manipulation of the intestinal epithelium by H. polygyrus

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Many parasitic helminths establish long-term chronic infections. This is thought to be due to their ability to alter the environment around them through secreted substances. Although a number of immunomodulatory proteins have now been defined that interfere with host immune cell responses, little research has been done to see if they impact the epithelial layer, which serves as the first point of contact and a critical barrier to infection; the epithelium also acts as an innate immune effector population through the products released by goblet cells and tuft cells which promote anti-helminth immunity. With both in vitro and in vivo techniques, we study this connection using *Heligmosomoides polygyrus*, a mouse helminth parasite that employs various immunomodulatory mechanisms to induce chronic infections. *H. polygyrus* excretory/secretory products (HES) were used to treat small intestinal cell organoid cultures (enteroids), revealing a wide range of effects on developmental pathways and the suppression of gene sets expressed by goblet, Paneth, and tuft cells. Furthermore, organoid morphology was drastically altered, with HES causing a spheroid, proliferative phenotype devoid of crypts and differentiated cells. In vivo, tuft cell induction by both succinate and the non-resident helminth *Nippostrongylus brasiliensis* was reduced in the presence of *H. polygyrus*. HES also reduced succinate-stimulated tuft cell expansion. Thus, chronic infection with *H. polygyrus* may prevent the development of a crucial epithelial cell for immune defence, allowing the parasite to survive. Our findings show that helminth parasites have an impact on their hosts that extends beyond the classical immune system, and that they can also alter the intestinal environment to their advantage.

### **Mast cells play a sexually dimorphic immunoregulatory role in ulcerative colitis**

Olivia Lenz - University of Pennsylvania School of Veterinary Medicine, Gauri Lahoti, Reilly Nakamoto, Elizabeth Lennon

Ulcerative colitis (UC) is a devastating immune-mediated condition that affects approximately 1 million people in the United States. Though prevalence is similar in males and females, UC sequelae differ greatly between sexes by unknown mechanisms. Colonic mucosal mast cells are dysregulated in UC and recent studies reveal a context-dependent immunoregulatory potential far more nuanced than their widely recognized pro-inflammatory role. The pro-inflammatory properties of mast cells are sexually dimorphic:

female mast cells contain greater numbers of granule-associated mediators, and in a mast cell-dependent anaphylaxis model, female mice exhibited a more severe response than males. However, whether mast cell immunoregulatory properties differ between sexes is unknown. Our previous work in a chronic, spontaneous colitis model demonstrated that mast cell-sufficient mice had milder colitis than mast cell-deficient mice, and that mast cell reconstitution ameliorated colitis. This suggests that mast cells suppress inflammation in this model, however sex differences were not investigated. Here, we hypothesized that mast cells play an immunoregulatory role in colitis in a sexually dimorphic manner. We applied the well-characterized oxazolone model of UC and discovered that female mast cell-deficient mice experienced more severe and prolonged colitis, as well as elevated colonic IL-13 compared to wild-type controls. Male mast cell-deficient mice did not show these differences. Reconstitution of female mast cell-deficient mice with female-derived mast cells partially rescued the colitis phenotype. Together, these results suggest that mast cells play a sexually dimorphic immunoregulatory role in UC, and that further studies are required to define downstream sex-based differences in UC pathogenesis.

### **Maternal EGF provides protection from pathogen translocation in the intestines of VLBW infants**

Kathryn Knoop - Mayo Clinic, Kara Greenfield, Olivia Harlow, Emily Levy, Jane Brumbaugh

The intestinal epithelial barrier is often a site of pathogen entry requiring protection from invading bacteria. Neonates, particularly those born prematurely or at a very low birth weight (VLBW), are at an increased risk for late-onset sepsis which can be caused by gut-resident pathogens. Amongst the many beneficial functions of breast milk is the reduction of risk of pathogen translocation through epidermal growth factor (EGF). We have previously shown maternal EGF is directly sensed by intestinal epithelial cells, including goblet cells, in the neonate to inhibit bacterial translocation and reduce incidence of disease in an animal model of late-onset sepsis. Sepsis can result from a variety of pathogenic bacteria and, unsurprisingly, through metagenomic sequencing of stool from VLBW infants we observed potential pathogenic organisms such as *Staphylococcus aureus*, *Enterobacter cloacae*, *Serratia marcescens*, and Group B *Streptococcus* (GBS). In mice, we

observed these gut-resident pathogens were able to utilize goblet cells to disseminate and cause disease. In a cohort of VLBW infants, we observed a strong correlation between concentrations of EGF in the diet and stool of infants. Formula-fed infants contained significantly less EGF in both diet and stool, and EGF concentrations in donor milk were reduced compared to maternal milk. Thus appropriate concentrations of EGF from the diet could protect from multiple pathogens disseminating from the intestine and the relationship of EGF in the diet and stool could be used to predict risk of disease of late-onset sepsis.

### The maternal microbiota during pregnancy modifies the neonatal epigenome to reinforce intestinal barrier integrity

Cristina Kalbermatter - University of Bern, Nerea Fernandez Trigo, Sandro Christensen, Hai Li, Aart Mookhoek, Mercedes Gomez de Agüero, Andrew J. Macpherson, Stephanie C. Ganai-Vonarburg

Birth represents a disruption from the sheltered environment in utero to the microbial-rich world. The innate immune system and breast milk provide crucial defense elements to protect the newborn from potential infections early in life. However, the starting point of gathering our immune strategies happens before birth. Herein, the maternal microbiota plays a vital role in priming fetal immune development by transferring microbial metabolites via the placenta. Using the auxotrophic *E. coli* strain HA107, it is possible to reversibly colonize germ-free pregnant dams during gestation and distinguish between the contribution of the maternal microbiota from the influence of postnatal colonization. Since changes in gene expression due to in utero priming with HA107 remained until adulthood, we hypothesize that microbe-host interactions during pregnancy shape neonatal immunity and epithelial maturation by altering the epigenome. Transient gestational colonization triggered a DNA hypomethylation in genes relevant for intestinal epithelial cell homeostasis in the offspring. In contrast, postnatal colonization of pups born to germ-free dams could not reestablish these effects, indicating an exclusive and long-lasting role of the maternal microbiota. Moreover, the modifications of the epigenetic landscape in the neonatal intestine accelerated epithelial maturation and rendered the gestationally exposed offspring more resilient to cellular stress and intestinal colitis. Our research will reveal essential insights into epigenetic mechanisms due to interactions between the

maternal microbiota, the embryo, and the neonate. It will strengthen the importance of a healthy maternal microbiota during pregnancy for neonatal health and display its durable consequences.

### Maternal Supplementation with Lansoprazole Protects Newborn Mice Against Necrotizing Enterocolitis via Aryl Hydrocarbon Receptor Activation

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Background: Necrotizing enterocolitis (NEC) is the most common gastrointestinal disease in preterm infants with high morbidity and mortality rates, and the development of NEC is mediated by elevated signaling via Toll-like receptor 4 (TLR4) in the intestinal epithelium. Aryl hydrocarbon receptor (AHR) is a transcription factor that regulates the immune system. We hypothesize that supplementation with lansoprazole during pregnancy and lactation activates AHR, limits TLR4 signaling and prevents NEC in the offspring. Methods: Mouse enteroids or human explant culture were treated with lipopolysaccharide (LPS) or/and lansoprazole, and the activation of AHR and TLR4 were determined by induction of Cyp1a1 and pro-inflammatory cytokines (Il6 and Tnf), respectively. Wild type C57Bl/6 and AHR deficient (AHR<sup>-/-</sup>) mice were used. Endotoxemia was induced in 11-day-old mouse pups by administering LPS via intraperitoneal injection. Experimental NEC was induced by hypoxia and gavage feeding of formula containing bacteria from a patient with NEC in the 7-day-old newborn mice. Lansoprazole was administered to newborn mice during NEC induction or to the female mice during pregnancy and lactation. The expression of Cyp1a1, Tlr4 and Il6 and Tnf was quantified using qRT-PCR, and the intestinal morphology was analyzed using H&E staining. Results: Lansoprazole activates AHR as revealed by induced expression of Cyp1a1 and limits TLR4 signaling as revealed by reduced LPS-induced expression of Il6 and Tnf in mouse enteroids in vitro and human explant culture ex vivo. Lansoprazole induces the expression of Cyp1a1 in the intestinal epithelium and reduces LPS-induced expression of Tlr4, Il6 and Tnf when fed to wild-type but not Ahr<sup>-/-</sup> newborn mice in vivo. Administration of lansoprazole significantly reduced NEC severity in wild type but not Ahr<sup>-/-</sup> newborn



mice, as revealed by improved histology and reduced expression of Il6 and Tnf. Administration of lansoprazole to pregnant female mice activates AHR in utero and prevents NEC in the offspring. Conclusions: We show that administration of lansoprazole during pregnancy and lactation reduces NEC severity via AHR activation and TLR4 inhibition, suggesting lansoprazole may serve as a novel agent that can be administered either during pregnancy or postnatally for the prevention and treatment of NEC.

### Measuring microbiota-directed IgA responses in a murine model of intergenerational undernutrition

Yadeliz Serrano Matos - University of Virginia, Carrie Cowardin, Jasmine Cano

Linear growth stunting due to undernutrition impacts over 150 million children under five in developing countries. The immature gut microbiota found in undernourished children is thought to play an important role in promoting stunting. Children with undernutrition also have altered intestinal immunity, including high levels of secretory Immunoglobulin A (IgA). Although stunted mothers are more likely to give birth to stunted children, the contribution of the gut microbiota transmitted from mother to baby in the development of stunting is not well understood. To address this knowledge gap, our lab has developed a mouse model of intergenerational undernutrition to study microbiota-dependent phenotypes in gnotobiotic mice. In this model, germ-free dams and sires are colonized with microbiota from a healthy or stunted human infant donor prior to breeding. Resulting pups are then weaned onto an undernourished diet until maturity. The pups born to dams colonized with the stunted microbiota ("SD pups") weigh significantly less than pups born to dams colonized with healthy donor microbiota ("HD pups"). Furthermore, IgA levels in serum and fecal samples were significantly higher in SD pups compared to HD pups. To determine the enrichment of specific taxa bound to IgA (IgA+) in stunted mice, we performed Bug-FACS/IgA-Seq. We observed increased targeting of the microbiota in fecal samples from SD pups compared to HD pups. This finding suggests microbes in the gut of stunted pups drive mucosal immune activation and IgA production, which could be used to identify critical bacterial species that cause reduced growth.

### Memory Th2s integrate into the tuft-ILC2 circuit to provide protective immunity to helminth infection

Alison Stanbery - University of Washington, Lily Webeck, Jakob von Moltke

Both helminth infection and allergic responses involve type 2 immune activation at barrier tissues. Type 2 immune activation is characterized by cell recruitment, mucus production, and tissue remodeling mediated by IL-13-secreting ILC2s and T helper 2 (Th2) cells. When activated chronically by allergens, tissue-resident Th2 cells (Th2 Trm) can cause morbidity, while ILC2s and circulating Th2s play important roles in clearing helminth infection. During acute helminth infection in the small intestine, epithelial tuft cells secrete IL-25 to activate ILC2s. ILC2-derived IL-13 acts on epithelial crypt progenitors to promote differentiation and increased frequency of tuft cells, thereby establishing a feed-forward tuft-ILC2 circuit that mediates epithelial remodeling and worm expulsion. It is unknown if Th2 cells can integrate into this circuit. Here we show that tuft cells contribute to Th2 Trm generation, and that Th2 Trm regulate tuft cell frequency. Using an in vivo model to permanently label and track cytokine-producing Th2 cells, we found distinct populations of lineage-traced Th2 Trm within intestinal and peripheral tissues. In particular, intestinal Th2 Trm express the receptor for IL-25, while Th2 Trm in adipose tissues express the IL-33 receptor. Loss of tuft cells results in a defect in the generation of Th2 Trm, leading to greater worm burdens and a defect in serum levels of IgE following reinfection. Finally, we found that helminth-induced Th2 are necessary for tuft cell hyperplasia during chronic primary infection and sufficient to induce tuft cell expansion and worm clearance during reinfection. Together, our data indicate that the tuft-ILC2 circuit can be rewired to incorporate Th2 cells and provide the first evidence that intestinal tuft cells contribute to adaptive immunity to helminths.

### MerTk and Axl efferocytic receptors have differential role in lung homeostasis and silicosis

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Efferocytosis is paramount to regulate homeostasis and inflammation, particularly in an environment with continuous cell turnover and colonized by microbiota, such as the lungs. The TAM receptor family (Tyro3, Axl and MerTk) mediates efferocytosis and inhibits pro-inflammatory pathways through Gas6 or Protein S binding to phosphatidylserine on apoptotic cells. Here we investigated how MerTk and Axl regulate lung homeostasis and inflammation, using silicosis as a study model. During homeostasis, lung cells and AMs from wild-type (WT) mice showed high expression of Axl, MerTk, and Gas6 by RT-PCR. BALFs from MerTk  $-/-$  mice had a higher frequency and numbers of AMs and neutrophils compared to Axl  $-/-$  or WT mice. Also, Lungs from MerTk  $-/-$  mice had higher expression of CXCL1, CXCL2, TNF- $\alpha$ , and IL-6, and enhanced frequency and number of AMs, monocytes, and neutrophils, impaired lung parenchyma structure, and higher number of MHCII $^{+}$  AMs compared to Axl  $-/-$  or WT mice. We also found an upregulation of MerTk in Axl  $-/-$  AMs, suggesting an important role of MerTk in regulating homeostasis of pulmonary mucosa. On the other hand, during silicosis, Axl seems to have an important role in regulating inflammation, since we found more cells in the BALFs and lungs of SIL-Axl compared to the SIL-MerTk or SIL-WT groups. Particularly, SIL-Axl had higher numbers of AMs and neutrophils, higher levels of CXCL1, TGF- $\beta$ , and lower levels of IL-10 compared to the SIL-MerTk or SIL-WT groups. Finally, we found that both SIL-WT and SIL-MerTk upregulated mRNA for Axl post silica exposure compared to the SIL-Axl group. Collectively, our data suggest that MerTk and Axl are dedicated to maintain homeostasis/tolerance and control inflammation in the lungs, respectively, pointing out that these functional differences must be taken into account in the design and application of TAM-targeted therapy. Financial support: FAPERJ, CNPq, CAPES

### Metabolic dysregulation induces impaired lymphocyte memory formation during severe SARS-CoV-2 infection

Sanjeev Gurshaney - University of Central Florida, Hung Nguyen, Anamaria Morales Alvarez, Hue Bui

Metabolic dysregulation accompanying SARS-CoV-2 infection is a key determinant of disease severity. In this study, we performed extensive data-mining of multiple existing single-cell RNA seq datasets of COVID-19 BALFs, in combination with high-dimensional immune cell profiling of PBMCs from COVID-19-infected patients, to get a comprehensive, systemic profile

of the immunometabolic regulation of adaptive immunity during severe COVID-19. Our results demonstrate that hypoxia, a hallmark of COVID-19 ARDS, elicits a global metabolic reprogramming in effector lymphocytes; in response to oxygen and nutrient-deprived microenvironments, these cells were observed to shift dependence from aerobic respiration to anaerobic processes including glycolysis, mitophagy, and glutaminolysis to fulfill bioenergetic demands. We demonstrate that metabolic dysregulation of ciliated lung epithelial cells is linked to significant increase of proinflammatory cytokine secretion and upregulation of HLA class 1 machinery. We link augmented epithelial HLA class-1 antigen stimulation to a significant increase in cellular exhaustion of metabolically dysregulated CD8 and NK cells, leading to impaired differentiation into multiple memory cell-types, including lung CD8 tissue-resident memory cells. Using unsupervised clustering techniques, along with a novel single-cell metabolomics assay, we reveal multiple distinct, differentially abundant CD8 and NK memory cell states that are marked by high glycolytic flux, mitochondrial dysfunction, and cellular exhaustion, further highlighting the connection between disrupted metabolism and impaired memory cell function in COVID-19. We validate multiple metabolic inhibitors as potential therapeutic agents to restore lymphocyte function and memory cell differentiation. Overall, our findings provide novel insight on how SARS-CoV-2 infection affects host immunometabolism and anti-viral response.

### Metabolically stressful MUC2 mucin biosynthesis increases pro-inflammatory cytokine release from colonic goblet cells

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The colonic mucus layer is composed of MUC2 mucin produced by goblet cells that forms the first line of innate host defense. High MUC2 mucin biosynthesis and production induces endoplasmic reticulum (ER) stress. We hypothesize that stressed goblet cells produce elevated levels of pro-inflammatory cytokines in response to pathogens, adding another layer of innate host defense in the gut. The expression of the pro-inflammatory chemokines, interleukin-8 (IL-8) and monocyte-chemoattractant protein-1 (MCP-1), were analyzed by RT-qPCR for mRNA, and 15-plex Luminex array for protein, basally and in response to the mucus secretagogues, phorbol

myristate acetate (PMA), the colonic pathogen, live *Entamoeba histolytica* (Eh), and lysed soluble Eh proteins (SAP). ER stress was measured by the expression of ATF4 and GRP78 by RT-qPCR and Western blotting and following alleviation of ER stress with IL-22. MAPK signalling was analyzed by Western blotting. WT goblet cells expressed high levels of ER stress proteins and pro-inflammatory chemokines compared to MUC2 KO constitutively and in response to Eh and PMA. Alleviation of ER stress abrogated pro-inflammatory responses. Eh enhanced the activation of ERK/p38 MAPK pathways in WT cells that were abolished with specific pathway inhibitors. These results demonstrate that goblet cells under high ER stress triggered by MUC2 mucin biosynthesis activate the ERK/p38 MAPK signalling pathways basally and in response to inflammatory agonists to produce elevated levels of pro-inflammatory chemokines. In contrast, MUC2 KO cells showed dysregulated pro-inflammatory responses and altered MAPK signalling that could dysregulate innate host defences. Supported by CIHR.

### MHCII expression on Goblet cells is required for small intestinal homeostasis

Bibiana Barrios - Washington University of Saint Louis, Vini John, Alexandria Floyd, Jazmine E. David, Sreeram Udayan, Ellen M. Schill, Keely McDonald, Rodney Newberry

As the primary barrier separating the host from trillions of microbes, intestinal epithelial cells (IECs) are positioned to promote tolerance and immunity against pathogens. MHCII is highly expressed on IECs, however the role of MHCII expression by IECs has been controversial. Goblet cells (GCs) are specialized epithelial cells that have critical role in barrier maintenance. GC can form Goblet cells associated passage (GAPs) and deliver luminal antigens to lamina propria (LP) antigen presenting cells (APCs) in a manner capable of inducing adaptive immune responses. However, the role of GC MHC II expression is largely unknown. We found that MHCII is highly expressed on small intestinal epithelial cells with GCs have significantly increased expression of MHCII when compared to other IECs; with ileum GCs showing the highest expression. Interestingly, the induction of GAPs resulted in increased MHC II specifically on GCs and not on other epithelial cells. Abrogation of MHCII on GCs resulted in significant reduction of TCRb + CD4 + CD8a + cells small intestine intraepithelial lymphocytes. Furthermore, the absence

of MHCII on GCs, resulted in a reduction of transferred ovalbumin specific T cells in MLN in a model of tolerance induction to luminal antigens. RNAseq analysis on mice lacking MHCII expression in GCs revealed an expansion of B cell responses in the LP and significant increase in IgA+ cells in Peyer's patches. This data suggest that GC expression of MHCII might have a crucial role in direct antigen-specific responses and in regulating intestinal homeostasis.

### Microbial-dependent B cell proliferation is ubiquitously induced in ileal Peyer's patches of piglets

Mutsumi Furukawa - Tohoku University, Ryota Hirakawa, Takuho Okada, Ayako Miyazaki, Yoshihiro Muneta, Haruki Kitazawa, Hisashi Aso, Tomonori Nochi

The morphological characteristics of Peyer's patches (PPs) differ among the species in mammals. Pigs develop two types of PPs, scattered and continuous, in the jejunum and the ileum, respectively. However, the knowledge of the organogenesis and function of the two PPs in pigs has been inadequate. In this study, using conventional (CV) embryos, and CV and germ-free (GF) neonates, the differentiation and maturation of B cells in jejunal PPs (JPPs) and ileal PPs (IPPs) were investigated in pigs by immunohistochemistry using anti-CD20, anti-IgM and anti-Ki67 antibodies. In the IPPs of CV piglets, the lymphoid follicles were divided into two regions based on the expression level of IgM as CD20 + B cells expressing no/little IgM and abundant IgM in the marginal and central regions, respectively. In contrast, such distinction was not seen in the JPPs regardless of the presence of CD20 + B cells express either no/little IgM or abundant IgM. In CV piglets, Ki-67 + proliferating cells may form germinal centers (GCs) in JPPs, which are quite different from IPPs developing no classical GCs. In GF piglets, such cell proliferation was also observed in JPPs of GF piglets; however, IPPs were extremely immature due to the lack of the marginal and central regions. The organogenesis of IPPs in CV prenatal embryos was still underdeveloped because of incomplete formation of two distinct regions. These findings indicate that the structural and functional maturation of IPPs (not JPPs) depends on stimulation from microorganisms that cohabit in the gastrointestinal tract after birth.

### The microbiome drives IL-1b expression by gingival Ly6Chi monocytes in health.

Kelly Wemyss - University of Manchester, Flora A. McClure, Siddharth Krishnan, Hayley M. Bridgeman, Melissa A. E. Lawson, Richard K. Grenics, John R. Grainger, Joanne E. Konkel

Our understanding of innate cells located at barrier sites has expanded dramatically, in particular with regard to tissue-specific monocyte and macrophage populations located in the skin, intestine and lung. Relatively little is known about tissue-specific functions of these innate mediators in the gingiva, the mucosal barrier surrounding the teeth. Better understanding of innate cells in the gingiva will enhance understanding of gingival immune homeostasis and may herald the development of new therapeutics for periodontitis, the most common chronic inflammatory condition of mankind. We have previously demonstrated that Ly6C<sup>hi</sup> monocytes are present in healthy gingiva at frequencies much greater than at other barrier sites. We probed the functions of this enlarged population to determine their contribution to maintenance of gingival health. We show that Ly6C<sup>hi</sup> monocytes express high levels of the cytokine IL-1b without additional ex vivo stimulation, indicating monocytes residing in healthy gingival tissue are primed to make IL-1b. RNAseq analysis of whole gingiva tissue identified an increase in expression of components of the IL-1 signalling pathway between 1 and 3 weeks of age. At this timepoint, teeth erupt and weaning leads to increased bacterial numbers and altered microbial composition, whilst introduction of solid food leads to increased damage from mastication. Analysis of germ free and antibiotic treated animals identified reduced IL-1b expression by gingival monocytes, indicating regulation by the microbiome. Mono-colonisation of germ free mice and specific antibiotic depletion suggests that IL-1b is not universally induced by bacterial species. Combined our data begin to outline a commensal-induced IL-1b pathway in healthy gingiva.

### Milk-derived osteopontin supports the development of the intestinal immune system and microbiota

Kathleen McClanahan - Vanderbilt University, Danyvid Olivares-Villagómez

Breast-feeding is important for the growth and development of infants, delivering nutritional support as well as a wealth of immunological factors that provide protection while the infant immune system matures. A critical role of breastmilk is to promote the development of a proper gut microbiota, which lays the foundation for intestinal development and maturation of the infant immune system. A prominent breastmilk factor supporting this role is osteopontin, a highly glycosylated phosphoprotein involved in a range of physiological processes including bone mineralization, inflammation, wound healing, and homeostasis of lymphoid cells in the intestinal epithelium. Osteopontin is a major protein component of breastmilk, and its levels fluctuate among women based on factors including BMI, diet, and geographical location. On the other hand, commercial infant formulas contain little to no osteopontin. The impact of such diverse levels of osteopontin intake on the developing infant intestine and microbiota are poorly understood. We show that milk-derived osteopontin plays a critical role in the development of the murine intestinal microbiota, and that lack of osteopontin intake during the post-natal period leads to an altered intestinal immune compartment persisting into adulthood, accompanied by increased risk of intestinal inflammation and disease. Therefore, intake of milk-derived osteopontin has potential implications for both short- and long-term health outcomes. This work lays the foundation for studies of the effects of osteopontin supplementation on the human infant microbiota and intestinal immune system.

### Modulation of Anti-viral Immunity in Neonates by Maternal Antibody

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Rotavirus (RV) induced diarrhea remains a leading cause of illness and death in young children in low-income countries, which is in part due to the poor efficacy of available vaccines specifically in developing countries. Various correlative studies have suggested a role for maternally derived antibodies in the poor vaccine performance in countries in which RV is endemic. We here explore the role of preconception maternal RV infection on the RV-induced immune response of the offspring. We found that previously RV-exposed (immune) dams delivered high amounts of RV-specific IgA via the breast milk and that suckling pups were efficiently protected from diarrhea and active fecal RV shedding. The protection was primarily



mediated by breast milk instead of placental transfer of antibodies. To delineate consequences for long-term immune protection from viral assault in the context of passive, mother-derived, immune protection in early life, we next investigated whether asymptomatic virus exposure of pups born to immune dams nevertheless elicited an immune response. Indeed, we detected a comparable RV exposure-induced induction of mesenteric lymph node (mLN) germinal center B cells and total IgA plasmablasts in pups derived from naïve and immune dams. However, the RV-specific IgA response was dramatically reduced in pups from immune dams in both mLN and small intestinal lamina propria. This project provides a framework for further analysis of the mechanisms through which prior maternal RV exposure influences the adaptive immune response to RV infection and/or vaccination in the suckling offspring and will hopefully inform improved vaccine development.

## Molecular mechanisms related with post COVID19 syndrome

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Post-COVID syndrome is defined as a wide range of chronic symptoms that appear after infection, persist for more than 12 weeks and cannot be explained by an alternative diagnosis. Symptoms can fluctuate or cause relapses; being the most common fatigue, dyspnea, anxiety, depression, etc. Their biological mechanisms are nevertheless unknown. Hence, cell memory, cytokines and immune phenotyping using flow cytometry, multiplex and mass cytometry respectively were carried out in 84 post-COVID patients and 25 post-FLU 3, both of them 3 months after hospital discharge, compared with 17 pre-pandemic controls. Several cytokines and immune cell subsets were altered in post-COVID patients, referred to both the post-FLU and the pre-pandemic controls, revealing a post-COVID-specific immune signature (Figure 1). Moreover, post-

COVID patients displayed a higher T-cell basal activation which became more evident following CD3/CD28 stimulation confirming that the immune alterations are also functional rendering a more activated immune system in these patients. Machine learning models (random forest, SVM and K-means) were used to classify post-COVID patients by age, gender and severity (days in hospital and oxygen need) but no differences have been found between them. In summary, we hereby have demonstrated that: i) Post-COVID patients have immune sequelae 3 months after infection being those changes specific to SARS-CoV-2 infection; ii) Post-COVID patients have a pro-inflammatory T-cell phenotype, both in resting conditions and after stimulation; iii) Immune changes were independent of post-syndrome symptoms; iv) Post-COVID individuals behave as a single entity, not being able to stratify them through the machine learning models used.

## Morphine induced microbial dysbiosis drives intestinal inflammation and potentiates Neutrophil infiltration at gut mucosal surface.

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Prescription opioids are considered as gold standard to treat pain and is an important component of treatment. Although, the co-morbidities associated with chronic morphine use on systemic immune function has been well demonstrated but its effects on mucosal immunity and gut homeostasis are less well defined. We recently demonstrated that morphine treatment induces gut microbial dysbiosis, disrupts intestinal epithelial barrier and drives inflammation at mucosal surface. Neutrophils are innate immune cells that provide first line of defense against bacterial infection. Neutrophils mount an anti-microbial attack via phagocytosis and through neutrophil extracellular traps, which serve to concentrate the neutrophil's antimicrobial agents and trap and kill bacteria. While these activities are desirable during active infections, unrestricted neutrophil activation and movement can cause significant tissue damage. Our result shows that morphine treatment significantly increases the number of infiltrating neutrophils into small intestinal lamina propria which correlated with increased expression of neutrophil recruiting chemotactic factors CXCL1, CXCL2, CXCL3 and CXCL5 by epithelial cells. The pattern of neutrophil infiltration into intestine after morphine treatment

paralleled closely with the expression of pro-inflammatory cytokines TNF $\alpha$  and IL1 $\beta$  as confirmed by qPCR. Our data show that increased secretion of bio-reactive substances from infiltrated neutrophils not only contribute to tissue damage but also lead to change in the homeostatic microbiome composition with an increase in pathogenic bacteria and depletion of commensal bacteria. Future work focuses on devising new strategies to restore the microbial - immune homeostasis at the mucosal surface so that opioid can be used safely and effectively.

## **MUC5AC SNP rs28737416 is associated with tuberculosis meningitis susceptibility, mortality and CSF cytokine responses**

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**Background:** The factors leading to tuberculosis meningitis (TBM) are incompletely understood. The lung mucins MUC5B and MUC5AC influence local and systemic immune responses, but their role in TB pathogenesis is unknown. **Methods:** The association between four haplotype-tagging single nucleotide polymorphisms (SNPs) in MUC5B and MUC5AC and CSF cytokine concentrations was determined using Wilcoxon rank-sum testing. Associations between tagging SNPs and TBM susceptibility was assessed using linear regression. Following this screening study, the association between the MUC5AC promoter SNP rs28737416 and TBM mortality was evaluated in two independent cohorts. Participants in these cohorts all received dexamethasone therapy. Survival distributions were generated by Kaplan-Meier estimates and compared using log-rank testing. The association between rs28737416 and MUC5AC expression was assessed using the Genotype-Tissue Expression Project portal. **Results:** The presence of the T allele in MUC5AC SNP rs28737416 was associated with lower CSF concentrations of TNF( $p=1.9 \times 10^{-8}$ ) and IFN $\gamma$ ( $p=2.3 \times 10^{-6}$ ) and higher TBM susceptibility (odds ratio=1.24,  $p=0.02$ ). Mortality from TBM was higher among participants with the rs28737416 T/T or T/C genotypes (35/119, 30.4%) compared to the C/C genotype (11/89, 12.4%; log-rank  $p=0.005$ ) in a discovery cohort (N=211) and an independent validation cohort (N=87; 9/87, 19.1% vs 1/20,

2.5%; log-rank  $p=0.02$ ). The T allele was associated higher lung expression of MUC5AC mRNA. **Conclusions:** The rs28737416 T/T and T/C genotypes were associated with a higher risk of death from TBM and lower concentrations of CSF TNF and IFN $\gamma$  compared to the C/C genotype, suggesting this SNP may contribute to immune changes that influence TBM outcomes.

## **Mucolytic bacteria mediate fiber deprivation-induced colitis in IL-10 deficient mice colonized with a human synthetic microbiota**

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Combined with host genetic predisposition, the low-fiber Western diet and the gut microbiome have been proposed as major environmental contributors of inflammatory bowel diseases (IBD), but the mechanisms underlying these interactions are still unclear. Mucolytic gut bacteria are enzymatically equipped to forage the host intestinal mucus layer, and their activities are regulated by the availability of dietary fibers. We hypothesized that, under a fiber-deprived diet, mucolytic bacteria catalyze important disease-promoting immune pathways of IBD. Using a tractable gnotobiotic mouse model with a defined human gut microbiota, we show that fiber deprivation induces colitis in genetically-susceptible IL10<sup>-/-</sup> mice and that this dietary effect depends on the presence of mucolytic bacteria. Diet-induced inflammation was characterized by an infiltration of NK, Th1 and Th17 cells in the cecal and colonic lamina propria. In the colon, this was preceded by a loss of IgA-producing cells, which was reflected by reduced IgA-coating of specific bacteria species, including the mucus-specialist *Akkermansia muciniphila*. Removal of mucus-degrading bacteria from the microbial community abrogated the Th1 response, however the Th17 and IgA responses were unchanged. Our results highlight a multi-hit framework to unravel the complex interactions of diet, host, and microbial factors that contribute to IBD development.

## **Mucosal fungi promote gut barrier function and social behavior via Type 17 immunity**

Irina Leonardi - WCMC, Iliyan Iliev

Fungal communities (the mycobiota) are an integral part of the gut microbiota. Changes in the composition of the mycobiota can contribute to both local and gut-distal pathologies. We characterized the biogeography of the fungal communities along the gastrointestinal tract and identified a subset of fungi associated with the intestinal mucosa in both laboratory mice and humans. We thus investigated whether mucosal and luminal associated fungi could have distinct effects in their mammalian host. We assembled two distinct consortia of mucosa- and lumen-associated fungi and assessed their local and systemic effect on the murine host. Using a chemically induced intestinal injury model we showed that intestinal colonization with mucosa-associated fungi, but not lumen-associated fungi, protected mice against intestinal injury. We showed that mucosa-associated fungi induced a strong Type 17 immune response and reinforced intestinal epithelial function. The local response was characterized by the production of the cytokines IL-17A, IL-17F, and IL-22 whereas the systemic response was dominated by IL-17A. We showed that mucosa-associated fungi protected mice against intestinal injury via the local production of IL-22 by CD4 + T helper cells. We further showed that gut colonization with mucosa-associated fungi promoted social behavior in mice. Using transgenic mouse models, we showed that the effect of fungi on social behavior was mediated through IL-17R-dependent signaling in neurons. Our work demonstrates that mucosa-associated fungi are associated with host-protective immunity, epithelial barrier function and might be a driver of neuroimmune modulation of mouse behavior through distinct type 17 immune mechanisms.

### Mucosal memory T cells in breastmilk are modulated by SARS-CoV-2 mRNA vaccination

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We compared the phenotype, diversity, and antigen specificity of T cells in the breastmilk and peripheral blood of lactating individuals who received SARS-CoV-2 mRNA vaccination. Relative to blood, breastmilk contained higher frequencies of T

effector and central memory populations that expressed mucosal-homing markers. T cell receptor (TCR) sequence overlap was limited between blood and breastmilk. Overabundant breastmilk clones were observed in all individuals, were structurally diverse, and contained CDR3 sequences with known epitope specificity including to SARS-CoV-2 Spike. Spike-specific TCRs were more frequent in breastmilk compared to blood and expanded in breastmilk following a third mRNA vaccine dose. Our observations indicate that the lactating breast contains a distinct T cell population that can be modulated by maternal vaccination with potential implications for infant passive protection.

### Mucosal organoids capture Innate Lymphoid Cells tissue development and disease associated functions

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Innate Lymphoid Cells (ILC) develop from ILC precursors (ILCP) to provide a potent, antigen-non-specific source of cytokines at mucosal sites. Deciphering what local stimuli drive the differentiation and function of ILC in these tissues remains a pressing question, as ILC frequencies can become dysregulated in disease. For example, Type-1 innate lymphoid cells (ILC1) are enriched in the mucosa of patients with active inflammatory bowel disease (IBD) and the impact of this accumulation remains elusive. Here, we develop and use co-cultures of murine lung and gut organoids with ILCP and human intestinal organoids with mature ILC isolated from intestinal biopsies. Harnessing these versatile models, we demonstrate that murine intestinal and lung organoids provide a complex niche capable of supporting the maturation of all ILC. Notably, organoid identity was sufficient to robustly recapitulate tissue-specific ILC phenotype and distribution, even in the absence of microbial stimuli or cytokine supplementation. In addition, we show that murine ILC1 drive expansion of intestinal epithelial cells through TGFβ1. We further show that human gut ILC1 also express TGFβ1, drive epithelial and mesenchymal expression of CD44v6 and regulate extracellular matrix remodelling, suggesting that ILC1 enrichment may promote fibrosis and cancer in IBD patients. Taken together, our work provides unprecedented insight into in situ ILC maturation and function. Moreover, our work

introduces a modular organoid platform, which provides exquisite control over both environmental stimuli and host genetics, making it a powerful tool for dissecting immune-epithelial interactions in health and disease.

## **Mucosal rAd5 Immunization against SARS-CoV-2 Spike Elicits Cross-Reactive Nasal and Serum Neutralizing Antibodies and Protects Against Beta Variant Challenge in Non-Human Primates**

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Mucosal vaccination may offer increased protection against SARS-CoV-2 compared to parental immunization. Here, we describe immunogenicity and efficacy following viral challenge in non-human primates (NHP) after intranasal delivery of three unique non-replicating adenoviral vector vaccine (rAd5) candidates. NHPs were prime boost immunized 29 days apart with vaccine candidates either expressing the parental spike protein alone (Wuhan-S), spike plus nucleocapsid (Wuhan-S-N), or the spike protein from the beta variant (beta-S). Serum and nasal swabs were collected every 14 days and humoral responses to full length spike (S) and receptor binding domain (RBD) were assessed. Mucosal immunization with Wuhan-S induced significant increases in serum IgG and IgA responses against the homologous parental lineage, as well as beta and delta variants, and generated substantial neutralizing activity. In nasal samples, Wuhan-S immunization elicited 1000-fold increases in cross-reactive IgA against multiple variants and had greater neutralizing activity compared to Wuhan-S-N and beta-S vaccination. While the beta-S vaccine candidate induced enhanced humoral responses to homologous S and RBD proteins, this approach resulted in less cross-reactive antibodies to other variants compared to Wuhan-S. Despite the differences in the ability to elicit cross-reactive antibody responses, all vaccinated NHPs challenged with SARS-CoV-2 B.1.351 (beta variant), had a significant reduction in viral titers by TCID50 in the nasal passages compared to unvaccinated controls. These results demonstrate mucosal administration of rAd5 clinical candidate vaccine, Wuhan-S, is immunogenic and offers broad cross protective humoral responses in both serum and nasal compartments against a mismatched SARS-CoV-2 challenge virus.

## **Mucosal vaccination provides protection from HSV-2 infection and disease**

Kiersten Tucker - Fred Hutch, Veronica Dave

Herpes simplex virus type 2 (HSV-2) is a sexually transmitted pathogen that is estimated to infect around 23 million people per year. Despite high global prevalence, there are not any approved vaccines that are therapeutic or preventative. Most vaccines that we use today rely on injecting antigens intramuscularly in order to elicit an adaptive immune response. However, given that most pathogens gain entry to the host across barrier surfaces, a focus on eliciting mucosal immunity may enhance protection; vaccine-induced local immunity at the site of first pathogen exposure may have the best chance at preventing the spread of infection beyond the pathogen portal of entry. We hypothesized that a mucosal immunization would prime memory T cells to reside in vaginal tissues and provide better protection against vaginal HSV-2 exposure than other routes of immunization. Our initial data suggests that intranasal immunization is effective in protecting mice from vaginal HSV-2 infection. Ongoing work focuses on characterizing the role of vaccine-elicited mucosal CD8+ T cells in preventing infection to provide insight into the mechanisms of protection induced by mucosal immunization for HSV-2. These findings contribute to the efforts to generate an effective vaccine to prevent HSV-2 infection and disease.

## **Mucus composition alterations in inflammatory bowel diseases**

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Introduction: Intact mucus layer is crucial in the intestinal mucosal barrier by inhibiting bacterial-epithelium interaction and thus intestinal immune responses. Mucins, mucus layer building blocks, are secreted by goblet cells and composed of glycans that are essential for resisting bacterial interactions. Inflammatory bowel diseases (IBD) pathophysiology may be related to mucins composition alterations. Aim: To assess intestinal mucus expression and composition in IBD, and inflammation-induced alterations. Methods: MUCs gene



expression in intestinal biopsies of patients with IBD and controls (Ben-Shachar S, 2013) were clustered to generate a heat-map. To study mucus composition, glycan-binding lectins were assessed in mucosal biopsies from patients with IBD, using immunofluorescence. Organoids obtained from surgically resected intestines of controls were differentiated to enrich goblet cells and increase mucus secretion. MUCs mRNA levels were assessed, following treatment with pro-inflammatory cytokines by RT-PCR. Results: Hierarchical clustering analysis revealed MUC1/MUC2/MUC4/MUC6 increase in patients with IBD with active inflammation compared to controls. N-acetyl-D-galactosamine (GalNAc) expression was higher, while Galactose expression was lower, in inflamed compared to the non-inflamed ileal samples (n=4). However, GalNAc and Galactose expression in inflamed compared to non-inflamed colonic samples, were comparable (n=1). MUC1/MUC4/MUC6 expression was upregulated in response to cytokines in organoids (n=4). Conclusion: Higher MUC1/MUC2/MUC4/MUC6 expression in inflamed mucosa suggests mucus contribution to intestinal inflammatory processes. Moreover, discordant expression pattern of glycans between ileal and colonic samples suggests that mucins composition is inflammatory site-related. Organoids may serve as a platform for studying mucus layer composition. Inflammation-induced alterations suggest that mucus layer modification may be immune-mediated.

### NAIP—NLRC4 inflammasome activation in Tuft cells contributes to host defense against bacteria

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Renate Bauer, Isabella Rauch

The small intestinal epithelium is exposed to various microbes, and intestinal epithelial cells (IECs) have developed an array of host defense mechanisms to distinguish commensals from pathogenic microbes and initiate an immune response against the later. NAIP—NLRC4 inflammasome induced-pyroptosis, which is activated when bacterial flagellin or type III secretion systems are detected in the cytosol of the host cell, is one such mechanism. Activation of NAIP—NLRC4 induces extrusion of IECs and release of the inflammatory mediators prostaglandin E2 and IL-18. Work on epithelial inflammasome activation thus far has not delineated if different subtypes of IECs have specific roles in the ensuing immune response. Tuft cells are a rare subset of IEC that is primarily known for its role

in sensing parasites, and it is unknown whether they are involved in promoting antibacterial host responses. Here we show that upon activation of the NAIP—NLRC4 inflammasome specifically in tuft cells, they uniquely among IECs release prostaglandin D2, and within the small intestine, tissue IL-22 increases. NLRC4 expression only in tuft cells leads to better control of *Salmonella Typhimurium* compared to NLRC4 null controls. Taken together, these data suggest that NAIP—NLRC4 inflammasome activation in tuft cells leads to lipid mediator induced downstream inflammatory responses that are complementary to signaling pathways that are activated by other IEC subsets. Understanding how NAIP—NLRC4 activation in different epithelial subsets affects downstream inflammatory responses will inform on the different signaling pathways that synergize together to mount pathogen specific host defense mechanisms.

### Neonatal Peyer's patch cDC activation as a pacemaker of postnatal immune maturation

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Marked differences exist between the mucosal immune system of the neonate and adult host. The pronounced influence of the enteric microbiota in adults suggests a causal relationship between postnatal colonization and immune maturation. However, using metagenomic, metaproteomic, and functional immunological analyses we demonstrate an early presence of bacteria and immunogenic microbial antigens preceding immune maturation in the small intestine, the primary inductive site of intestinal immunity. Instead, transcriptomic, flow cytometric and histological analysis indicated neonatal Peyer's patch (PP) mononuclear phagocytes (MNP) as rate limiting factor of postnatal immune maturation. Despite the early presence of MNPs, conventional dendritic cells (cDC) of type 1, 2a and 2b exhibited significant age-dependent differences in tissue distribution and cellular composition. Single cell transcriptional profiling and functional assays revealed decreased antimicrobial and antigen processing/presentation capacity, an overall retarded cell maturation and reduced

antigen uptake. In cDC2a this resulted in a reduced proportion of CCR7 + migratory cells and a consequent defect in CD4 T cell priming. Interestingly, transcriptional profiling of neonatal DC subsets identified reduced expression of type I interferon (IFN)-stimulated genes (ISG). Type I IFN induction by oral administration of the TLR7 agonist R848 accelerated MNP maturation and enhanced cognate antigen CD4 T cell priming. However, humoral responses to oral vaccination in the presence of R848 were significantly reduced. Together, our results identify PP MNP maturation as pacemaker of postnatal mucosal immune priming, indicate the biological role of delayed maturation and demonstrate that targeted interventional strategies allow manipulation of mucosal responses in early life.

### NKG2D expression on Intraepithelial lymphocytes from the human gastrointestinal tract

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Intraepithelial lymphocytes (IELs) are the first line of surveillance along the gastrointestinal tract and therefore their phenotype and function may be affected early on disease. Hence, we aimed to perform an exploratory study of IELs on resting and proinflammatory conditions through the human gut. IELs were obtained from the stomach (body, incisura and antrum from 6 controls), duodenum (37 controls, 19 non-coeliac inflamed, 21 inflamed coeliac and 6 non-inflamed coeliacs) and paired ileum and colon (14 controls and 6 patients with Crohn's disease). IEL were further analysed by flow cytometry. The proportion of  $\gamma\delta$  (CD3 +  $\gamma\delta$  +), classical T cells (CD3 +  $\gamma\delta$  -) or NK-like cells (CD3 -  $\gamma\delta$  -) within total IEL were not altered through the length of the human gut in controls. Indeed, the expression of IL15R $\alpha$  or IL2R $\beta$  was not altered either in the different subsets through the human gut. NKG2D expression, on the contrary, was dependent on tissue location as it was increased on duodenal  $\gamma\delta$ , classical T cells and CD7 - NK-like cells but decreased on CD7 + NK-like cells. NKG2D expression was also dependent on the inflammatory status as non-inflamed coeliacs had higher expression on  $\gamma\delta$  and classical T cells, while the affected ileum of Crohn's

patients showed lower levels of NKG2D on  $\gamma\delta$ , classical T cells and CD7 + NK-like IELs. All together these data suggest that duodenal IELs are more likely to initiate an inflammatory response. This predisposition is exacerbated on coeliac patients that are not exposed to gluten but surprisingly decreased on affected tissue from Crohn's disease patients.

### Nociceptor neuron regulation of the host response to influenza A virus infection

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The sensory nervous system densely innervates the respiratory tract, mediating breathing, cough, and bronchoconstriction. Nociceptor neurons are a major sub-type of peripheral neurons that sense noxious/harmful stimuli and protect organisms from danger. We recently found that nociceptor neurons from the vagal ganglia play a critical role in suppressing innate immunity against *Staphylococcus aureus* lung infection and lethal bacterial pneumonia. However, the role of the peripheral nervous system and its crosstalk with the immune system in host defense against viral pathogens has not been well defined. Influenza A virus is a major cause of human disease and is responsible for more than 50,000 deaths per year in the US. Our preliminary data show that nociceptor neurons play a protective role against influenza A virus infection. We find that mice deficient in nociceptor neurons show an increase in mortality and lung immunopathology after intranasal infection with PR8 strain (H1N1). These results are correlated with decreased core body temp. and oxygen saturation among the neuron-ablated animals. We are characterizing the mechanisms of this regulation now at the immunological and physiological levels. Enhancing sensory neuron activation could potentially offer a novel treatment modality for influenza A infection.

### Non-hematopoietic IL-33 negatively regulates IFN $\gamma$ - and IL-22 dependent antibacterial immune responses in pneumonia

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The innate immune response to infections is vital for preserving lung function during pneumonia as it fights the invading microbes. An unrestricted inflammation, however, can also lead to excessive tissue damage. In this study, we tested the hypothesis that alarmins are released during *Streptococcus pneumoniae* infection to regulate immune responses during pneumonia. Our data demonstrate that *S. pneumoniae* infection leads to the release of several alarmins including uric acid, ATP and IL-33. Experiments with specific knock-out mice, inhibitors and degrading enzymes revealed that IL-33, but not uric acid or ATP, negatively regulates antibacterial immunity through its receptor ST2. Bone-marrow chimera and scRNAseq experiments identified non-hematopoietic cells - most likely type 2 alveolar epithelial cells - as the relevant source of IL-33. IL-33 negatively regulates IFN $\gamma$  and IL-22 production, resulting in impaired control of pneumococcal infection. Moreover, single nucleotide polymorphisms in IL33 and IL1RL1 (encoding ST2) were found to be associated with pneumococcal community-acquired pneumonia. Ongoing work aims to further unravel the cellular and molecular mechanisms of how IL-33 controls IL-22 and IFN $\gamma$  production.

## Non-optimal bacteria species induce neutrophil-driven inflammation and epithelial barrier disruption in the female genital tract

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The mucosal surface of a healthy female genital tract (FGT) is comprised of physicochemical, immunological and microbial components that serve as a rapid, first line of defense against infections. Alterations of these components have been associated with higher HIV acquisition risk. Analysis from >700 women from the CAPRISA004 cohort show that women with a non-*Lactobacillus* dominant vaginal microbiome were at significantly higher risk of sexual HIV acquisition, and that this strongly correlated with loss of barrier integrity, inflammation and neutrophil accumulation. However, how FGT barrier function is impacted by changes in the

vaginal microbiota, and a mechanistic understanding of mucosal neutrophils in this process, remain unclear. Here, we utilized microscopy and proteomic approaches to better define the interplay between vaginal microbial species, epithelial barrier function and neutrophil activation *in vivo*. Balb/c mice intravaginally inoculated with *Lactobacillus crispatus* (optimal bacteria species) had little impact on FGT biology, whereas inoculation with *Mobiluncus mulieris* or *Gardnerella vaginalis* (non-optimal bacteria species) induced inflammation, increased cytokine release and upregulation of neutrophil-related signatures in vaginal secretions. We found that the presence of non-optimal bacterial species causes substantial damage to the vaginal epithelium and results in high neutrophil recruitment along with an increase in the release of extracellular matrix-modifying enzymes shortly after challenge. Excitingly, we also show that neutrophils response to these non-optimal bacteria species directly impacts FGT barrier function *in vivo*. Non-optimal bacteria also caused substantial damage to the vaginal epithelial barrier in humanized BLT mice, also accompanied by high neutrophil influx. We are currently addressing whether changes in vaginal barrier integrity directly impact HIV acquisition *in vivo* using humanized BLT mice. Together, our work provides a mechanistic understanding of how composition of the vaginal microbiome can alter epithelial barrier function and innate immune responses to modulate HIV risk.

## The novel role of the m6A-demethylase Fat-mass and obesity related (FTO) protein in the tumor microenvironment in colorectal cancer.

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Colorectal cancer (CRC) is the second most frequent and third most deadly cancer worldwide showing high mutational heterogeneity and poor therapy response with many molecular factors [MH1]. The role of the m6A-demethylase FTO has recently been described in CRC cell stemness, and epithelial / mesenchymal transition, however, its function in the tumor microenvironment (TME) and progression remains unclear. The study aim was evaluating FTO protein expression in tumor and healthy tissue from CRC patients (n=22) with different histological TNM stages (hTNM) by immunohistochemistry.

Tumor and stromal FTO localization was classified and associated with clinical and histopathological features to provide a more precise perspective of FTO in the TME. Our study shows that the localization of FTO is highly present in lamina propria cells in healthy mucosa, in lymphoid infiltrates and fibro-immune compartments in the TME, corresponding mostly to CD4+ T cells and Iba+ macrophages. Additionally, FTO+ tumor cells are increased in early carcinogenic stages (hTNM I vs healthy tissue\*) and in moderately differentiated tumors\*. Furthermore, stromal FTO+ cells are increased in more invasive stages\*(T3) and with low desmoplasia\*. Our preliminary results indicate that after exposure to cancer cell-derived conditioned media, nuclear FTO expression is enhanced in normal T cells and fibroblasts, suggesting FTO activity in the TME interaction following a paracrine signal. Together, our findings highlight essential role of FTO in early CRC stages suggesting a TME component interaction. Lastly, research is presently on-going to find new FTO functions in the CRC-TME. (\*p<0.05, Funding: FONDECYT 3190931).

### NTM mucosal-induced immune response enhances the protective effect of BCG against Mycobacterium tuberculosis infection.

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Tuberculosis (TB) continues to increase worldwide despite vigorous attempts to control it. Bacillus Calmette-Guerin (BCG) is the only licensed vaccine currently available for protection against TB. However, its efficacy is highly variable between countries. Some studies have revealed that BCG's variable protection is due, amongst others, to immunological interference by environmental, non-tuberculous mycobacteria (NTM). However, a definitive mechanism has not been identified so far. Considering the previous, we developed a murine model closely resembling the natural history of human exposure to different mycobacterial species, including 1) BCG vaccination at an early age; 2) exposure to viable NTMs (*Mycobacterium avium* subsp. *avium*) via the oral route and 3) maintaining continuous NTM exposure even after TB infection, as occurs in endemic regions. Surprisingly, we found that a low dose of NTM via the oral route enhanced BCG-mediated protection for up to 120 days post-infection, as determined by

decreased *Mycobacterium tuberculosis* (Mtb) burden and in lungs and spleens of infected mice and improved pathology scores. This reduction in Mtb is directly correlated to increased numbers of B220+ B2 B-cells and CXCR5+ follicular helper T cells in the lungs. Intestinal Peyer's Patches likewise had increased numbers of B cells as early as 3 months post NTM exposure, before Mtb aerosol infection. Reduced bacterial counts correlated also with higher numbers of CD8 cytotoxic T cells and NK cells positive for perforin in the lungs of these mice. Interestingly, Immunohistopathology and spatial transcriptomic results demonstrated developed of ectopic germinal centers (eGC) in the lungs of mice immunized with BCG taking NTMs in the drinking water. Furthermore, animals receiving NTMs in the drinking water had increased quantities of IgA and IgG against Mtb in bronchoalveolar lavage and serum. These results suggest that chronic live NTM exposure via the oral route elicits a protective mucosal immune response against Mtb. Ongoing experiments are testing the difference between cytotoxic T cells and antigen-specific B cells from the different groups evaluated.

### OCA-T1 and OCA-T2 are coactivators of OCT11 in the tuft cell lineage

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Tuft cells are rare chemosensory cells found on mucosal layer and play an important role in type II immunity. Tuft cells have suggested as a potential cell-of-origin for small cell lung cancer (SCLC). Despite this importance, the transcriptional mechanisms that generate tuft cells are poorly understood. Here, we show that binding of tuft cell master regulator POU2F3 to the uncharacterized proteins C11orf53 and COLCA2 (renamed here OCA-T1 and OCA-T2) is critical for both normal and malignant tuft cells. OCA-T proteins are paralogs of the B cell-specific coactivator OCA-B, which are encoded in a gene cluster and harbor a conserved peptide that binds to OCT proteins on DNA. We demonstrate that binding between OCT11 and OCA-T is essential for growth and cell survival of SCLC. OCA-T1 knockout mice are viable but selectively deficient in tuft cells and deficient in type II immunity response. These findings reveal a protein-protein interaction



required for specifying tuft cell lineage with therapeutic potential in SCLC.

### Oral mucosal immune dysfunction during viral infections

Pushpa Pandiyan - Case Western Reserve University

Understanding the mechanisms of mucosal dysfunction and persistent inflammation during chronic viral infections will lead to improved immune-targeted interventions, extending the health span and life span. Here we show an altered immune landscape of the oral mucosa of HIV-positive patients on therapy involves increased TLR- and inflammasome signaling, localized CD4 + T cell hyperactivation, and counterintuitively, enrichment of regulatory T cells. HIV infection of oral tonsil cultures in vitro causes an increase in FOXP3 + cells expressing PD-1, IFN- $\gamma$ , Amphiregulin and IL-10. These cells persist even in the presence of anti-retroviral drugs, and further expand when stimulated by TLR-2 ligands and IL-1 $\beta$ . Mechanistically, IL-1 $\beta$  upregulates PD-1 expression via AKT signaling and PD-1 stabilizes FOXP3 and Amphiregulin through a mechanism involving Asparaginyl Endopeptidase, resulting in FOXP3 + cells that are incapable of suppressing CD4 + T cells in vitro . Phenotypically similar cells are abundant in HIV-positive patients, and their presence strongly correlates with CD4 + T cell hyper-activation, suggesting diminished CD4 + T cell regulation in the oral mucosa in vivo . Taken together, this study provides unprecedented insights into the mechanism of FOXP3 + cell dysregulation and its potential role in mucosal dysfunction in HIV patients.

### Oral Tablet Vaccination to SARS-CoV-2 Induces Pan-coronavirus Nasal IgA Responses in Humans

Sean Tucker - Vaxart, Susan Johnson, Becca Flitter, Clarissa Martinez, Colin Lester, Sarah Tedjakusuma, Clara Jegede, Josefina Martinez, Shaily Garg

Covid-19 has morphed to a multi-virus disease where highly-transmissible variants are able to circumvent vaccine antibodies. While variant-specific vaccines might improve performance, variants are appearing faster than an injection-based campaign can be conducted. A different approach would be to develop an easily distributed vaccine that induces cross-reactive mucosal IgA that not only inhibits infection but

also reduces viral shedding. In this study, we examined whether a pan-coronavirus mucosal IgA response could be induced in humans following oral tablet administration. A vaccine candidate (VXA-CoV2-1) was constructed using recombinant adenovirus expressing the SARS-CoV-2 Spike(S) and Nucleocapsid(N) proteins from SARS-COV-2 wuhan-1, as well as a molecular dsRNA adjuvant. An open-label phase-1 clinical study with 35 healthy subjects was conducted. Mucosal samples were taken, and measured for antibody responses to several different coronaviruses. VXA-CoV2-1 tablets were well tolerated. 50% of subjects had increased antibody responses to the S protein either in the saliva or nasal samples. Of the subjects that had a 2-fold or greater increase in the SARS-CoV-2 S specific responses in the nasal swabs, 100% of these subjects had increases in the S specific responses to all the coronaviruses tested including SARS-CoV-1, MERS-CoV, and NL63. Furthermore, these subjects displayed cross-reactivity to both delta and omicron variants. In summary, the pan-coronavirus IgA response appears to be the preferential immune response, rather than the generation of strain specific IgA, suggesting that mucosal memory B cells may be available in the vaccine inductive sites. A phase-2 human study is now enrolling a more advanced candidate.

### Oral vaccination with APN-targeted FedF triggers protective immunity against E. coli infection in piglets.

Hans Van der Weken - Ghent University, Raquel Sanz Garcia, Eric Cox, Bert Devriendt

Many pathogens enter the host via the gut, causing disease in animals and humans. A robust intestinal immune response is necessary to protect the host from these gut pathogens. Despite being best suited for eliciting intestinal immunity, oral vaccination remains a challenge due to the gastrointestinal environment, a poor uptake of vaccine antigens by the intestinal epithelium and the tolerogenic environment pervading the gut. To improve uptake, efforts have focused on targeting antigens towards the gut mucosa. Previous research identified aminopeptidase N (APN), a conserved membrane protein present on small intestinal epithelial cells, as an interesting target due to its ability to mediate epithelial transcytosis after binding by antibodies. Here, we developed an oral vaccination strategy by targeting a clinically relevant antigen towards APN using monoclonal antibody-antigen fusion constructs. To this end, we genetically linked the FedF

tip adhesin from F18 fimbriated *E. coli* to the Fc-domain of an APN-specific chimeric mouse-pig IgA antibody and evaluated its ability to trigger immune responses in piglets after oral administration. Upon oral delivery of these recombinant antibodies, both mucosal and systemic immune responses were elicited. Furthermore, a significant decrease in bacterial excretion was observed after oral challenge infection with an F18+ *E. coli* strain. Altogether, these findings show that targeted delivery of molecules to epithelial aminopeptidase N results in their transcytosis and delivery to the gut immune system and provides a solid foundation for the development of oral subunit vaccines to protect against gut pathogens.

### Orally-dosed *Prevotella histicola* induces a population of CD4+ T cells that can mediate resolution of skin inflammation

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The gut is connected to the peripheral immune system through a network of lymphatic, cellular, and molecular components. EDP1815 is a non-living pharmaceutical preparation of a single strain of *Prevotella histicola* isolated from the duodenum of a human donor. It is gut-restricted after oral delivery, is non-colonizing, and does not modify the host gut microbiota. It can engage this gut-peripheral network to resolve Th1 and Th17-mediated inflammation in various murine models of inflammation as well as psoriasis in a Phase IIb human clinical trial. We have investigated the mechanism of action of resolution of peripheral inflammation by EDP1815 in a murine delayed-type hypersensitivity (DTH) model. The data reveal a 3-step process: i) stimulation of TLR2 receptors; ii) interaction of circulating lymphocytes with cells in mesenteric lymph nodes; iii) anti-inflammatory effects of these conditioned lymphocytes in the periphery. This modulation of the peripheral immune system is not antigen-specific, does not involve direct immunosuppression, and can be adoptively transferred via CD4+ T cells from inflamed EDP1815-treated mice into untreated recipient mice undergoing a DTH response. We have used this adoptive cell transfer model to identify receptors, cells, and cytokines which are involved in this

network. These data demonstrate that oral dosing of EDP1815 induces peripheral immune changes, modifying CD4+ T cells within the mesenteric lymph node giving them the capacity to modulate systemic inflammation outside of the gut.

### Osteopontin Plays A Critical Role in the Induction of Corneal Neovascularization Following Herpes Simplex Virus Type 1 Infection

Dan Carr - University of Oklahoma Health Sciences Center, Adrian Filiberti, Grzegorz Gmyrek

Herpes simplex virus type 1 (HSV-1) is a highly successful neurotropic virus that is associated with a severe inflammatory condition in the eye of patients known as herpetic stromal keratitis (HSK) that often includes opacity, neovascularization, and in some instances, loss of sensation of the cornea. The present study explored further the relationship between OPN and HSV-1-induced corneal pathology using OPN-deficient (OPN KO) mice. HSV-1-infected OPN KO mice displayed significantly reduced neovascularization compared to infected C57BK/6 (WT) mice including a reduction in blood and lymphatic vessels residing in the central cornea. This change was also reflected by a reduction in corneal opacity and a significant reduction in the infiltration of neutrophils (CD45 + CD11b + F4/80 - Ly6C - Ly6G +). The near absence in pathology was not due to a reduction in infectious virus as OPN KO mice possessed significantly more virus in the cornea compared to WT mice during lytic infection. Likewise, OPN KO mice maintained the same level of activated and HSV-specific CD4 + and CD8 + T cells in the draining lymph node as their WT counterparts. Rather, the change in virus-induced neovascularization was due to a temporal reduction (delay) in VEGF-A and IL-6 expression in the cornea following infection compared to WT animals in which OPN levels doubled within 12 hours pi at a time that also showed a peak in expression of VEGF-A and IL-6. Collectively, OPN is a critical component involved in HSV-1-mediated cornea pathology with the absence delaying the expression of pro-angiogenic and pro-inflammatory factors in mice.

### The overlap of microbial changes to different mucosal tumor microenvironments

Catherine Huynh - Brown University

A hallmark of cancer progression is the Warburg effect, a metabolic switch that results in a reliance on the less efficient aerobic glycolysis. This shift triggers many intracellular and extracellular changes, including excessive production of reactive oxygen species and nucleotides, and the nutrient microenvironment. Such metabolic shifts in the tumoral microenvironment likely result in significant changes in the composition and behavior of proximal microbes. While some work has been done on individual cancers, there has been limited study in the overlap between different cancers of sites that harbor a microbiome. We hypothesize that during cancer progression in the lung, stomach, and cervix, there are recognizable and overlapping patterns of change in tumor environment taxa that likely impact disease progression in cancer. In our analysis, we utilized two 16S RNA-sequencing datasets of non-small cell lung carcinoma, colorectal cancer, and cervical cancer. Then using the DADA2 pipeline and phyloseq visualization methods, we explored the impact each cancer had on the respective organ microbiomes. We found that modulation of the tumoral sites during cancer progression begets corollary changes to the microbiome across the lung, stomach, and cervix. This work highlights the importance of considering local and systemic changes to the microbiome during cancer, as well as potentiating its role tumor dynamics.

## Physiological translocation of gut-residing bacteria in early life

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Gut-resident microbiota regulate host health via multiple mechanisms. Some of the most striking effects microbiota exert on hosts are time-dependent, and occur in extraintestinal tissues and at infancy. However, how gut residing microbes affect extraintestinal tissues and the reason for time-specific effects remains unclear. Here, we sought to investigate if live gut-residing bacteria physiologically and purposefully translocate to extraintestinal tissues of preweaning mice. To assess if gut-resident bacteria translocate spontaneously in early life, we harvested MLNs and spleens from pre/post weaning mice, homogenized and inoculated them onto BHI agar, and identified bacteria from plated tissue homogenates by Sanger sequencing of full length 16s rDNA. Live gut-

resident bacteria, predominantly *Lactobacillus*, were identified in the MLN and spleens of preweaning, but not adult mice. Orally administered *Lactobacillus animalis* translocated to the MLN and spleen of preweaning, but not adult mice. *L. animalis* translocated via colonic goblet-cell associated antigen passages (GAPs) and required sphingosine-1-phosphate receptor expressing host cells. *L. animalis* translocation was physiological, as it did not trigger cytokine responses or neutrophilia, or inflammatory pathways in the MLN of preweaning mice. *L. animalis* exhibited antimicrobial activity against *E. coli* ST69, a blood isolate from a late onset sepsis infant, and *L. animalis* translocation protected preweaning mice from *E. coli* induced mortality. Whole genome sequencing of *L. animalis* identified coding sequences for a tyrocidine-gramicidin antibiotic-synthesizing gene cluster, which may confer antimicrobial activity against other bacteria. We propose that the translocation of select gut-resident bacteria is physiological and protective to the mammalian host in early-life.

## Pneumococcal pep27 Mutant-Upregulated Regulatory T Cells Inhibit Caspase-14 Expression and Attenuate Experimental Colitis and Gut Microbial Dysbiosis

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Inflammatory bowel disease (IBD) is a highly prevalent gut inflammatory disorder. Complicated clinical outcomes prolong the use of conventional therapy and often lead to compromised immunity followed by adverse events and high relapse rates. Thus, a profound medical intervention is required. Previously, intranasal immunization of pneumococcal pep27 mutant ( $\Delta$ pep27) exhibited long-lasting protection against immune-related disorders. System biology analysis has predicted an inverse correlation between  $\Delta$ pep27 immunization and gastroenteritis. Therefore, we evaluated whether  $\Delta$ pep27 can alleviate IBD.  $\Delta$ pep27 dose-dependent response was analyzed in dextran sulfate sodium-induced mice using transcriptome analysis. Pro- and anti-inflammatory signatures were cross-correlated by quantitative PCR and western blot analyses. To address the hierarchy regulating the activity of caspase-14, an undefined marker in IBD, and regulatory T cells (Tregs), antibody-based neutralization studies were conducted. Fecal microbiome profiles were analyzed by 16S rRNA pyrosequencing.  $\Delta$ pep27 significantly attenuated dextran

sulfate sodium-induced oxidative stress parameters, proinflammatory cytokines, caspase-14 expression level, and upregulated tight junction, anti-inflammatory genes IL-10 and TGF- $\beta$ 1 via upregulation of Tregs to restore healthy gut microbiota. Neutralization studies unveiled that  $\Delta$  pep27 had a remedial effect via Treg upregulation. Caspase-14, being important mediator in the pathogenesis of IBD, can be an alternate therapeutic target in IBD.  $\Delta$ pep27-increased Tregs repressed caspase-14 expression and reversed gut microbial dysbiosis aiding to re-establish immunological tolerance.

### Primary Human-Derived Macrophages in Co-culture with Bronchial Epithelial Cells Cause a Medium-Dependent and Macrophage Phenotype-Dependent Reduction in RSV Infection

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Respiratory syncytial virus (RSV) infection is common in young children and, in some cases, can progress to severe bronchiolitis and pneumonia. Despite tremendous progress in understanding RSV pathogenesis, there are no RSV-specific therapies available for clinical use. Disease pathology may in part be related to host immune responses, and therefore in vitro co-culture models offer the opportunity to better understand cell-cell interactions. In this work, we used an in vitro co-culture model with primary human bronchial epithelial cells (HBECS) to investigate the role of M0-, M1-, and M2-like human blood monocyte-derived macrophages in RSV infection. HBECS were cultured in PneumaCult™-ALI Medium, and macrophages were generated with ImmunoCult™-SF Macrophage Medium. The epithelia were co-cultured without contact with macrophages in either medium and infected with RSV immediately after initiation of co-culture. Flow cytometry and cytokine secretion analyses indicated that the phenotype of M0-, M1-, and M2-like macrophages was maintained after 72 hours of co-culture with HBECS in both media. Barrier function of the HBECS was also stable after incubation in ImmunoCult™-SF Macrophage Medium. Compared to HBECS without macrophages, RSV infection was decreased by the presence of M1-like macrophages and enhanced by M0- or M2-like macrophages. The co-culture medium also impacted the severity of the infection, as co-cultures with PneumaCult™-

ALI Medium reduced the protective effect of M1-like macrophages. These results demonstrate that macrophage phenotypes may perform differential roles during epithelial RSV infection and provide further understanding of macrophage-epithelial cell interactions during viral infections.

### Protective neutrophil-, IL-12- and IFN $\gamma$ -dependent antibacterial immunity is compromised in a murine diabetes model

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Metabolic comorbidities such as obesity and diabetes are associated with increased risk for lower respiratory tract infections, but the mechanisms underlying these associations are poorly understood. In order to examine how these factors affect antibacterial immunity in the lung, we employ leptin receptor-deficient db/db (LepR db/db) mice, which spontaneously develop obesity and type 2 diabetes-like disease, and heterozygous db/+ control animals. Following intranasal infection with *Streptococcus pneumoniae* or *Legionella pneumophila*, LepR db/db mice had higher bacterial loads in the lungs as compared to control animals, and preliminary bone-marrow chimera experiments suggest that this difference in infection susceptibility is independent of hematopoietic LepR expression. Pulmonary recruitment of neutrophils was reduced, and production of IL-12, IFN $\alpha$  and IFN $\gamma$  was impaired in infected LepR db/db mice. scRNAseq analysis revealed an IL12 $\alpha$ -expressing neutrophil-like population which was absent in LepR db/db mice, and NK cells from this group expressed lower levels of Ifng. Moreover, IL-12 and IFN $\gamma$  levels positively correlated with neutrophil counts. Finally, differences in antibacterial defense between diabetic and non-diabetic mice were almost absent after neutrophil depletion or IFN $\gamma$  blockage. Collectively, our data indicate that LepR deficiency-associated diabetes compromises neutrophil-, IL-12- and IFN $\gamma$ -dependent antibacterial immunity in the lung.

### Re-emergence of neonatal bacteria TCRs during the intestinal damage in adult life

Jaeu Yi - Washington university in St. Louis, Jaeu Yi, Patricia Chyi, Chyi-Song Hsieh



While both self-antigens and commensal bacteria are important for early host immune system development, how each of them contributes the TCR repertoire is unclear. Here, we conducted TCR repertoire analysis with intestinal T cells from 2-week-old SPF and GF mice and found that microbiota is more important than self-antigens in shaping the colonic TCR repertoire. Notably, microbiota dependent TCRs are diverse but most of them disappear in adult life. The gut bacteria composition is significantly different between adults and neonates, with *Lactobacillus* being a dominant neonatal genus. To study neonate bacteria-specific T cell responses, we analyzed the TCR repertoire of *Lactobacillus* mono-colonized GF mice. The TCR J1L2 was cloned and found to be reactive to several species of *Lactobacillus* and neonate luminal antigens but not adult luminal antigens. This TCR becomes undetectable in adult mice during homeostasis but can be expanded during *Citrobacter* infection. We speculate that this maybe one mechanism by which T cell interactions to bacteria predominantly found during early life may contribute to host immunity much later in ontogeny.

## Regulation of antigen presenting cells by retinal pigment epithelial cells

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The retinal pigment epithelial cells (RPE) produce soluble immune regulators that drive the activation of antigen-specific Treg cells. One effect of this immune regulation is the ability of RPE to induce APC to activate Treg cells in an antigen-specific manner in vivo. This ability of RPE is not seen under conditions of experimental autoimmune uveitis (EAU) but recovers following treatment with the anti-inflammatory neuropeptide alpha-melanocyte stimulating hormone (alpha-MSH). To further understand the effects of RPE regulation of APC, we treated APC processing antigen in vitro with the condition-media of RPE-eyecups from the eyes of naive, EAU, and alpha-MSH-treated EAU mice. These APC were used to activate in vitro T cells from antigen-immunized mice. We assayed for APC and T cell cytokine production and by flowcytometry CD25+FoxP3+ Treg cells. The RPE-eyecup conditioned-media from the alpha-MSH-treated EAU mice did not suppress or enhance APC stimulation of T cell production of IFN-gamma, IL-17, or IL-10 production. There was suppression of both proliferation and expansion of

CD25+FoxP3- T cells. There was no change in the CD25+FoxP3+ Treg cell population. In contrast, the APC treated with the RPE-eyecup conditioned media treated-APC had significantly induced IL-10 production with suppressed TNF-alpha production. These results suggest that the effects of RPE on the immune response may not be about inducing Treg cell activity but inducing an anti-inflammatory environment that minimizes effector T cell expansion. In addition, the RPE-induced APC may have to locate into a specific tissue space (i.e., spleen) to induce retinal antigen-specific Treg cells.

## Regulation of cell death in mucosal-associated Invariant T cells is distinct from that of naive conventional T cells

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Mucosal-associated invariant T (MAIT) cells are innate-like T cells which recognize vitamin B metabolite antigens. MAIT cells become activated and dramatically expand during bacterial infection, contributing to control of bacterial load. Interestingly, after an infection is cleared, MAIT cells are retained at high numbers while the conventional T cell populations contract. Since expanded MAIT cells can both boost immunity and drive immune pathology, it is important to understand the signals controlling their abundance and longevity. While cell death in conventional T cells is well studied, its regulation in MAIT cells, which exhibit an effector-memory phenotype (CD62-L lo CD44 hi), is uncharacterised. Receptor-interacting protein kinase (RIPK)1 and RIPK3 are regulators of cell death, initiating apoptosis and necroptosis via the activation of caspase-8 and mixed lineage-kinase like protein (MLKL) respectively. We report here that MAIT cells express an abundance of both RIPK1 and RIPK3, and high levels of MLKL similar to conventional effector/memory T cells, but distinct from naive subsets. We find that the loss of RIPK3 results in increased MAIT cell frequencies in mice. Additionally, we dissect the contribution of caspase-8 and MLKL to MAIT cell accumulation at steady state, and MAIT cell expansion and contraction in a *Francisella tularensis* infection model.

Cumulatively, our data demonstrate a role for RIPK3 in restraining MAIT cell numbers at steady state, distinguishing this subset from conventional T cells. Understanding the pathways regulating MAIT cell survival throughout development, homeostasis, and infection will be critical to developing immune interventions seeking to deplete, expand or otherwise manipulate MAIT cells.

### Regulation of microbiota specific T cell responses in inflammatory bowel disease.

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In the human body, dynamic interactions between the host and microbiota shape the development and responsiveness of the host immune systems, allowing for the induction of protective immunity against pathogens while also limiting aberrant responses against the microbiota. A breakdown of tolerance to the microbiota can lead to the development of chronic inflammatory disorders such as inflammatory bowel disease (IBD). We sought to understand the mechanisms that limit the development of inflammatory T cell responses against the microbiota. We previously demonstrated that colonization with select members of the microbiota induced macrophage production of the anti-inflammatory cytokine IL-10. This limited pathologic inflammation by restraining Th1 but promoting Treg immunity in murine models of colitis or after infection with pathogens like Salmonella. In the absence of host IL-10, we found Th1 cell responses against this bacteria. This anti-inflammatory effect required microbial adherence to epithelial cells as mutants with reduced adherence did not induce IL-10 production and worsened pathology in models of colitis. We also found expansion of microbe specific T cell responses. Together this demonstrates that individual members of the microbiota can induce an anti-inflammatory environment that limits the induction of inflammatory immune responses against the microbes themselves. Understanding this regulatory loop will allow us to develop novel ways to limit intestinal inflammation and protect from IBD.

### Regulation of Nucleic Acid Vaccine Responses By The Microbiome

Andrew Johnson - Fred Hutch Cancer Center, Nicole Potched, Kevin Hager, Drew Weissman, Mahamad-Gabriel Alameh, Jim Kublin

Vaccines are among the most effective tools for preventing human morbidity and mortality from infectious diseases. Despite this, inter-individual variability in vaccine response represents a significant barrier that makes vaccine effectiveness difficult to predict and limits overall clinical efficacy. Knowledge of the mechanisms regulating vaccine response and the specific determinants of protective immunity remains limited, however recent data has indicated that the microbiome may modulate immune responses induced by some existing vaccine platforms. Here, we interrogated the contribution of the microbiome to the immunogenicity of subcutaneously administered protein-adjuvant vaccines, as well as emerging DNA-prime protein-boost and mRNA-lipid nanoparticle (LNP) vaccines using germ-free and specific-pathogen-free (SPF) C57BL/6J mice. While the endogenous mouse microbiome has a limited role in regulating protein-adjuvant vaccine approaches, we discovered that the microbiome is a significant factor in determining DNA-prime protein-boost and mRNA-LNP vaccine immunogenicity. Specifically, we observed that the SPF microbiome suppresses humoral and CD4+ T cell responses to DNA-prime protein-boost immunization, and enhances CD8+ T cell responses to mRNA-LNP immunization. We further report on the innate immune responses initiated by mRNA-LNP immunization in these mouse models. Future work will seek to determine the underlying mechanisms by which the microbiome regulates nucleic acid vaccine response and interrogate associations with immune endpoints in human vaccine cohorts.

### Regulation of tonic type I interferon signaling by spatial regulation of intestinal mononuclear phagocytes

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Barrier mucosae are continuously exposed to sterile and non-sterile immune cues. Such exposure leads to the constitutive expression of type I interferons (IFN), which under tight immune regulation confer a protective state. The mononuclear phagocyte (MP) compartment, including monocytes, macrophages, and dendritic cells (DC), has been implicated in

orchestrating tonic IFN signaling. However, the exact mechanisms and spatial regulation of these processes remains elusive. To investigate the spatial distribution, diversity, and involvement of MPs in the regulation of constitutive type I IFN response, we employed a high dimensional 50-plex Co-Detection by Indexing (CODEX) imaging approach onto the Mx1-gfp reporter mouse model. Unsupervised cell classification identified various clusters of MPs in large and small intestine, differentiated them from cells of the lymphoid compartment, and revealed their microanatomical location within the intestinal wall. GFP expression (surrogate marker for type I IFN signaling) indicated a major contribution of the MP compartment, specifically of monocyte and macrophage populations, to the tonic IFN production in the gut. To further characterize the niche of these IFN-producing MPs, cellular neighborhood analyses were performed and revealed longitudinal as well as layer-dependent cellular associations. The latter were altered early during acute dextran sulfate sodium (DSS)-induced colitis, but not the source of IFN. Taken together, the workflow presented here allows for the identification of MP cellular neighborhoods and their involvement in constitutive IFN production, shedding light on the regulation of these responses and their biological function.

### Regulatory T cell coordination of the mucosal NK cell response during viral infection

Sarah Vick - Fred Hutchinson Cancer Research Center,  
Jennifer Lund

In many infections the mucosal barrier is the site of pathogen exposure, yet the regulation of mucosal immunity is not well understood. Herpes simplex virus-2 (HSV-2) is one of the most prevalent mucosal infections, making this disease an ideal model to study mucosal anti-viral immune response. My goal is to define how regulatory T-cells (Tregs) shape anti-viral immune responses through modulation of natural killer (NK) cells during virus infection. Utilizing a mouse model of intravaginal HSV-2 infection, I examined NK cell phenotypes using high parameter flow cytometry. To define how transient Treg depletion during HSV-2 infection impacts NK cells, I utilized Foxp3 DTR mice. Treg depletion led to increased maturation of NK cells in the vaginal tract (VT). KLRG1 is associated with NK cell maturation and is a marker of terminally mature NK cell subsets. I found a significant increase in the frequency and total number of KLRG1+ NK

cells in the VT two days after HSV-2 infection in the absence of Tregs. I further analyzed NK cell maturation using the expression of the two surface markers CD11b and CD27 and found a significant increase in CD11b+CD27+ NK cells after Treg depletion. This subset of NK cells is known to be significant producers of pro-inflammatory cytokines. My data suggests that Tregs have a modulatory effect on NK cell development in the VT as Treg depletion leads to altered NK cell maturation and development. This demonstrates the role of Tregs in mediating an effective immune response while limiting immune pathology.

### Relationship between group 3 innate lymphoid cells (ILC3) and Th17 activation in human nasopharynx-associated lymphoid tissue and their association with pneumococcal carriage

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Background : Bacterial carriage including *S. pneumoniae* (Spn) in human nasopharynx is common, and is considered a prerequisite of invasive disease. Innate lymphoid cells (ILC) are considered critical in mediating host mucosal homeostasis with local microbiome and controlling inflammation, and ILC3 were shown to regulate T cell response in intestinal tract. It is unknown whether ILC3 play any role in regulating bacterial carriage in human nasopharynx. Methods : We characterized ILC3 in nasopharynx-associated lymphoid tissue (NALT) from children and adults, and analyzed the relationship between ILC3 and Th17 cells in NALT and their relationship with Spn carriage. ILC3 and Th17 frequencies were examined by flow-cytometry following staining for lineage markers, CD127, NKP44 (NCR), c-kit, RORgt, IL17A and IL-22. Results : A higher frequency of NCR+ILC3 in NALT was shown in children as compared to adults, which was in contrast to the lower Th17 frequency in children than in adults. The abundance of ILC3 inversely correlated with the lower Th17 in children as compared to adults. A higher frequency of ILC3 was shown in children who were Spn carriage +ve than in those who were Spn carriage -ve. Spn stimulation of tonsillar MNC induced increases in ILC3 number and expressions of HLA-DR and IL22. ILC3 HLA-DR expression was positively correlated with RORgt expression. Conclusion : ILC3 may play a critical role in regulating Th17 response in the nasopharynx, and in mediating Spn carriage during childhood. Understanding local

interactions between bacteria, ILC and Th17 cells in the nasopharynx may lead to novel therapeutics against inflammation and disease.

### RelB and C/EBPa critically regulate the development of Peyer's patch dendritic cells

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To establish the protection against harmful foreign antigens, the small intestine harbors guardian sites called Peyer's patches (PPs) where mucosal immune responses is evoked. PPs take up antigens through microfold (M) cells distributed in follicle-associated epithelium (FAE) covering PP lymphoid follicles, from which the antigens are transferred to dendritic cells (DCs) located in subepithelial dome (SED) for subsequent T cell-priming. Accumulating evidence indicates that SED-DCs have unique functions other than priming T cells to facilitate mucosal immune responses. We have previously shown that SED-DCs express IL-22 binding protein (IL-22BP) to suppress barrier functions of FAE, allowing an easier access of bacterial antigens to M cells for an efficient antigen-uptake into PPs. However, the crucial factor regulating the development of SED-DCs has not been determined. Here we performed digital transcriptome analysis of SED-DCs, and identified hallmark genes of SED-DCs. Further data interpretation with weighted parametric gene set analysis (wPGSA) predicted several transcription factors (TFs) responsible for the development of SED-DCs. Among them, we identified that RelB and C/EBPa were preferentially expressed by SED-DCs in murine PPs. To evaluate the role of these TFs in SED-DCs, we examined the phenotypes of RelB- or C/EBPa-conditional knockout mice. RelB-deficiency silenced the expression of IL-22BP by SED-DCs. On the other hand, C/EBPa-deficiency decreased the expression of Lysozyme by SED-DCs, causing the increased invasion of orally administered pathogenic bacteria into PPs and mesenteric lymph nodes. Our findings demonstrate that RelB and C/EBPa regulate the development of SED-DCs, and contribute to the mucosal host defense.

### The role of capsular polysaccharides and O-antigen variation in *E. coli* immune evasion in the gut

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Antimicrobial resistance, caused by antibiotics overuse, is becoming a major issue worldwide, especially in food production animals. While major efforts are underway to develop new antibiotics, and alternative is to expand our armory of bacteria-targeting vaccines. Non-toxicogenic bacteria are best targeted by immunogenic responses against polysaccharides also called glycans, present on the bacterial surface. Whole cell-inactivated oral vaccines made from *E. coli* or *Salmonella* induce strong intestinal antibody responses [IgA] against O-antigens. However, many pathogenic enterobacteria produce abundant polysaccharide capsules that are poorly targeted by these vaccines. We are studying the importance the importance of *E. coli* surface glycans in evading intestinal IgA responses. This first includes deciphering the importance of polysaccharide capsules during the colonization of *E. coli* in the gut; as well as understanding the variation of the O-antigen in some pathogenic strains, in order to design an oligovalent vaccine capable of preventing immune evasion of virulent *E. coli*.

### The role of chemokines and the glycocalyx in driving normal tissue toxicity in radiotherapy induced bowel inflammation.

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Although radiotherapy (RT) is an effective anti-cancer treatment strategy, in pelvic and abdominal cancers, it also results in gastrointestinal tract damage; limiting the dose that can be safely administered. Leukocyte recruitment is mediated by chemokines and the extracellular matrix glycocalyx and drives acute RT-induced toxicity, which can develop into chronic inflammation and fibrosis. Following RT, locally produced chemokines diffuse and are transported to the luminal side of the endothelium where they bind to their receptors on circulating leukocytes, facilitating their recruitment. The glycocalyx is a thick barrier that lines blood vessels that, in its intact state, inhibits leukocyte adhesion to



the endothelium and ultimately, recruitment. However, how RT alters chemokine production, glycocalyx structure and function, and how this regulates RT toxicity is poorly understood. A radiation platform was used to deliver X-rays using CT-guided images. The chemokine and glycocalyx component concentrations in irradiated C57BL/6 mice were determined using Luminex and ELISAs. Small intestine analysis revealed that at acute time points following RT, chemokines that mediate innate immune cell recruitment were elevated. Flow cytometric analysis supports this cellular recruitment within the small intestine. Plasma analysis demonstrates a significant increase in the glycocalyx components, syndecan-1, heparan sulphate and hyaluronan post-RT. We reveal potential chemokines and immune cells responsible for driving RT-induced acute bowel inflammation and suggest that RT induces glycocalyx shedding. Experiments inhibiting chemokine receptors that mediate cell recruitment will help confirm their role in RT-induced toxicity. Further studies will focus on identifying mechanisms to therapeutically target and progress cancer treatments.

### Role of invariant natural killer T (iNKT) cells on immune responses to influenza A virus (IAV).

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Respiratory infections are serious health concerns worldwide. During IAV infection iNKT cells are thought to enhance early immune response. iNKTs are a subset of  $\alpha\beta$ T cells that modulate innate and adaptive immunity, and are prevalent in mucosal tissues. The functional relevance of different iNKT subsets to this effect, remains unknown. Using ET-2;CD4cre mice, we demonstrated that sustained E protein activity results in changes in subsets representation, with a shift from iNKT1 cells towards iNKT2/iNKT17 cells, and an expansion of a novel iNKT subset (Ly108 + CD44<sup>low</sup> T-bet - ROR $\gamma$ T - GATA3<sup>low</sup> PLZF<sup>low</sup>), that occur naturally in the mLN and lungs of WT C57BL/6 mice. This change in NKT subset representation correlates with better outcomes to IAV challenge. A caveat of the ET-2;CD4cre mouse model is that conventional T cells also express ET-2, so the effect could be partially T cell-dependent. To overcome this, we developed a new mouse model that has large number of iNKT cells and few conventional T cells. NKT ET-2 transgenic mice are ET2;CD4-cre;TCR $\alpha$  -/- ;V $\alpha$ 14tg, and our NKT WT mice are CD4-cre;TCR $\alpha$  -/- ;V $\alpha$ 14tg mice. When

challenged with IAV PR8, NKT ET-2 mice showed less weight loss and faster recovery compared to NKT WT mice. We can't correlate morbidity with viral load differences, but we observed changes in myeloid cell recruitment across 10 days challenge. NKT ET-2 mice have decreased lung neutrophilia and lower activation markers in inflammatory monocytes starting at day 4 post-infection. These results suggest that NKT ET-2 mice regulate better early immune response to IAV via the NKT cell-neutrophils-inflammatory monocytes axis.

### The role of regulator of G-protein signaling (Rgs)-1 in CD8<sup>+</sup> TRM-cell mediated intestinal immunity

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The gene encoding regulator of G-protein signaling 1 (Rgs1) is one of the most up-regulated genes in tissue-resident (T<sub>RM</sub>) cells. Rgs1 inhibits signal transduction by increasing the GTPase activity of the G $\alpha$ i protein subunit, which may attenuate chemokine receptor-mediated immune cell trafficking. Intriguingly, there is a striking genetic association of Rgs1 SNPs with T cell-mediated autoimmune disorders in patients (e.g. celiac disease, multiple sclerosis). The precise effects of Rgs1 on T cell differentiation, however, remain ill-defined. Herein, we generated a Rgs1-tdTomato reporter mouse, and confirmed the remarkable Rgs1 signature in intestinal T<sub>RM</sub>-cell subsets during homeostasis. Following local infection with *Listeria monocytogenes* (Lm)-Ova, Rgs1 expression is rapidly induced in Ova-specific T cells in the intestine. To assess the impact of Rgs1 on the generation and maintenance of CD8<sup>+</sup> T<sub>RM</sub> cells in the intestine, we used an adoptive co-transfer of congenic Rgs1<sup>-/-</sup>, and Rgs1<sup>+/+</sup> OT-I CD8<sup>+</sup> T cells into Lm-Ova-infected recipient mice. During the acute phase of an intestinal infection with Lm-Ova, Rgs1<sup>-/-</sup> OT-I T<sub>RM</sub> cells became underrepresented in the small intestine when compared to transferred Rgs1<sup>+/+</sup> OT-I cells. The lower abundance of Rgs1<sup>-/-</sup> OT-I cells persisted throughout the memory phase. Upon reinfection with Lm-Ova, intestinal Rgs1<sup>-/-</sup> OT-I T<sub>RM</sub> cells showed an attenuated production IFN $\gamma$ , and, in contrast to Rgs1<sup>+/+</sup> OT-I cells, failed to mediate clearance of the pathogen. These experiments

reveal the critical requirement of Rgs1 for the local accumulation of CD8 + T RM cells, and for the efficient T cell-dependent immunoprotection from systemic dissemination of pathogens upon reinfection.

### The role of RORgt+ Tregs locally and systemically upon vaccination following oral antigen pre-exposure

Nicole Potchen - University of Washington, Andrew Johnson, Kevin Hager, Jennifer Lund, James Kublin

Many promising vaccine candidates fail to elicit protective immune responses against critical human pathogens. Exposure to antigens in the gut leads to reduced systemic responses upon vaccination with the same antigen as a result of immune tolerance, which could contribute to reduced efficacy of vaccines. Tolerogenic mechanisms are necessary to avoid inappropriate inflammation and is partly achieved by the high abundance of regulatory T cells (Tregs) in the gut. Specifically, RORgt+ Tregs can be induced by gut commensals and can control type-2 responses in the small and large intestines. While Tregs are necessary for maintaining balance in the gut, it is unclear which Treg subsets lead to local and systemic changes that could affect antibody subclasses upon immunization. We have used a conditional knock-out (cKO) mouse model of RORgt+ Tregs to examine the role of this population in suppression of systemic antibody titers after oral exposure to vaccine antigen. Our data suggest that lack of RORgt+ Tregs leads to similar responses upon exposure and vaccination to the model antigen OVA as control groups with this subset intact. Both cKO groups and controls exhibited a reduction in OVA-IgG and OVA-IgG1 upon OVA exposure prior to vaccination compared to mice that were not previously exposed to OVA. cKO mice exhibited altered immunophenotypes at baseline and upon vaccination, specifically an increase in IL-33R+ Tregs, but did not contribute to an altered systemic tolerance response. This data suggests that RORgt+ Tregs are not solely responsible for maintaining gut homeostasis in a mouse model.

### The Role of Tuft Cell-Expressed KIT in Intestinal Immunity and Regeneration

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The small intestinal (SI) epithelium has two vital roles: acting as a barrier against pathogens and absorbing nutrients. This monolayer can rapidly adapt to a wide range of demands as is highlighted in the type-2 immune response. Acting as type-2 sentinels, tuft cells detect helminths and succinate, a protist secretion, and then release IL-25 to promote production of IL-13 and other canonical type-2 cytokines by group 2 innate lymphoid cells (ILC2). IL-13 acts back on the epithelium promoting expansion of tuft cells by the proliferative crypt cells thereby completing a feed-forward circuit important to effective worm clearance. However, how IL-13 induces tuft cell hyperplasia remains unclear. Our data show that tuft cells express cellular KIT (cKIT/KIT) and that IL-13 signaling through IL-4RA is both necessary and sufficient to induce KIT. Why tuft cells express KIT remains to be elucidated. KIT is a receptor tyrosine kinase required for cell proliferation and survival in a variety of cell types. KIT activation has been linked to intestinal regeneration within another intestinal epithelial lineage, Paneth cells, but this signaling has not been studied in tuft cells. I hypothesize that IL-13 stimulated tuft cells utilize KIT signaling to promote their survival and this signaling on progenitor cells helps regenerate the helminth-disrupted epithelial barrier.

### Salivary cytokines reveal a type-2 inflammatory profile in eosinophilic esophagitis

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Rationale: Eosinophilic esophagitis (EoE) is an increasingly common immune-mediated disease in which food antigens lead to dysphagia and eosinophil infiltration in the esophagus causing inflammation and tissue damage. There are currently no FDA-approved therapies for EoE or noninvasive biomarkers that can be used to diagnose or monitor the disease. Instead, patients must follow strict empiric diet eliminations that can take months up to a year and undergo frequent upper endoscopies to monitor disease status and response to therapy. A noninvasive method of identifying biomarkers for EoE would significantly lessen the financial and emotional burden placed on patients. Salivary proteomes have been previously characterized in many diseases including Sjogren's Syndrome and Schizophrenia. In this study, we investigate cytokine expression in saliva from patients with active and resolved EoE, as well as non-EoE control patients. Methods:

Whole saliva was collected in 23 patients with EoE (active EoE n=9, resolved EoE n=8) and six non-EoE controls. Twenty-three cytokines/chemokines were measured in saliva using the Milliplex MAP Human Cytokine/Chemokine Magnetic Bead Panel. Cytokine data were analyzed with Belysa Immunoassay Curve Fitting Software 1.1. Standard-curve-interpolated cytokine concentrations were normalized to total protein concentrations and compared across active and resolved EoE groups and active and control groups using Mann-Whitney U testing. Results: The levels of the eosinophil chemoattractant cytokines eotaxin-3 ( $p=0.0206$ ), MCP-2 ( $p=0.0274$ ), and MCP-4 ( $p=0.0274$ ), were significantly elevated in the saliva of patients with active compared to resolved EoE. I-309, a monocyte and eosinophil chemoattractant, was also significantly higher in active EoE specimens ( $p=0.0148$ ), compared to resolved. Detection levels of TSLP ( $p=0.0072$ ), IL-33 ( $p=0.0172$ ), and TRAIL ( $p=0.0256$ ) were higher in patients with active EoE versus control patients. Conclusions: In this study, we demonstrate successful measurement of salivary cytokines from patients. The elevated levels of eosinophil chemoattractants and inflammatory cytokines such as MCPs, Eotaxin-3, TSLP, and IL-33 correspond to previous work. Our findings suggest that salivary cytokines can be used as biomarkers for EoE to distinguish disease status and activity. This is a relevant and noninvasive modality that should be investigated further for both characterizing disease and assessing treatment response.

## Salmonella subverts T cell activation during bacterial cancer therapy by inducing metabolic paralysis

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Bacterial cancer therapy (BCT) is a promising therapeutic for solid tumours. *Salmonella enterica* Typhimurium (STm) is well-studied among bacterial vectors due to advantages in genetic modification, metabolic adaptation, and motility. A longstanding paradox has been the redundancy of T cells for treatment efficacy; instead, STm BCT depends almost exclusively on innate phagocytes for tumour control. Here, we used TCR reporter mice (Nr4a3<sup>-Tocky</sup>-Ifng<sup>-YFP</sup>) and a colorectal cancer (CRC) model to interrogate T cell activity during BCT with an attenuated STm mutant. We found TILs produced IFN- $\gamma$  predominantly in absence of recent TCR

activity (IFN- $\gamma$  + Timer neg), exhibiting reduced polyfunctionality and TCR responsiveness. Modelling T-cell:tumour interactions with a tumour organoid platform revealed that soluble signals from the infected tumour could potentially modulate TCR-driven T cell activation, but not by cytokine activation. Investigating TCR signalling showed intact nuclear NFAT/NF- $\kappa$ B translocation, but severe disruption to the metabolic signalling networks required for full T cell activation. Our work shows for the first time that T cells are metabolically impaired during STm BCT—elucidating a decades-long enigma—and providing a fundamental target for augmenting treatment efficacy. This study also lends insight into *Salmonella* evasion of T cell immunity during gastrointestinal infection.

## SARS-CoV-2-specific CD8+ T cells recognize infected respiratory epithelial cells

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CD8 T cells are thought to protect against coronavirus disease by recognizing infected respiratory epithelial cells, secreting antiviral cytokines or killing infected cells, and ultimately reducing local viral replication. SARS-CoV-2 has been reported to reduce HLA class I expression, but few studies detail interactions between specific CD8 T cell effectors and infected antigen presenting cells. We have developed an effector-target interaction model with the human bronchial epithelial cell line HBEC3-KT-ACE2. Infection with GFP-SARS-CoV-2 based on strain WA1 results in cytopathic effect, syncytia formation, and apoptosis of target cells. Importantly, HBEC3-KT-ACE2 maintains expression of HLA-A\*03:01 protein on the plasma membrane. Quantitative PCR confirms viral RNA increase from  $1.0 \times 10^4$  copies/ $10 \mu$ l supernatant at 24 h to  $3.2 \times 10^6$  and  $5.6 \times 10^6$  at 48 h and 72 h, respectively. HLA-A\*03:01-restricted SARS-CoV-2 Spike AA 378-386-specific CD8 + T cells from a COVID-19-convalescent donor were obtained by re-stimulation, tetramer sorting, and further polyclonal expansion. These effector T cells secreted interferon- $\gamma$  in response to SARS-CoV-2-infected HBEC3-KT-ACE2s, reducing supernatant SARS-CoV-2 RNA levels by 2.4-, 4.2- and 8.9-fold at effector-to-target cell ratios of 5:1, 15:1 and 45:1, respectively. Specific recognition of SARS-CoV-2

infected, renal epithelial-origin 293T-ACE2 cells by HLA-matched Spike and SARS-CoV-2 ORF9B-specific T cell lines was also noted. We are also developing primary human nasal epithelial cell culture models. Our studies show that SARS-CoV-2-infected epithelial cells can stimulate virus-specific CD8 T cells in vitro resulting in a net anti-viral effect and provide a system for detailed studies of immune recognition and evasion. (XW and TYH contributed equally.)

## Secondary bile acids expand granulocyte monocyte progenitors by interacting with hematopoietic progenitor cells via the vitamin D receptor

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The microbiome and its associated metabolites have been shown to interact with immune cells and can alter the severity of infection with intestinal parasite *Entamoeba histolytica*. We have demonstrated that host-derived, microbially-metabolized, secondary bile acids can increase bone marrow myelopoiesis, and provide protection from amebic infection. Treatment of bone marrow cells in-vitro with deoxycholic acid (DCA), during colony-forming assays as well as fibroblast co-culture experiments showed that DCA preferentially expanded both immunophenotypic and functional myeloid progenitors (CFU-GM) as well as granulocyte monocyte progenitors (GMPs). Interestingly, we were also able to show that treatment with another secondary bile acid, lithocholic acid (LCA), but not primary bile acid, cholic acid (CA) also expanded CFU-GMs and GMPs. Treatment of FACS sorted LSK (lin-, SCA-1+, C-KIT+) cells in-vitro increased CFU-GMs suggesting that DCA interacts directly with hematopoietic stem and progenitor cells (HSPCs). To better understand how DCA communicates with HSPCs we sought to determine the contribution of bile acid receptors in the bone marrow. We demonstrated that the secondary bile-acid sensing vitamin D receptor (VDR) was required for the DCA-induced increase in CFU-GMs. Moreover, analysis of vitamin D receptor-deficient (VDR -/-) mice provided evidence that VDR was required for DCA to expand GMPs. Finally, treatment of whole marrow with known VDR agonist 1,25 dihydroxy vitamin D3 dose-dependently expanded CFU-GMs. The action of DCA on HSPCs to expand GMPs in a VDR-dependent manner suggests a mechanism for how host-microbiome interactions can alter the fundamental

processes of hematopoiesis and thus the severity of *E. histolytica* infection.

## Sharing of TRB Sequences

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HSV-2 infects epithelial cells and establishes life-long latency in a sensory nerve ganglion. Viral reactivation can lead to asymptomatic shedding or symptomatic outbreaks; our current understanding is that host defense, in particular resident memory T cells (TRM), determines the outcomes of these events. By clonal tracking of TRB sequences from 3-4 serial genital skin biopsies spanning 2-8 weeks after resolution of an HSV-2 lesion (quiescent biopsies) and HSV-2-reactive T cells from blood, we identify a resident population of T cells. A resident TRB was a clonotype identified in each of an individual's quiescent biopsies. In 10 persons, a median of 598 resident clonotypes/person were consistently detected (range 121-1032). These represented a median of 9.0% of all quiescent TRB sequences (range 2.0-24.4%). In a subset of 6 persons, HSV-2-reactive CD4 and CD8 T cell clonotypes from peripheral blood were enriched by activation-induced marker (AIM) sorting. AIM+ CD4 clonotypes overlapped with a median of 1.1% quiescent TRB sequences (range 0.2-2.1%), increasing to 16% (range 2.9-48.9%) resident TRB sequences ( $p = 0.03$  by paired Wilcoxon). AIM+ CD8 T cells overlapped with a median of 0.2% (range 0.04-0.45%) quiescent TRB sequences, compared to 1.9% (range 0.6-7.1%) resident TRB sequences ( $p = 0.03$  by paired Wilcoxon). Using GLIPH2 software, a median of 17.5% (range 7.0-96.0%) resident TRB sequences were identified by sequence similarity as potentially HSV-2-reactive. In summary, healed genital skin after an HSV-2 outbreak demonstrate TRB clonal stability in serial biopsies, and is enriched for persistent, virus-specific T cells that overlap with circulating clonotypes.

## Single cell RNA-sequencing: a tool to study the diversity of the lamina propria CD45+ cells in the rabbit caecum

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Rabbits are reliable animal models used in immunological research, in particular to study gut mucosal immunity. The lamina propria, underlying the epithelium in the mucosa, is considered the primary effector site of the intestinal immune response and is composed of a heterogeneous immune cell population. A detailed characterization of the diversity of mononuclear hematopoietic cells (CD45<sup>+</sup>) in the rabbit caecal lamina propria is lacking, even though this gut segment has a key role in the interaction with the gut microbiota. The present study aimed to fill that void. We isolated caecal lamina propria mononuclear cells by physical, chemical and enzymatic dissociation followed by a purification in a Percoll gradient. Single live CD45<sup>+</sup> cells were sorted by flow cytometry (16% of total) and droplet-based single cell RNA-sequencing was performed. Data were analyzed using the Cell Ranger software and the R package Seurat. After applying quality filters, 4,246 cells were kept and the expression of 29,587 genes was detected. 13 cell clusters were identified, with the majority affiliated to B, T, NK and dendritic cells and macrophages based on the expression of canonical markers (JCHAIN, RORA, KIT, NKG7, C1QB, CD14). Our method thus enabled us to clearly identify the main mononuclear immune cell types present in the lamina propria with little contamination with other cell types (e.g. fibroblast). This study provides the first single cell phenotyping data on rabbit caecal mononuclear hematopoietic cells and offers great perspectives for the study of microbiota gut mucosal immunity regulations.

## Single-cell RNA sequencing unveils intestinal eosinophil development and specialization

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The gastrointestinal (GI) lamina propria is home to the largest population of tissue-resident eosinophils in the body, yet the contribution of these cells to local homeostatic and inflammatory processes is still incompletely understood. Recent evidence indeed implicates eosinophils in the regulation of intestinal immune cell populations, maintenance of epithelial barrier integrity and promotion of tissue morphogenesis, but also highlights their contribution to the pathogenesis of chronic intestinal disorders such as inflammatory bowel diseases (IBD). To date, the transcriptional programs and the gene regulatory networks governing eosinophil pleiotropic functions remain largely unexplored. Using single cell transcriptomics, high-dimensional flow

cytometry and functional genomics, we found that eosinophils exist as five discrete cell states along their maturation from bone marrow progenitors to intestinal resident cells. Within GI tissues, "basal" eosinophils differentiate into a highly specialized "active" subset that localizes at the luminal extremity of the mucosa and is characterized by PD-L1 and CD80 expression. Basal-to-active subset conversion is driven by NF- $\kappa$ B signaling in response to local tissue and microbial cues. Upon bacterial challenge infection or inflammation, the number of "active" eosinophils dramatically increases, while "basal" eosinophils remain unaltered. This subset further exhibited strong immune regulatory properties and re-activated systemic anti-microbial gene programs, thus enhancing their cytotoxic arsenal and promoting pathogen clearance. Both "basal" and "active" eosinophils were readily detected in the human colonic mucosa, while the "active" subset was significantly enriched in IBD patients. Together, our work dissects eosinophil's heterogeneity and plasticity, highlights their crucial contribution to intestinal homeostasis, and provides a framework for their functional characterization in human gastrointestinal diseases.

## Site-specific immune responses of tissue resident memory T cells following live attenuated oral typhoid vaccine, Ty21a in humans

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The human gastrointestinal tract constitutes a major reservoir of memory T cells (T<sub>M</sub>), especially tissue-resident memory T (T<sub>RM</sub>) cells which are central to mediate protective immunity. T<sub>RM</sub> are phenotypically different from circulating T<sub>M</sub> subsets and expressed CD69 and integrin  $\alpha$ Eb7 (CD103). Recent findings on the distribution and T<sub>RM</sub> antigen-specific responses in human tissues have indicated that the organization, differentiation and maintenance of human T<sub>RM</sub> are strikingly tissue-intrinsic. However, very limited data is available in humans regarding site-specific characterization of T<sub>RM</sub> and their antigen-specific responsiveness following oral immunization. Here, we examined duodenal (Duo) and terminal ileum (TI) immune responses of lamina propria mononuclear cells (LPMC) CD4<sup>+</sup> and CD8<sup>+</sup> T<sub>RM</sub> obtained from biopsies of Ty21a-vaccinated and unvaccinated

individuals. We found no significant differences in the frequencies of CD4<sup>+</sup> and CD8<sup>+</sup> T RM between the two tissues regardless of vaccination. Next, the baseline level of cytokines secreted by LPMC CD4<sup>+</sup> and CD8<sup>+</sup> T RM subsets was compared between Duo and TI and significant differences were observed. For example, CD103<sup>+</sup> CD69<sup>+</sup> CD4<sup>+</sup> T RM spontaneously produced significantly higher levels of IFN $\gamma$  in TI than Duo following Ty21a immunization. Importantly, we determined and compared Duo and TI S . Typhi-specific CD4<sup>+</sup> and CD8<sup>+</sup> T RM subset responses following Ty21a immunization. Preliminary data indicate that there are significant differences in S . Typhi responsive cells between the two tissues. For example, CD103<sup>+</sup> CD69<sup>+</sup> CD8<sup>+</sup> T RM exhibited significantly higher S . Typhi-specific IL-17A production in TI than Duo following Ty21a immunization. These comparisons provide unique insights into the role of tissue specificity on the generation of S . Typhi specific responses in the human intestine.

### Site-Specific Immunity and Cellular Clustering Regulate Th2 Differentiation

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T helper 2 (Th2) cells orchestrate diverse immune responses, ranging from protection against parasites to inappropriate inflammation during allergy and asthma. Substantial efforts have gone into identifying the mechanisms leading Th2 differentiation, yet how Th2 cells are generated in tissue context remains poorly defined. Here, we used highly-multiplexed quantitative imaging to investigate the early generation of Th2 immunity within skin-draining lymph nodes (LNs) after immunization with the allergen protease Papain. We found that Papain immunization elicited the formation of extensive LN regions composed of highly activated and tightly clustered T cells undergoing early Th2 differentiation that were in association with migratory dermal dendritic cells (dDCs). Th2 cells in these clusters were characterized by enhanced expression of IRF4 and BATF transcription factors, homotypic LFA-1 / ICAM-1 integrins, as well as of phosphorylated STAT-5 and -6, and these were collectively tightly correlated with GATA3 expression. Surprisingly, formation of these Th2 microenvironments was highly dependent on the specific site of cutaneous immunization, as papain administration in different skin sites resulted in dramatically non-equivalent T

cell clustering and differentiation. Consistent with this, mRNA sequencing of antigen-bearing migratory dDCs in different draining LNs revealed distinct transcriptional profiles associated with cellular maturation, with the dDCs in Th2 promoting LNs expressing much higher levels of several key costimulatory molecules. Timed in vivo blockade of co-stimulatory molecules or disruption of integrin-driven T cell clustering resulted in marked abrogation of Th2 differentiation, revealing that these processes are critical for early Type-II responses. Collectively, we identify dedicated microenvironments within LNs that support Th2 differentiation through enhanced T cell clustering driven by activated dDCs, and reveal that formation of these microenvironments is highly context-dependent, with the existence of dermal site-specific responses in the generation of Th2 immunity.

### Small intestinal TCR repertoire is mainly regulated by non-antigenic dietary ingredient via shifting microbiota composition.

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Dietary antigens (Ags) have been thought to be major stimuli recognized by small intestinal T cells based on changes in effector and regulatory T cell populations. However, the impact of dietary protein on small intestinal TCR repertoire has not been examined. Here, we performed a fixed TCR $\beta$  chain model TCR repertoire analysis of small intestinal T cells from mice fed with a normal diet vs protein-free diet under specific pathogen-free (SPF) or germ-free (GF) environment. Unexpectedly, we found dietary Ags make a minor contribution to small intestinal TCR repertoire. Rather, the majority of TCR repertoire changes were associated with the presence of intestinal microbiota. More importantly, such microbiota-associated TCR repertoires were dependent on normal diet under SPF condition. Hence, natural ingredients in normal chow diet are essential for supporting growth of T cell-stimulatory microbiota. In the line with this notion, feeding purified protein of normal diet did not recover microbiota composition nor normal diet/microbiota co-dependent TCR in vivo reactivity. Therefore, diet-dependent microbiota is the primary driver of mucosal T cell responses different from conventional notion that dietary Ags make bigger contribution.

## Spatial analysis of $\gamma\delta$ T-cells in mucosal Chronic Graft-Versus-Host Disease (cGVHD)

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Chronic Graft-Versus-Host-Disease (cGVHD) is an autoimmune-like disease following allogeneic hematopoietic stem cell transplantation (HSCT). cGVHD targets multiple organs, including the oral cavity where oral mucosa (OM) and minor salivary glands (MSG) are frequently affected. Our prior work in the human OM identified that most IL17-producing cells in the cGVHD patients are non-T cells, which differ from other mucosal cGVHD sites suggesting a differential immune milieu at distinct effector sites. Gamma-delta T cells ( $\gamma\delta$  T) are uniquely conserved population of lymphocytes that, as a first line of defense, mediate innate immunity against infections playing a unique role in immune surveillance and tissue homeostasis. Single-cell RNA sequencing (scRNA seq) analysis of human OM and MSG identified an increase in IFN $\gamma$  and IL17 producing  $\gamma\delta$  T-cells in cGVHD versus unaffected post-transplant patients. These cells had upregulated expression of transcription factor EOMES which plays crucial role in activation of cytotoxicity related genes like GZMA, GZMB and PRF1 in cGVHD patients. Spatial transcriptomic analysis on human OM and MSG sections revealed  $\gamma\delta$  T-cell distribution at the basement membrane and deep submucosa.  $\gamma\delta$  T-cells were frequently co-localized with GZMA, GZMB and PRF1 clusters at the basement membrane, which is a frequent site of tissue damage in cGVHD. These data suggest a direct cytotoxic role of  $\gamma\delta$  T-cells in local production of IFN $\gamma$  and IL17 cytokines contributing to chronicity of GVHD. In future, use of genetic mouse models with  $\gamma\delta$  T-cell alterations will help elucidate their function in cGVHD pathogenesis.

## Spatial compartmentalisation of pathobiont-reactive T cell differentiation in gut tissue

Yisu Gu - University of Oxford, Raquel Bartolome-Casado, Cornelia Heuberger, Sarah A. Teichmann, Emily Thornton, Fiona Powrie

Spatial compartmentalisation of pathobiont-reactive T cell differentiation in gut tissue Yisu Gu, Raquel Bartolome-Casado, Cornelia Heuberger, Sarah Teichmann, Emily Thornton\* and Fiona Powrie\* \*Senior co-authors T regulatory

cells (Tregs) are key players in maintaining gut homeostasis by controlling aberrant immune responses towards microbial antigens. Our lab has previously shown intestinal infection by the pathobiont *Helicobacter Hepaticus* (H.h) induces tolerance through the expansion of IL-10 producing Tregs. The murine gut tissue contains hundreds of solitary isolated lymphoid tissue (SILTs) structures, which include cryptopatches and isolated lymphoid follicles. Controversy remains with regards to the functional contribution of SILTs to tissue immunity. How Treg spatial positioning within SILTs or the surrounding tissue relates to their function is currently unclear. We thus aimed to assess the role of SILTs in the education of microbial antigen-reactive Treg responses in support of barrier immunity. Through adoptive transfer of naive H.h-specific T cells (HH7-2tg) [1] into colonised mice, we show Treg enrichment occurs primarily within gut tissue, and not secondary lymphoid structures. In vivo live imaging of gut tissue demonstrates IL-10 producing HH7-2tg T cells to be predominately positioned within the lamina propria, spatially distinct from SILTs. To identify which signals in the lamina propria may drive IL-10 production in response to H.h, we next performed niche-seq [2] of lamina propria and SILT compartments and found unique antigen presenting cell subsets at the two sites. We are currently exploring receptor/ligand interactions found on distinct Treg and myeloid cell subsets to establish a previously unrecognised spatial mechanism of tolerance at barrier sites. Detailed mapping of unique antigen presenting cell subsets within gut tissue micro-niches could be of therapeutic benefit if potential druggable targets are identified. 1. Xu, M., et al., c-Maf-dependent regulatory T cells mediate immunological tolerance to intestinal microbiota. bioRxiv, 2017. 2. Medaglia, C., et al., Spatial reconstruction of immune niches by combining photoactivatable reporters and scRNA-seq. Science, 2017.

## Spontaneous Remission of Experimental Eosinophilic Esophagitis

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Rationale: Eosinophilic Esophagitis (EoE) is a chronic, immune-mediated type 2 allergic disease characterized primarily by the infiltration of eosinophils into the esophagus. Herein, we seek to characterize the remission of the disease in an experimental EoE murine model. Methods: Wild type (WT)

mice were skin sensitized by the application of oxazolone (1%, OXA), followed by 5 challenges (0.5% OXA). From day 18, mice were challenged intraesophageally (1% OXA) for a total of 8 oral challenges using a modified feeding tube to enhance intraesophageal administration. Mice were euthanized twenty-four hours after the last challenge (day 36) and on day 64, and the presence of eosinophils as well as lamina propria and epithelial thickness were determined. Results: Intraesophageal challenges of OXA to skin-sensitized mice resulted in experimental EoE that highly resembles human EoE including epithelial thickening, edema, basal cell proliferation, intraepithelial eosinophilia, fibrosis and substantial transcriptome overlap with human disease. One month following cessation of intraesophageal OXA challenges, increased esophageal edema, lamina propria thickening and epithelial cell proliferation were completely abrogated and were comparable to naive mice. Interestingly, although scattered eosinophils were still detected in the esophagus of OXA-challenged mice on day 64, the levels of eosinophils was dramatically reduced on day 64. Conclusions: We report the establishment of a self-resolving experimental model of EoE. This model can be used to characterize molecular and cellular mechanisms of remission in EoE.

### Strain-variation within *Bacteroides fragilis* species shapes immunomodulatory functions

Marvic Carrillo Terrazas - UCSD, Chia-Yun Hsu, Luke Loomis, Hiutung Chu

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract and involves host genetics, environmental factors, and an inappropriate immune response to gut microbes. Previous studies have demonstrated the commensal bacterium, *Bacteroides fragilis*, is able to induce regulatory T cells (Tregs) and protect during experimental colitis, suggesting that this tolerogenic bacteria may play a protective role in IBD. However, it is unknown if the inflammatory intestinal environment of IBD alters the anti-inflammatory function of *B. fragilis* strains. Notably, the current body of literature on *B. fragilis* immunomodulation largely use the type strain NCTC 9343, originally isolated from an abscess. Thus, it remains unclear if this anti-inflammatory property is universally conserved among *B. fragilis* strains in the human population. This study aims to examine how bacterial diversification within individuals elicits a host-specific

immune response. Our work takes a novel approach by investigating the functional variation of *B. fragilis* at the strain-level, in healthy and inflammatory conditions. We hypothesize that the inflammatory condition in the gut may drive adaptation of *B. fragilis*, impairing its ability to induce anti-inflammatory Tregs necessary in controlling intestinal inflammation during IBD.

### Stromal cells control the maturation of myeloid cells and maintain intestinal mucosal tolerance via MyD88-dependent mechanisms

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Introduction: Intestinal mesenchymal cells, in particular myofibroblasts, in a healthy gut are among the key players in mucosal tolerance. Microbiota sensing and microenvironment signals in fibroblasts activate MyD88-depending pathways and produce essential molecules that require homeostasis. Fibroblasts and macrophages are located in the proximity of each other in colonic mucosa. However, the impact of fibroblast on macrophages under intestinal mucosal tolerance remains unclear. We hypothesized that MyD88-mediated signaling in fibroblasts regulates macrophage activity/differentiation under colonic mucosal tolerance. Results: In vivo deletion of MyD88 in myofibroblasts and fibroblasts by using cell-specific B6ACTA2CreMyD88 fl/fl, B6Col1a2CreMyD88 fl/fl mice, respectively, resulted in the development of microcolitis. Using mice B6RagCol1a2CreMyD88 fl/fl that lack T and B cells we demonstrated that disruption of fibroblast specific MyD88 mediated regulation of innate immune cells is the key in the induction of microcolitis. Dramatically weight decrease and diarrhea were observed in this model. The destructive processes in the mucosal tissue of myo-/fibroblasts specific MyD88KO mice were associated with increasing pro-inflammatory genes and the accumulation of immature monocyte-derived macrophages. RNA-Seq analysis showed that in vitro deletion of MyD88 from fibroblasts resulted in changes of basal and TLR4-mediated pathways involved in myeloid cell migration/maturation as well as inflammation and extracellular matrix remodeling. The co-culture system of fibroblasts with bone marrow-derived macrophages showed



that MyD88-mediated signaling pathways in fibroblasts are required to suppress myeloid cell inflammatory activity. Conclusion: Our data suggest that myo-/fibroblasts intrinsic MyD88 signaling is critical to macrophages maturation and controls the inflammatory activity of monocytes/macrophages under intestinal mucosal tolerance.

### The structures and functions of dimeric and secretory Immunoglobulin A

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Secretory (S) Immunoglobulin (I) A is the predominant mucosal antibody, which mediates host interactions with pathogenic and commensal microbes using a polymeric structure and associated effector functions including antigen coating, crosslinking and Fc receptor mediated processes. Typically, two copies of IgA assemble with one joining-chain (JC) to form dimeric (d) IgA, which is bound by the polymeric Ig-receptor ectodomain, called secretory component (SC), to form SIgA. Despite significance, the dIgA and SIgA structures remained elusive until 2020, when our group and others published the cryo-electron microscopy structures of these crucial antibodies. Our structures revealed two IgAs conjoined through four heavy-chain tailpieces and the JC. The two IgAs were bent and tilted with respect to each other, forming distinct concave and convex surfaces. In SIgA, SC was bound to one face, asymmetrically contacting both IgAs and JC; SC was largely solvent accessible suggesting potential to bind host or microbial factors. Unpublished structure-based mutational analysis provides insights on mechanisms of SIgA assembly and computational modeling indicates that the bent and tilted arrangement of complex components will limit the possible positions of both sets of antigen binding fragments (Fabs) and preserve steric accessibility to receptor binding sites. Together data provide new insights on long-outstanding SIgA structure-function relationships relevant to understanding SIgA's diverse mucosal functions, including how it shapes host microbiota and eliminates pathogens, and relevant to engineering SIgA-based therapeutics.

### The synergistic role of a Crohn's disease-associated pathobiont and NSAID in promoting inflammation and cell death in susceptible host via the caspase-8/NLRP3 axis

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Non-steroidal anti-inflammatory drugs (NSAIDs) are believed to exacerbate inflammation in patients with inflammatory bowel disease (IBD), but the mechanisms regulating NSAID-induced symptoms are unknown. Pathobionts such as adherent-invasive *Escherichia coli* (AIEC) are widely prevalent in the mucosa of patients with Crohn's disease (CD) and considered relevant to CD pathogenesis. The inflammasomes such as NLRP3 are implicated in the maintenance of gut immune homeostasis and involvement in gut injury. Caspase-8 is a protein regulating programmed cell death, intestinal homeostasis, and inflammation. We hypothesise that the presence of AIEC might explain the NSAID-induced symptomatic worsening in IBD. Using IL-10 <sup>-/-</sup> mice, we show an aggravation of colitis in AIEC-colonised mice fed on an NSAID supplemented diet accompanied by activation of NLRP3 inflammasome, caspase-8 and cell death executors, e.g., caspase-3, PARP and Gasdermin D. However, IL-10 <sup>-/-</sup> mice colonised with AIEC alone or fed an NSAID supplemented diet alone did not develop colitis, highlighting the synergistic effect of both AIEC and the NSAID. Using small-molecule inhibitors targeting NLRP3 and caspase-8, we show an amelioration in colitis due to a reduction in pro-inflammatory cytokines, M1 macrophages, cell death (apoptosis/pyroptosis) and improved histology. 16S analysis identified an increased fecal abundance of *Clostridium* XIVa species in both inhibitor-treated groups. In conclusion, our findings provide evidence and mechanistic insights into how NSAIDs and an opportunistic CD-associated gut pathobiont can synergise to worsen IBD symptoms and inflammation. The data suggest that targeting the caspase-8 and NLRP3 axis could be a potential therapeutic strategy for IBD patients with NSAID-worsened inflammation.

### Targeted gut microbiome modulation alters autoimmune neuroinflammation

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Given the impact of the intestinal microbiome on autoimmune diseases, such as multiple sclerosis (MS), microbiome modulation has gained considerable attention as a potential therapy-supporting approach for the benefit of affected patients. However, little is known about how specific constellations of bacterial species within an intestinal microbial community affect disease pathophysiology. We investigated the impact of different microbiome compositions on the development of experimental autoimmune encephalomyelitis, a preclinical model of MS, in gnotobiotic mice containing a characterized 14-member synthetic human microbiome. We performed multiple species-drop-out experiments based on the microbial functional implications. Our readouts included the disease course in mice harboring such targeted modulation of the microbiome as well as thorough characterization of T cell- and B-cell subsets, microbial metabolic profiles from cecal contents and various mucosal barrier-associated readouts under homeostatic and neuroinflammatory conditions. We identified a combination of cellular, microbial and metabolic risk factors, which allowed prediction of the disease course. Our experiments revealed that the disease-promoting properties of such risk factors are based on the presence rather than the relative abundance of a given species, are not determined by one hallmark species alone and are crucially influenced by the composition of the remaining community members. These results underscore the potential of microbiome modulation for therapy-assisting purposes in the gut-brain axis and suggest that the personalized approaches of the modulation are more likely to succeed than a one-size-fits-all approach.

## T-cell and colorectal cancer organoid cocultures in an organoid killing assay provides an effective in vitro model for immunotherapy

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Colorectal cancer (CRC) organoids provide one of the most physiologically and clinically relevant in vitro cancer models available for research and drug discovery. However, organoids

alone capture only part of the complex nature of tumors in vivo. By coculturing CRC organoids with patient-matched tumor-infiltrating lymphocytes (TILs), we can capture the immune component of the tumor and open the door to new models for immunotherapy in vitro. A major challenge of a coculture model is maintaining both the viability and relevant activity of both tissue types for the duration of the assay. We optimized a combination of IntestiCult™ Organoid Growth Medium (OGM) and ImmunoCult™-XF T cell expansion medium as a base medium for each culture type and found that a 1:1 ratio of each was optimal for maintaining a high degree of viability for up to 5 days. Furthermore, activated TILs secreted  $2981 \pm 160$  pg/mL IFN $\gamma$  (n = 3), indicating they remain active in the coculture medium. When the CRC organoids are maintained in coculture with TILs for 3 days, we evaluated TIL-induced apoptosis in the organoids via Caspase 3/7 activity. A 4.40 - fold increase in apoptosis of CRC organoids cocultured with TILs as compared to CRC organoids alone demonstrated significant killing activity of the TILs in coculture. Furthermore, TIL viability and IFN $\gamma$  secretion remained high during the coculture. In conclusion, this assay can quantify the activity of TILs within a tumor in an in vitro model, thereby providing a robust tool for developing patient-specific immunotherapies.

## TGFbR signaling in squamous epithelial cells plays a fundamental role in controlling tissue specific allergic inflammation.

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Allergic diseases are becoming a global health crisis. Individuals harboring loss-of-function variants in transforming growth factor beta receptor (TGFbR) have an increased prevalence of allergic disease, but the mechanisms responsible are poorly understood. We demonstrate that mice harboring a variant identified in patients spontaneously develop disease that clinically, immunologically, histologically, and transcriptionally recapitulates eosinophilic esophagitis, a condition characterized by allergic inflammation in the esophagus. Diminished TGFbR signaling impairs epithelial cell maturation, resulting in local expression of inflammatory mediators that drive accumulation and activation of

eosinophils, mast cells, T cells, and innate lymphoid cells. Our work reveals a fundamental, non-redundant, cell-intrinsic role for TGF $\beta$ R signaling in epithelial cells needed to maintain immune homeostasis and prevent allergic inflammation, independent of barrier function or adaptive immune tolerance. Moreover, we demonstrate that epithelial dysfunction alone is sufficient to initiate and perpetuate allergic inflammation, which has implications for the prevention and treatment of allergic diseases.

### TIGIT expression differentiates regulatory from inflammatory Th1\* gut-homing effector CD4+ T cells in inflammatory bowel disease patients

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Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is characterized by intestinal infiltration of pathogenic effector CD4 + T cells. The defects driving loss of T-cell regulation in IBD vary between patients and remain undefined. Previously, we have shown that 20-40% of intestinal effector (CD62L neg CD4 + ) T cells expressed TIGIT, an inhibitory receptor modulating dendritic cell and T-cell function. In peripheral blood of healthy individuals, TIGIT expression was enriched in gut-homing CD38 + effector T cells while in a subgroup of IBD patients, frequencies of TIGIT + CD38 + effector T cells were decreased and associated with earlier relapse of disease. This raised the question whether gut-homing effector T cells lacking TIGIT are pathogenic mediators of intestinal inflammation in IBD. We monitored TIGIT + and TIGIT neg cells in peripheral blood and intestinal resection tissue of pediatric IBD patients. At diagnosis, approximately 50% of CD patients had strongly reduced frequencies of circulating TIGIT + CD38 + effector T cells compared to UC patients and age-matched healthy controls. As anticipated, TIGIT neg CD38 + effector T cells showed high expression of chemokine receptors associated with inflammatory IFN $\gamma$  high -IL17 low -producing cells (Th1\*). Moreover, intestinal TIGIT neg CD4 + T cells of IBD patients contained higher frequencies of IFN- $\gamma$ - and IL-17A-producing

cells than TIGIT + CD4 + T cells. Interestingly, in an in-vitro assay, among multiple IBD-related cytokines, only IL-12p70, a known driver of IFN- $\gamma$ , could convert inhibitory TIGIT + CD38 + effector T cells of healthy individuals into their TIGIT neg proinflammatory counterpart. In conclusion, we identify TIGIT neg gut-homing effector T cells as potential drivers of intestinal inflammation in a subgroup of CD patients.

### Towards a translational understanding of colonic Bacteroides species in Crohn's disease pathogenesis

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Crohn's disease (CD) is a chronic inflammatory condition of unknown aetiology, although the microbiota is a major contributor to the disease. An increased abundance of Bacteroides species was identified in inflamed colon biopsies from an Irish CD-cohort. This study aims to identify mechanisms regulated by Bacteroides species and their relevance to CD-pathogenesis. Human colon epithelial cells (C2Bbe1) exposed to whole Bacteroides cells and conditioned-media (CM) were assayed for viability (LDH), gene expression (RT-qPCR) and cytokine response (ELISA). Murine intestinal organoids were differentiated in media supplemented with CM-from B. vulgatus and B. fragilis for 7 days and assayed for epithelial differentiation genes, Wnt/ $\beta$ -catenin and BMP-pathways. Antibiotic-depleted-microbiota IL10 -/- mice were orally gavaged with B. vulgatus ( $1 \times 10^9$  CFU, 3-days) at 3-weeks intervals for three months, followed by the collection of faeces (16S analysis) and colon samples (RT-qPCR, MSD, histology). Whole Bacteroides species and Bacteroides -CM have pro-inflammatory potential by activating NF $\kappa$ B/STAT-JAK pathways, with B. vulgatus being the most pro-inflammatory. Bacteroides- CM had a low impact on organoid development but altered BMP and Wnt/ $\beta$ -catenin-associated genes, potentially affecting epithelial cell proliferation. Male IL10 -/- mice colonised with B. vulgatus developed worsened colitis based on altered inflammatory and epithelial gene profile, microbiota and bile acid composition and histology. Notably, female IL10 -/- mice with antibiotic-depleted-microbiota developed intestinal inflammation, not seen in male counterparts or female IL10 -/- mice colonised with B. vulgatus. Collectively, our data indicate that B. vulgatus has a significant effect on host and microbial

responses, especially in males, supporting a role for *B. vulgatus* in CD-pathophysiology.

### Transcriptional inhibitor of bacterial origin to moderate mucosal inflammation

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Mucosal pathogens, elicit inflammation and pathology, reflecting their virulence factor repertoire, while commensal bacteria create a state of friendly coexistence. Here, we identified mechanisms of bacterial adaptation to the host niche, through the secretion of inhibitors of gene expression. Asymptomatic carrier strains were shown to inhibit RNA Polymerase II (Pol II) in host cells by targeting Ser2 phosphorylation; a step required for productive mRNA elongation. Assisted by a rare, spontaneous loss-of-function mutant from a human carrier, the bacterial NlpD protein was identified as a Pol II inhibitor. After internalization by host cells, NlpD was shown to target constituents of the Pol II phosphorylation complex (RPB1 and PAF1C), attenuating host gene expression and suppressing pathogen-specific innate immune signaling. Therapeutic efficacy of treatment with recombinant NlpD protein was demonstrated in a urinary tract infection model, by reduced tissue pathology, accelerated bacterial clearance and attenuated Pol II-dependent gene expression. The findings suggest an intriguing, evolutionarily conserved mechanism for bacterial modulation of host gene expression, with a remarkable therapeutic potential. Ambite I et al. Active bacterial modification of the host environment through RNA Polymerase II inhibition. *Journal of Clinical Investigation* 131(4): e140333 (2021). doi: 10.1172/JCI140333.

### The tuberculosis resistance protein TOLLIP prevents immune pathology by resolving the integrated stress response in alveolar macrophages.

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A functional polymorphism responsible for Toll-Interacting Protein (TOLLIP) deficiency is associated with increased tuberculosis (TB) risk. However, the fundamental mechanisms underlying TOLLIP's role in TB pathogenesis and lung-resident macrophage function are unknown. To understand how TOLLIP influences Mtb-macrophage interactions, we infected mice lacking the Tollip gene (Tollip<sup>-/-</sup>) with *Mycobacterium tuberculosis* (Mtb) and evaluated lung-resident macrophage function. Tollip<sup>-/-</sup> mice developed worsened Mtb disease characterized by lipid-laden foamy macrophages in their lungs. This phenotype was intrinsic to alveolar macrophages (AM), as Tollip<sup>-/-</sup> AM from mixed bone marrow chimeras selectively harbored excessive Mtb bacilli and intracellular lipid. Global gene expression analysis of Mtb-infected, Tollip<sup>-/-</sup> AM, followed by immunohistochemistry, demonstrated alteration in dozens of genes consistent with increased EIF2 signaling, a master activation signal for the integrated stress response that adapts cells to variable environmental conditions. Administration of mycolic acid (MA), an Mtb cell wall lipid, to Tollip<sup>-/-</sup> macrophages induced increased lipid accumulation, EIF2 signaling, and intracellular Mtb burden, defining a role for TOLLIP in preventing MA-induced macrophage dysfunction. Pharmacologic EIF2 inhibition restored host defense and reduced bacillary burden in both Tollip<sup>-/-</sup> and WT mice. In humans, TOLLIP and EIF2 kinase mRNA expression was selectively increased in caseous TB granulomas, providing translational relevance for our observations. TOLLIP deficiency in Mtb-infected AMs induces EIF2 signaling, increased bacterial replication, and immunopathology. These findings illustrate a major mechanism by which TOLLIP-deficient individuals are susceptible to TB disease and suggest EIF2 inhibition as TB host-directed therapy.

### Tuft cell-derived acetylcholine regulates epithelial fluid secretion during homeostasis and Type 2 immunity

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The intestinal epithelium maintains a barrier against microbiota, pathogens, and environmental insults in part through the secretion of fluid and mucus. During the Type 2 immune response to parasitic helminths, IL-13 signaling drives dramatic epithelial remodeling, resulting in goblet cell hyperplasia and increased mucus production that contribute to the "weep and sweep" required for helminth clearance. Tuft cells, rare chemosensory epithelial cells that initiate this Type 2



response upon sensing of helminths and certain microbial metabolites, also express the enzyme ChAT required for synthesis of acetylcholine (ACh). ACh is a potent inducer of epithelial fluid and mucus secretion but is typically thought to be produced by enteric neurons. We find that at homeostasis the microbial metabolite succinate induces rapid fluid secretion in the distal small intestine dependent on tuft cell-derived ACh. Secretion also requires tuft cell chemosensing and is independent of neuronal involvement. Fluid secretion is enhanced during Type 2 inflammation, consistent with the observed increase in ChAT<sup>+</sup> tuft cells. Upon infection with the hookworm *Nippostrongylus brasiliensis*, tuft-specific ChAT-deficient mice suffer delayed worm clearance despite robust epithelial remodeling. Our findings suggest that upon sensing of luminal signals produced by helminths and microbes, tuft cells stimulate an epithelium-intrinsic effector unit to rapidly respond with fluid secretion. This response is amplified by epithelial remodeling that occurs during the Type 2 response, contributing to anti-helminth immunity.

### Type 3 innate lymphoid cells instructs the differentiation of gut microbiota-specific regulatory T cells

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The mutualistic relationship of gut-resident microbiota and cells of the host immune system promotes homeostasis that ensures maintenance of the microbial community and of a poised, but largely non-aggressive, immune cell compartment. Consequences of disturbing this balance, by environmental or genetic factors, include proximal inflammatory conditions, like Crohn's disease, and systemic illnesses, both metabolic and autoimmune. One of the means by which this equilibrium is achieved is through induction of both effector and suppressor or regulatory arms of the adaptive immune system. In mice, *Helicobacter* species induce regulatory (iTreg) and follicular helper (T<sub>fh</sub>) T cells in the colon-draining mesenteric lymph nodes under homeostatic conditions, but can instead induce inflammatory Th17 cells when iTreg cells are compromised. How *Helicobacter hepaticus* and other gut bacteria direct T cells to adopt distinct functions remains poorly understood.

Here, we investigated which cells and molecular components are required to convey the microbial instruction for the iTreg differentiation program. We found that antigen presentation by cells expressing ROR $\gamma$ t, rather than by classical dendritic cells, was both required and sufficient for iTreg induction. These ROR $\gamma$ t<sup>+</sup> cells, likely to be type 3 innate lymphoid cells (ILC3), require the MHC class II antigen presentation machinery, the chemokine receptor CCR7, and a  $\alpha$ 4 $\beta$ 1 integrin, which activates TGF- $\beta$ , for iTreg cell differentiation. In the absence of any of these, instead of iTreg cells there was expansion of microbiota-specific pathogenic Th17 cells, which were induced by other antigen presenting cells (APCs) that did not require CCR7. Thus, intestinal commensal microbes and their products target multiple APCs with pre-determined features suited to directing appropriate T cell differentiation programs, rather than a common APC that they endow with appropriate functions. Our results illustrate the ability of microbiota to exploit specialized functions of distinct innate immune system cells, targeting them to achieve the desired composition of equiposed T cells, thus maintaining tolerance.

### Type I and III IFN Drive Non-Redundant and Airway Site-Specific Antiviral Immune Responses in Human Metapneumovirus Infection

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Human metapneumovirus (HMPV) is a leading cause of acute respiratory infection. Although nearly every person is infected during childhood, re-infections occur often throughout life, highlighting difficulty in building long-term immunity. Early host responses to HMPV are poorly characterized, and further understanding could identify important therapeutic targets. Type I (IFN- $\alpha/\beta$ ) and III interferons (IFN- $\lambda$ ) display potent antiviral activity against many respiratory viruses. However, their functions in HMPV infection remain largely unknown. Here, we identify distinct roles for type I and III IFN in HMPV. Mice lacking IFN- $\alpha/\beta$  receptor (IFNAR<sup>-/-</sup>) exhibit less disease and reduced lung inflammatory cytokine levels, but no difference in lung HMPV titer. In contrast, mice lacking IFN- $\lambda$  receptor (IFNLR<sup>-/-</sup>) show moderate clinical disease, high lung cytokine levels, and increased lung HMPV titer. IFN-driven control of virus replication is airway site-dependent, as both IFNAR<sup>-/-</sup> and IFNLR<sup>-/-</sup> show increased HMPV titer in the nose. Site-specific IFN-driven innate immune responses were

also observed. Loss of lung M2 macrophages in HMPV infection was abrogated in IFNAR  $-/-$  mice, while both IFNAR  $-/-$  and IFNLR  $-/-$  abrogated nose M2 macrophage loss. IFN-specific differences, including IFN- $\lambda$ -dependent epithelial cell and monocyte recruitment and type I IFN-dependent plasmacytoid dendritic cell recruitment, were present in the lung but not nose. These data suggest type I IFN is necessary for HMPV pathogenesis, while IFN- $\lambda$  is required to limit HMPV lung replication. Differences in virus control and immune responses in upper vs. lower airways of IFNAR  $-/-$  and IFNLR  $-/-$  mice suggest type I and III IFN exert non-redundant antiviral activity through site-specific signaling.

### Understanding how maternal IgA affects bacterial composition and immune activation in the small intestine

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The postnatal intestinal immune system undergoes significant development during initial bacterial colonization following loss of sterility postpartum. Due to their immature immune system, neonates are at significant risk for enteric infection and diseases associated with invasion by the microbiome, such as necrotizing enterocolitis (NEC). The developing immune system, particularly the T cell population, is tightly regulated as aberrant T cell responses early during development can predispose individuals to allergy and autoimmune disorders. A key predictor of early life intestinal health is breastfeeding which supplies components of the maternal immune system in the form of antibodies and antimicrobial peptides. The most abundant maternal antibody in breast milk is Immunoglobulin A (IgA), generated by plasma cells trafficked from the maternal intestine during gestation. We hypothesize that maternal IgA in breastmilk shapes intestinal colonization by limiting the ability of adherent bacteria to colonize the mucus layer and epithelium, limiting infant inflammatory immune responses. Adherent bacterial species would be highly susceptible to colonization interference from maternal antibodies and many are proven inducers of IgA. Using tetramers and transgenic mouse models, we will examine the phenotype, function and longevity of adherent microbiota specific T cells generated during the preweaning period. These experiments will characterize the function of maternal IgA in controlling early colonization events and subsequent immune activation within the fetal intestine. Understanding the interaction between

maternal antibodies and the developing neonatal T cell compartment will be crucial in addressing the pathologies associated formula feeding and early cessation of breastfeeding, such as NEC.

### Understanding the immunogenicity of different components of the mouse intestinal microbiota

Swapan Preet - University of ETH Zurich, Prof. Emma Slack, Yagmur Turgay, Prof. Klaus Eyer, Olivia Bucheli

Understanding the immunogenicity of different components of the mouse intestinal microbiota . Swapan Preet , Yagmur Turgay, Olivia Bucheli, Klaus Eyer & Emma Slack Institute of Microbiology and Immunology Vladimir-Prelog-Weg, 1-5/10, 8093 Zurich While some microbiota components, such as *E. coli*, are highly immunogenic in the intestine, others, such as *Eubacterium rectale*, seem to be not at all recognised by intestinal antibodies (IgA). My project aims to understand why some microbiota species are weakly targeted by IgA, despite being highly abundant . In particular, we will ask whether these species evade immune presentation per se, whether IgA is induced, but against irrelevant epitopes not accessible on the bacterial surface, or whether active mechanisms present in group XIVa Clostridia prevent immune system activation. This information will then be used to design vaccines that artificially overcome these immunological barriers, allowing us to test the consequences of high-affinity IgA for colonization of these functionally important microbiota members. Approaches include biophysical/biochemical characterisation of surface antigen structures reactive to IgA, picodroplet-based; approaches to test the affinities of antibody responses and within-host population dynamics to understand the influence of high-affinity IgA on intestinal colonization by targeted strains.

### Understanding the influence of CD4 T cell intrinsic hypoxia-response pathways on gut homeostasis

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The gastrointestinal (GI) tract is home to a rich flora of microbes as well as a highly diverse and specialized immune system. The lymphocytes that reside in the gastrointestinal tract must balance tolerance to the microbiota with the ability to

mount inflammatory responses against pathogens in order maintain healthy host tissue. The GI tract is hypoxic at steady-state, promoting the maintenance of a healthy and diverse microbiota. However, inflammation and crypt hyperplasia can disrupt homeostatic hypoxia within the GI tract, creating an opportunity for select pathogens to colonize and translocate across the epithelium. Despite the importance of maintaining hypoxia in the GI tract, little is known about role of hypoxia genes within intestinal lymphocytes and their contribution to maintaining healthy gut homeostasis. Using cell-specific inhibition and activation of hypoxia-response pathways in CD4 T cells, we aim to understand how intracellular regulators of hypoxia may promote or dampen homeostatic immune responses to the microbiota and pathogens in a T cell-intrinsic manner.

### Unraveling the molecular basis of *B. thetaiotaomicron* induced anti-inflammatory immune responses

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Intestinal homeostasis is achieved through a dynamic interplay between the microbiome and the intestinal immune system. Specific microbiome members, such as the prominent gut symbiont *Bacteroides thetaiotaomicron*, contribute to the development of intestinal homeostasis through the production of specific anti-inflammatory molecules that induce immune tolerance. However, the molecular mechanisms that underlie these beneficial interactions are poorly defined. Using an in vitro model system we found that outer membrane vesicles (OMVs) produced by *B. thetaiotaomicron* can elicit the production of the anti-inflammatory cytokine IL-10 in a TLR2 dependent manner. Using a transposon mutagenesis library, we identified mutants of *B. thetaiotaomicron* that have hindered ability to induce IL-10. Mapping of the location of transposon insertion in one such mutant demonstrated that disruption of gene BT1160, which encodes for Na(+)-translocating NADH-quinone reductase subunit A (*nqrA*), reduced the capacity of *B. thetaiotaomicron* to induce IL-10. Construction of a deletion mutant, where the entire gene is removed (*B. thetaiotaomicron*  $\Delta$ BT1160), recapitulated the findings with the transposon mutant without major effects on expression of other genes in the same locus, confirming that gene BT1160 is essential for the ability of *B. thetaiotaomicron* to promote IL-10 expression. Mechanistically, the diminished

IL-10 induction capacity of *B. thetaiotaomicron*  $\Delta$ BT1160 was associated with impaired outer membrane vesicle (OMV) biogenesis and altered OMV composition. Collectively, our data show that gene BT1160 is a critical coordinator of the immune-stimulatory potential of *B. thetaiotaomicron*, and a key regulator of host-bacterial mutualism by favoring the development of an anti-inflammatory milieu.

### The Upper Airway and Brain are Protected by Mucosal Plasma Cells but not by Circulating Antibodies

Sebastian Wellford - Duke University, Ashley Moseman

While blood antibodies mediate protective immunity in most organs, their ability to protect nasal surfaces in the upper airway is currently unclear. This is important for protection against the spread of respiratory microbes, but also against neuroinvasive pathogens, as the olfactory system provides an opportunity for severe CNS infection. Our data from multiple viral infection models indicate that blood-borne antibodies sufficiently defend respiratory tissue but cannot prevent infection of the olfactory epithelium. Consequently, despite high levels of serum antibody, airway pathogens infect the upper nasal turbinates, with pronounced replication in the olfactory epithelium. Furthermore, this olfactory replication allows neurotropic microbes to disseminate into the brain via the olfactory nerves, even in the presence of circulating neutralizing antibody. We found that circulating antibodies are excluded from the olfactory mucosa by a restrictive blood endothelial barrier. To circumvent this endothelial barrier and achieve sterilizing immunity in the nasal airway, we demonstrate that extravascular plasma cells must reside within olfactory tissue to directly secrete antibodies to the mucosal surface. Following infection, these plasma cells are recruited to the olfactory mucosa in a manner dependent on the chemokine receptor CXCR3. We also determined that many vaccine adjuvants were incapable of targeting plasma cells to the olfactory mucosa, but immunizations supplemented by a mucosal adjuvant were able to establish humoral protection of the olfactory surface. Our identification of a size exclusionary blood-olfactory barrier and subsequent requirement for tissue-derived antibody production has implications for mucosal vaccinology, respiratory and CNS pathogen transmission, and B cell fate decisions.

### Vaccination and Niche Competition for Targeted Exclusion of Unwanted Bacterial Species from the Gut Microbiota

Verena Lentsch - ETH Zurich, Claudia Moresi, Stefan Fattinger, Wolf-Dietrich Hardt, M  d  ric Diard, Emma Wetter Slack

Diarrheal disease is still among the leading causes of morbidity and death worldwide. Moreover, gut pathogens associated with these diseases - such as *E. coli* and non-Typhoidal *Salmonella* - can cause invasive infections and are increasingly resistant to antibiotics. Therefore, an alternative strategy for treatment independent of antibiotics is urgently needed. Live-attenuated vaccines against non-Typhoidal *Salmonellosis* (NTS) exist for the use in livestock but translation to humans is unlikely as these vaccines pose a major safety concern in exactly those groups that are most susceptible to severe disease caused by NTS. To tackle this problem, we follow a two-pronged strategy that combines inactivated oral vaccines with bacterial competitors. We have validated this approach in a murine NTS model and compared it to the classical live-attenuated vaccines. Oral vaccination induces a strong specific IgA response and leads alone to a significant decrease in *Salmonella*-driven intestinal inflammation. In combination with a niche competitor, we could reach complete elimination of pathogenic *Salmonella* from faecal samples and clearly provided better protection from intestinal disease than with the live-attenuated vaccination alone. We are currently working on the characterization of the intestinal immune response that is necessary to prevent invasive disease and an extension of this technique to other Enterobacteriaceae. Taken together, oral vaccination with niche competition holds great promise in the prevention, elimination and/or treatment of antimicrobial resistant infections. An extension of this technique may soon also allow specific strain-replacements to be carried out within the intestinal microbiota, i.e. could deliver targeted microbiota engineering.

**VEGFR3-driven, pulmonary lymphangiogenesis exacerbates induction of bronchus associated lymphoid tissue in allergic airway disease.**

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Asthmatic lung samples present with a higher density of pulmonary lymphatic vessels, and a higher incidence of bronchus associated lymphoid tissue (BALT). Here, we asked whether lymphangiogenesis, stimulated by the VEGF-C/VEGFR-3 signaling axis in lymphatic endothelial cells (LECs), plays a role in promoting BALT in mouse models of asthma. We first determined that chronic intratracheal instillation of house dust mite (HDM), a clinically relevant allergen, recapitulates both lymphangiogenesis and BALT induction. Interestingly, we found that stimulation of VEGFR-3 in LECs exacerbated BALT, while pharmacological inhibition of VEGFR-3 ameliorated BALT. Furthermore, in transgenic mice with an expanded pulmonary lymphatic network (induced prior to allergen challenge), we found an exacerbated BALT response upon chronic HDM inhalation. Recent studies have determined that LEC-derived CXCL13 plays an important role in secondary lymphoid structure organogenesis and maintenance, and thus we pondered whether LEC-derived CXCL13 would play a role in the development of tertiary lymphoid structures. Indeed, we observed an increase in lung infiltration by CXCR5+ cells when we used VEGF-C to modulate the chronic allergic response. Finally, we found that the VEGF-C exacerbated BALT phenomenon was indeed CXCL13 dependent. Altogether, these results suggest a causative role for pulmonary lymphatics in mediating induction of BALT in chronic allergic airway inflammation.

### $\gamma\delta$ + T cell Responses During Gut-Originating Late-Onset Neonatal Sepsis

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Late-onset neonatal sepsis (LOS), often the result of a bloodstream bacterial infection occurring during the first two months of life, remains a significant contributor to neonatal mortality. Clinical observations suggest neonates exhibit excessive inflammation during sepsis, however, major mechanisms of disease during LOS remain poorly understood. A common route of pathogen entry for neonates is the translocation of a gut-resident pathobiont. We have developed a mouse model of LOS that disrupts goblet cell homeostasis during early life to increase gut permeability and facilitate the translocation of bacterial species such as *E. coli*. Oral



infection recapitulates key features of clinical LOS including systemic translocation of bacteria to the spleen and liver, increased pro-inflammatory serum cytokines, organ failure, and death in 5 day-old pups. In 15-day-old pups, *E.coli* did not disseminate past the mesenteric lymph node. Following oral infection, we observed robust activation of  $\gamma\delta^+$  T cells in the spleen and liver of 5 day-old pups, accompanied by increased IL-17, IFN- $\gamma$ , and TNF- $\alpha$  production from  $\gamma\delta^+$  T cells. 5 day-old TCR $\delta^-$  pups were protected from mortality during LOS, suggesting that  $\gamma\delta^+$  T cells may also contribute to mortality in this model. Strikingly, we observed reduced activation of  $\gamma\delta^+$  T cells from 15-day-old pups and limited cytokine production, even when pathogens were administered systemically. Thus, neonatal mice are at risk for age-dependent systemic bacterial translocation and increased  $\gamma\delta^+$  T cell-mediated inflammation contributing to LOS. This study sheds light on the unique responses of neonatal  $\gamma\delta^+$  T cells to bloodstream infections resulting from gut-resident pathogens.