SOCIETY OF MUCOSAL IMMUNOLOGY LOCAL CHAPTER SYMPOSIUM - MICHIGAN

HOSTED BY:

THE UNIVERSITY OF MICHIGAN MEDICAL SCHOOL
ANN ARBOR, MI

MARCH 6, 2020

MEDICAL SCHOOL
UNIVERSITY OF MICHIGAN

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"A Career in Science: How to Do What You Love"

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"Neutrophil Extracellular Traps (NETs) in IBD: Friend or Foe?"

10:40 am - **Ann Decker, DMD, PhD,** *University of Michigan Medical School* 5
"Innate Immunity and Wound Healing in the Periodontium"

11:00 am - **Miguel Quiros, PhD,** *University of Michigan Medical School* 5
"The Molecular Balance of Intestinal Homeostasis: Role of Soluble Mediators During Inflammation and Repair"

11:20 am - **Yuan He, PhD,** *Wayne State University* 5
"Mechanism of NLRP3 inflammasome activation"

11:40 am - **Andrew Olive, PhD,** *Michigan State University.* 5
"Dissecting IFN-gamma-dependent Regulatory Networks Using Functional Genetic Approaches"

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"Maternal gut microbiome alteration regulates offspring immunity to RSV infection through gut microbial modification and metabolic reprogramming"

1:20 pm - **Karen Racicot, PhD,** *Michigan State University* 6
"Chronic heightened maternal corticosterone during pregnancy has sexually dimorphic programming effects on allergic inflammation in offspring"

1:40 pm - **Sho Kitamoto, PhD,** *University of Michigan Medical School* 6
"The intermucosal connection between the mouth and gut in commensal pathobiont-driven colitis"

2:00 pm - **Anny-Claude Luissint, PhD,** *University of Michigan Medical School* 6
"CAR-Like Membrane Protein (CLMP): A New Regulator of Intestinal Mucosal Homeostasis and Repair After Injury"

2:20 pm - **Sunil Tomar, PhD**, *University of Michigan Medical School* 6
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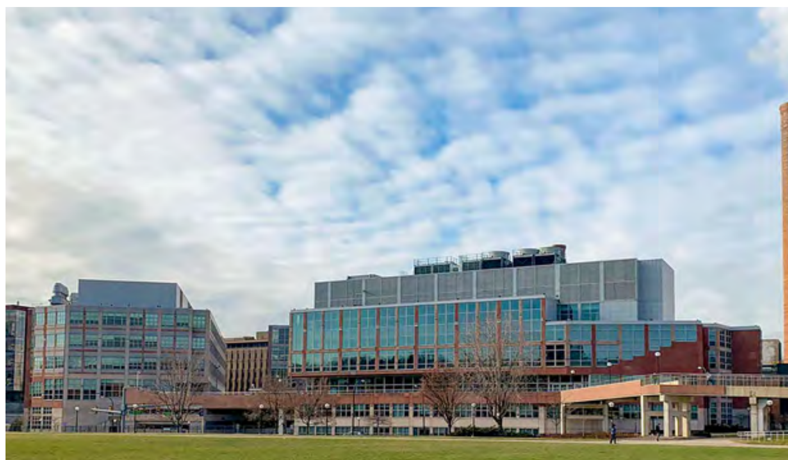

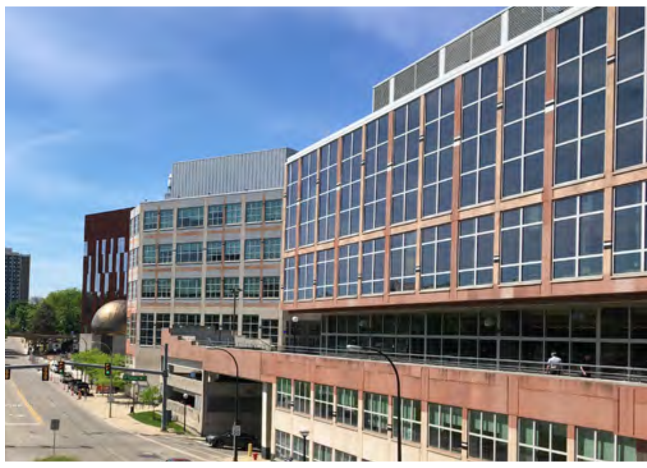

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Welcome

We are happy to welcome you to the Mucosal Immunology Local Chapter 2020 - Michigan, organized and hosted by the Pathology Department of the University of Michigan, and sponsored by the Society for Mucosal Immunology. Michigan harbors excellent institutions with a rich mucosal immunology community that includes many junior investigators, post-doctoral fellows and graduate students. Our goal today is to bring together the many outstanding groups in the area and provide the opportunity for our researchers to share their work and ideas, to connect with each other, and to promote the benefits of the Society for Mucosal Immunology for career advancement.

Today's program includes two oral presentation sessions featuring invited junior faculty members from the University of Michigan, Michigan State University, Wayne State University and the University of Toledo (OH). We also encourage you to attend the exciting sessions of oral and poster presentations from Ph.D. students and postdoctoral fellows highlighting the cutting-edge research in the field of mucosal immunology. We hope to have significant interactions between our students and faculty during the presentations, poster viewing and networking reception.

Our keynote speaker is Nicholas W. Lukacs, Ph.D., the Godfrey D. Stobbe Professor of Pathology and Scientific Directory of the Mary H. Weiser Food Allergy Center at the University of Michigan Medical School. Dr. Lukacs has also served as an Assistant Dean for Faculty Affairs at the University of Michigan, including actively advising faculty in achieving promotion and tenure. The Lukacs Lab research is presently focused on the innate and acquired immune responses in allergen- and respiratory virus-induced acute and chronic diseases, as well as the role that the gut microbiome has on development of allergic diseases. Dr. Lukacs will provide insights into career development for the new generations of mucosal immunologists.

We want to thank Dr. Charles Parkos, Chair of the Pathology Department, and Dr. Asma Nusrat, Director of Experimental Pathology, for providing departmental support. We also would like to thank Angela Suliman, Robin Kunkel, Brent Temple, and Elizabeth Walker. Without all their hard work, this symposium would not be possible. Lastly, we would like to acknowledge Dr. Simon Hogan as a member of the Education and Career Development Committee within the Society for Mucosal Immunology for bringing this opportunity to Michigan and the SMI for its sponsorship.

Enjoy the symposium!

The organizing committee,

Veronica Azcutia, PhD

Jennifer Brazil, PhD

Roberta Caruso, MD, PhD

Taeko Noah, PhD

Catherine Ptaschinski, PhD

SOCIETY OF MUCOSAL IMMUNOLOGY LOCAL CHAPTER SYMPOSIUM - MICHIGAN

March 6, 2020

Palmer Commons - Forum and Great Lakes Room

Ann Arbor, Michigan

Program Schedule

9:00 am Coffee and Registration (Atrium)

9:25 am Welcome: **Jennifer Brazil, PhD,** *Assistant Professor of Pathology, University of Michigan Medical School*

9:30 am **Career Development Keynote Presentation:**
Nicholas W. Lukacs, PhD, *Godfrey Dorr Stobbe Professor of Pathology, Scientific Director, Mary H. Weiser Food Allergy Center, Dept. of Pathology, University of Michigan Medical School*

"A Career in Science: How to Do What You Love"

Session I: **Invited Presentations**
Chairs: **Veronica Azcutaia, PhD,** *Research Assistant Professor of Pathology, University of Michigan Medical School*
Kathryn Michels, PhD, *Postdoctoral Fellow, Department, of Pathology, University of Michigan Medical School*

10:20 am **Matam Vijay-Kumar, PhD,** *Associate Professor, Department of Physiology & Pharmacology, University of Toledo College of Medicine*
"Neutrophil Extracellular Traps (NETs) in IBD: Friend or Foe?"

10:40 am **Ann Decker, DMD, PhD,** *Assistant Professor, Dept. of Periodontics & Oral Medicine, University of Michigan Medical School*
"Innate Immunity and Wound Healing in the Periodontium"

11:00 am **Miguel Quiros, PhD,** *Research Investigator, Department of Pathology, University of Michigan Medical School*
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11:40 am **Andrew Olive, PhD,** *Assistant Professor, Department of Microbiology & Molecular Genetics, Michigan State University.*
"Dissecting IFN-gamma-dependent Regulatory Networks Using Functional Genetic Approaches"

12:00 pm **Lunch Break (**Atrium and Great Lakes Room**)**

Session II: **Invited Presentations**
Chairs: Catherine Ptaschinski, PhD, *Research Assistant Professor of Pathology, University of Michigan Medical School*
Roberta Caruso, MD, PhD, *Research Investigator of Pathology, University of Michigan Medical School*

1:00 pm **Wendy Fonseca, DVM, PhD,** *Research Investigator, Department of Pathology, University of Michigan Medical School*
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"CAR-Like Membrane Protein (CLMP): A New Regulator of Intestinal Mucosal Homeostasis and Repair After Injury"

2:20 pm **Sunil Tomar, PhD**, *Postdoctoral Fellow, Department of Pathology, University of Michigan Medical School*
"IL-4 signaling directly regulates IL-9 producing intestinal mast cell precursor (iMCP9) in experimental food allergy"

2:40 pm **Coffee break** (Atrium)

Session III: **PhD Student & Postdoctoral Fellow Presentations**
Chairs: Taeko Noah, PhD, *Research Investigator of Pathology, University of Michigan Medical School*
Miguel Quirós, PhD, *Research Investigator of Pathology, University of Michigan Medical School*

3:00 pm **Jazib Uddin, BS,** *PhD student, Department of Pathology, University of Michigan Medical School*
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"Phenotypes and functional differences: how profibrotic effects of M2 macrophages "Change the game" in lung fibrosis"

4:30 pm: **Poster session** (Great Lakes Room)

5:00 pm: **Reception and Networking session** (Great Lakes Room)

Keynote Speaker:

Dr. Nicholas W. Lukacs

Godfrey Dorr Stobbe Professor of Pathology,
Scientific Director, Mary H. Weiser Food Allergy Center,
Department of Pathology,
University of Michigan Medical School

"A Career in Science: How to Do What You Love"

Nicholas W. Lukacs, Ph.D. is the Godfrey D. Stobbe Professor of Pathology at the University of Michigan Medical School in Ann Arbor, Michigan. He received his Ph.D. from Wayne State University in 1992 and was first appointed as a faculty member at the University of Michigan in 1994. He is presently the Scientific Director of the Mary H Weiser Food Allergy Center at the Medical School. His research funding includes numerous National Institute of Health grants as PI and Co-I. The Lukacs Lab research has previously examined leukocyte migration and chemokine biology during acute and chronic inflammatory diseases. The lab research is presently focused on the innate and acquired immune responses in allergen- and respiratory virus-induced diseases, as well as the role that the gut microbiome has on development of the early life immune system. Studies in the laboratory have been focused on the function and activation of DC and T cells during infections and the differential modulation of the immune and pathologic responses that lead to exacerbated disease progression. Translational collaborations include studies examining severe respiratory syncytial virus (RSV)-induced disease with infants in the Pediatric ICU as well as the development of food and airborne allergen responses in inner city birth cohorts linked to alterations in microbiome and metabolic profiles. In addition to research, he spends a significant amount of time mentoring Faculty and Trainees as a PI of a NHLBI post-doctoral T32 training grant, with his work with the University of Michigan CTSI grant, and as a member of numerous thesis and faculty mentoring committees.

INVITED SPEAKERS

Dr. Matam Vijay-Kumar is an associate professor and director of the University of Toledo-Microbiome Consortium (UT-MiCo). His research interests include host metabolic adaptions to inflammation especially as it pertains to the interplay between intestinal inflammation and metabolic disorders. His research has shown that Toll-like receptor 5 (TLR5)-deficient mice are prone to develop spontaneous gut inflammation. To cope up with gut inflammation, these mice adapt alternate metabolic pathways which protect against inflammation but culminate in metabolic diseases. In addition, Dr. Kumar is interested in studying the dynamics between innate immunity, gut microbiota and gastrointestinal pathogens. His ongoing studies focus on the role of neutrophil extracellular traps (NETs) in gastrointestinal physiology, gut microbiota homeostasis and gastrointestinal infections by employing PAD4/NET deficient mice models.

Dr. Ann Decker joined the Department of Periodontics and Oral Medicine at the University of Michigan School of Dentistry in July 2019 as an assistant professor. Her current research projects examine the effects of osteoimmunology on bone regenerative capacity and ways to develop successful therapeutic bone regenerative strategies for patients with poor oral wound healing. Dr. Decker received her BS in biomedical engineering from the University of Wisconsin-Madison, DMD from the University of Florida, and most recently her PhD in Oral Health Sciences/certificate in periodontics from the University of Michigan School of Dentistry. Ann pursued her PhD research on the interactions of skeletal metastases within the bone marrow in the laboratory of Dr. Russell Taichman. Dr. Decker has shown an outstanding track record of accomplishment and national distinction for her studies through receipt of awards such as the Henry M. Thornton/SCADA Fellowship Award, the American Academy of Periodontology Foundation Educator Award, and the Donald Kerr Distinguished Periodontics Award.

Dr. Miguel Quiros did his undergrad studies in Microbiology at the University of Costa Rica. He obtained his PhD in Molecular and Cellular Physiology at the Center for Research and Advanced Studies in Mexico City, Mexico. During his PhD he specialized in epithelial cell junctions and their physiological regulation. His research focuses on the resolution phase of intestinal inflammation. His current projects include: (a) establishing a defined matrix to grow mini-guts with the potential to be engrafted into ulcerated tissue from IBD patients and promote epithelial recovery; (b) characterizing the kinetics of cytokine release during the resolution phase of inflammation and describe the cascade of events that lead to recovery of homeostasis; (c) studying the effect of specialized pro-resolution mediators on intestinal epithelial wound healing. He is a member of the American Society of Investigative Pathology where he has been recognized with multiple travel awards to attend scientific meetings to present his work. Dr. Quiros also has a Crohn's and Colitis Foundation (CCF) career development award and was the 2016 recipient of the CCF Shanti Sitaraman Young IBD Investigator Award for his research on the role of Resolvin E1 on intestinal epithelial wound healing.

Dr. Yuan He is an Assistant Professor in the Department of Microbiology and Immunology for the Wayne State University School of Medicine. He earned his Ph.D. in Cell and Developmental Biology at the University of Illinois at Urbana-Champaign in 2011. His postdoctoral training was in the laboratory of Dr. Gabriel Núñez in the Department of Pathology and Immunology Program at the University of Michigan. He joined the faculty of Wayne State University School of Medicine in 2017. His research focus is on innate immunity. Dr. He is a recipient of the two-year NIH Research Career Development Award K22 (July 2017-July 2019).

Dr. Andrew Olive is an Assistant Professor in the Department of Microbiology and Molecular Genetics and Michigan State University. He received his Ph.D. in 2014 from Harvard University, and completed his postdoctoral studies at the University of Massachusetts Medical School. Since starting his own lab at Michigan State, Dr Olive's research has combined genetic approaches with *in vivo* disease models to study chronic infections from both the host and bacterial perspective, focusing on *Mycobacterium tuberculosis* and *Chlamydia trachomatis*, the leading causes of infection related death and preventable blindness worldwide, respectively. By defining effective resistance and tolerance mechanisms, he aims to identify targetable aspects of protective immunity that can ultimately be used to develop effective vaccines or host directed therapies.

Dr. Wendy Fonseca As a student, Wendy worked on the generation of recombinant Influenza virus that carried the F protein of Respiratory Syncytial Virus (RSV) and used it as a live attenuated vaccine. During postdoctoral training, she has studied the lung immune response to RSV, allergic asthma, and the role of the gut microbiome during viral lung infection. Currently she is examining the role of metabolites, like uric acid, during Respiratory Syncytial Virus infection and its impact on pulmonary immune responses. Future plans involve using neonatal models with RSV research in neonatal lung and immune systems.

Dr. Karen Racicot received a BS from the University of Florida in 2002 and graduated with a PhD from The Pennsylvania State University in 2009, majoring in Animal Science with a focus on Reproductive Biology and Immunology. Afterwards, she completed post-doctoral training in the Department of Obstetrics, Gynecology and Reproductive Sciences at the Yale School of Medicine, primarily working with murine models of polymicrobial infections during pregnancy. She is currently an Assistant Professor in the Department of Obstetrics, Gynecology and Reproductive Biology within the College of Human Medicine at Michigan State University. The main objective of her research program is to understand the regulation of inflammation and innate immune cells at the maternal-fetal interface and determine how they affect fetal development and maternal health. The lab uses human clinical samples, primary cells, immortalized cell lines, and has developed multiple animal models of pregnancy and infection(s) that are used to dissect the mechanisms of placental and maternal responses to pathogens and other environmental challenges.

Dr. Sho Kitamoto is a Research Investigator in the Department of Internal Medicine at the University of Michigan. He earned his Ph.D. in the field of cancer biology at Kagoshima University, Japan. Since he joined Dr. Kamada's laboratory as a Research Fellow in 2014, he has been studying the complex interplay between host immunity and microbes during the development of intestinal inflammation and tumorigenesis. During that time, he has been continuously funded by competitive fellowship awards including the Research Scholar Award from the Prevent Cancer Foundation (2018-2020) and the Postdoctoral Translational Scholar Program of the Michigan Institute for Clinical & Health Research (2018-2020).

Dr. Anny-Claude Luissint is a junior faculty (Research Investigator) in the Department of Pathology at the University of Michigan. Her research interest focuses on understanding the mechanism of regulation of intestinal barrier function and pathogenesis of chronic inflammation in the context of Inflammatory Bowel Disease. More precisely, she studies the contribution(s) of epithelial transmembrane cell adhesion molecules of the immunoglobulin superfamily in the regulation of intestinal mucosal barrier function, leukocyte transepithelial migration and the intestinal mucosal wound healing process.

Dr. Sunil Tomar completed his doctoral studies in the Department of Pathology, Microbiology and Immunology at the University of South Carolina. With the laboratory focus on inflammatory diseases, Sunil's PhD work identified protective effects of plant derived indole compounds and cannabinoid receptor 2 agonists in ameliorating acute liver failure, in particular showing a role for miRNAs regulating TLR4 signaling.

Currently with a focus on type 2 inflammation, Sunil is leading projects to identify the mechanistic role of IL-9 secreting mast cells in driving effector food allergic responses. In addition, Sunil is adopting a combination of murine *in vivo, in vitro* human iPSC derived models, and multi-omics tool to gain novel insights about development and function of these cells.

Paired Immunoglobulin-like Receptor B Regulates Inflammation and Histopathology in T-Cell Mediated Colitis

Jazib Uddin[1,2], Lisa Waggoner[3], Simone Vanoni[3], Kasper Hoebe[4], Senad Divanovic[4], Ariel Munitz[5], and Simon P. Hogan[1,6]

[1]Department of Pathology, University of Michigan, Ann Arbor, MI.
[2]Graduate Program in Immunology, University of Michigan, Ann Arbor, MI.
Divisions of [3]Allergy and Immunology and [4]Immunobiology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH.
[5]Department of Human Microbiology, Faculty of Medicine, Sackler School of Medicine, Tel Aviv University, Ramat Aviv, Israel.
[6]Mary H. Weiser Food Allergy Center, University of Michigan, Ann Arbor, MI

Rationale: The inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis are chronic relapsing gastrointestinal (GI) inflammatory diseases that are driven by an aberrant intestinal inflammatory response. We have previously identified a role of the inhibitory receptor, Paired Immunoglobulin-like Receptor (PIR) B in the negative regulation of the innate activating signal associated with inflammatory responses in IBD1. The aim of this study is to define the involvement of PIRB in T-cell mediated colitic phenotype.

Results: We demonstrate that $PirB^{-/-}$ $Il10^{-/-}$ mice were protected from development of $Il10^{-/-}$ spontaneous colitis phenotype (Clinical Score, $Il10^{-/-}$ vs $PirB^{-/-}$ $Il10^{-/-}$: 3.3 ± 0.6 vs 0.14 ± 0.08, $p < 0.01$; Histological Score: 2.3 ± 0.4 vs 1.2 ± 0.2; mean ± SEM, $p < 0.01$). The reduced colitic phenotype was associated with reduced $CD4^+$ Th17 cells in the mesenteric lymph nodes (% IL-17a$^+$ cells, $Il10^{-/-}$ vs $PirB^{-/-}$ $Il10^{-/-}$: 2.5 ± 0.3 vs 1.2 ± 0.1; mean ± SEM, $p < 0.001$). Employing a Th17-dependent model, we show that $PirB^{-/-}$ $Il10^{-/-}$ mice were also protected from αCD3-mediated colitis compared to $Il10^{-/-}$ mice (Clinical Score, $Il10^{-/-}$ vs $PirB^{-/-}$ $Il10^{-/-}$: 4.5 ± 0.2 vs 0.7 ± 0.4; mean ± SEM, $p < 0.001$) and this was associated with diminished systemic IL-17a levels (IL-17a, $Il10^{-/-}$ vs $PirB^{-/-}$ $Il10^{-/-}$: 27.9 ± 5.7 ng/ml vs 2.7 ± 1.1 ng/ml; mean ± SEM, $p < 0.05$). To test whether these effects were intrinsic to the $CD4^+$ T-cell compartment, we performed the $CD4^+$ CD45RBhi T-cell transfer model of colitis. Consistent with our previous observations, $Rag^{-/-}$ which received $PirB^{-/-}$ naïve $CD4^+$ T-cells were protected from T-cell mediated colitis (% Change in Body Weight: -3.5 ± 4.9 % WT; 20.7 ± 4.6 % $PirB^{-/-}$; $p < 0.001$; Histological Score: 3.8 ± 0.2 WT; 1.0 ± 0.4 $PirB^{-/-}$; mean ± SEM, $p < 0.001$). *In vitro* polarization of naïve $CD4^+$ T-cells revealed an intrinsic deficiency of $PirB^{-/-}$ Th17 cell proliferative response; specifically, polyclonal activation of $PirB^{-/-}$ Th17 cells lead to increased cell death (# of Dead Cells: 17.6 ± 2.3 x 10^3 WT; 38.5 ± 5.8 x 10^3 $PirB^{-/-}$; mean ± SEM, $p < 0.05$) as well as enhanced Caspase 3/7 activation (# of Caspase 3/7$^+$ cells: 9.8 ± 1.8 x 10^2 WT; 19.7 ± 1.2 x 10^2 $PirB^{-/-}$ mean ± SEM, $p < 0.05$).

Conclusions: These data support the concept that PIRB regulates $CD4^+$ Th17 differentiation and development of T-cell-dependent colitis phenotype and provides insight into divergent functions for inhibitory receptors in regulating innate and adaptive inflammatory responses.

References:
Munitz, A. et al. Paired immunoglobulin-like receptor B (PIR-B) negatively regulates macrophage activation in experimental colitis. Gastroenterology 139, 530-541, doi:10.1053/j.gastro.2010.04.006 (2010).

This work is supported by the NIAID Training Grant T32-AI007413.

Aggravated colitis in Farnesoid X Receptor deficient mice is associated with altered immunological responses and a reshaped gut microbiota

Rachel M. Golonka, Piu Saha, Ahmed A. Abokor, Beng San Yeoh, Neejad Chidiac, Matam Vijay-Kumar

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Inflammatory bowel disease (IBD) is associated with dysregulation of the gut microbiota and dysfunction in appropriate immunological responses. More recently, alterations in bile acid metabolism has been introduced as another factor that modulates both gut microbiota and inflammatory responses in IBD pathogenesis. The farnesoid X receptor (FXR) is the major bile acid sensor and regulator involved in tuning the bile acid pool, where deficiency of this nuclear receptor causes aggravated intestinal inflammation; yet, how FXR is beneficial in protecting against colitis is less clear. The objective of this study was to explore whether the reshaped gut microbiota and altered immunological responses due to FXR deficiency could be contributing to colitic pathogenesis. To pursue this endeavor, both wild-type (WT) and FXR deficient (FXRKO) mice were administered 1.8% dextran sulfate sodium (DSS) for 6 days, monitored for body weight daily, and feces collected every other day. We observed that FXRKO mice displayed aggravated DSS-induced colitis, as evident by splenomegaly, colon thickening, shortened colon length, and rapid body weight loss. Additionally, FXRKO mice had increased levels of inflammatory markers (i.e. serum amyloid A), along with elevated myeloperoxidase activity and neutrophil infiltration in the colon, compared to their WT counterparts. Intriguingly, immunosuppressive FoxP3$^+$RORγT$^+$ Tregs were significantly decreased in FXRKO mice treated with DSS. Furthermore, FXRKO mice had alterations in their gut microbiota and microbiome, where they had increased bacterial load, but less bile salt hydrolase (BSH) producing bacteria, diminished *bsh3c/e* gene expression, and absent fecal BSH activity. Overall, this study highlights that aggravated colitis in FXRKO mice is associated with a reshaped gut microbiota and lessened immunosuppressive cells.

Microbial metabolite signaling is required for systemic iron homeostasis

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Iron is a central micronutrient that is needed for all living organisms. We harbor a diverse group of microbial populations in our intestines that also rely on host diet for their iron. It is unclear if and how our gut commensal microbiota competes with the host intestinal iron absorption pathways. Germ free (GF) and control mice fed with iron-sufficient (350 ppm), moderately iron-containing (35 ppm) and iron-deficient (<5 ppm) diets for 2 weeks demonstrated a significant resistance to iron deficiency anemia in the GF compared to the controls. This provides the first evidence of a reciprocal competition between host and commensals for limiting dietary iron. The GF mice on all three iron diets (350, 35 and <5 ppm) exhibited significant induction of duodenal iron transporters, divalent metal transporter 1 (DMT1), duodenal cytochrome ferric reductase (Dcytb1), and ferroportin (Fpn1). The expressions of DMT1, Dcytb1 and Fpn1 are maximally induced during iron deficiency (<5 ppm diet), but GF mice on iron deficient diet showed even higher level of duodenal expression of these three transporters. Systemic iron homeostasis is tightly regulated via three distinct yet integrated systems: hepcidin, a liver-derived peptide hormone, controls iron mobilization through ferroportin (FPN), the only known mammalian iron exporter; hypoxia-inducible factor (HIF)-2a regulates the intestinal absorptive response and intracellular iron storage is mediated by ferritin (FTN). We demonstrate that gut microbiota possess an active iron-dependent mechanism that inhibits host iron transport and storage. Microbial community analysis by 16sRNA sequencing of fecal and duodenal isolates from wild-type mice revealed that iron deficient diet favored growth of *Lactobacillus* species, *L. johnsonii* and *L. reuteri* being the most abundant. Using a high-throughput microbial metabolite screen, we demonstrate that gut microbiota produce metabolites that suppresses HIF-2a and increase FTN to decrease intestinal iron absorption. Specifically, we identified 1,3-diaminopropane (DAP) and reuterin as inhibitors of HIF-2a. These bacterial metabolites interact with the pseudo-ligand binding pocket of HIF-2a to inhibit heterodimerization with aryl hydrocarbon receptor nuclear translocator (ARNT) and modulate the expression of host iron absorptive machinery. Furthermore, DAP and reuterin effectively ameliorated systemic iron overload, whereas the gut-specific antibiotic Rifaximin improved anemia in mouse models. This work provides evidence of intestine/microbiota metabolic crosstalk that is essential for host systemic iron homeostasis, and suggest the utility of a probiotic approach to treat iron-related disorders.

Uncovering novel regulators of MHCII using chemical and genetic approaches

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Intracellular pathogens have developed mechanisms to evade host immune strategies by targeting the class II major histocompatibility complex (MHCII). MHCII is crucial for the activation of CD4+ T cells that are needed to induce cytokine production, macrophage activation, B cell responses, and immune cell recruitment needed for successful pathogen clearance. Despite its central role in eliciting the adaptive immune response, the regulation of MHCII during infection is not fully understood. We hypothesize that the level of MHCII expression directly correlates with the level of protection provided by CD4+ T cells. By investigating how MHCII is regulated we can identify new regulatory pathways that can be targeted to improve antigen presentation and immune response activation during persistent infections. Dysfunctional MHCII expression increases susceptibility to lifelong chronic infections and autoimmune diseases; our findings can be directly applied to test new strategies to improve these conditions through MHCII modulation. Using a genome-wide CRISPR screen in immortalized macrophages we have identified regulators of IFNy-dependent MHCII expression, including Glycogen Synthase Kinase-3 (GSK3) and Mediator subunit 16 (Med16). GSK3 is a highly conserved protein kinase that is associated with numerous physiological processes such as development, intracellular signaling, and apoptosis. Med16 has been associated with hormone receptors and cyclin dependent kinase 8. By combining genetic and chemical approaches we have uncovered compelling evidence that both GSK3 and Med16 have a major impact on both MHCII antigen presentation and CD4+ T cell activation. These candidates are representative of multiple targets we are investigating to better understand MHCII regulation.

Interaction between *Staphylococcus aureus* Agr virulence and neutrophils regulates pathogen clearance and skin inflammation

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Summary

The epidermis located on the skin surface can serve as a physical and immunological barrier to protect the host from harmful invasive microbes. *Staphylococcus aureus*, a Gram-positive bacterium, is a leading cause of bacterial infections capable of invading most tissues of the human body via the production of virulence factors. One of the major *S. aureus* virulence programs is the accessory gene regulatory (Agr), which is a two-component quorum-sensing system regulated by bacterial population density. Upon activation, the *agr* locus controls the expression of multiple virulence factors including toxins and enzymes that are important for the *S. aureus* colonization. Using epicutaneous and intradermal *S. aureus* inoculation models, we found that Agr virulence was required for both epicutaneous and intradermal pathogen growth and induction of inflammation. In the epicutaneous model, phenol-soluble modulin (PSM)α, a group of secreted peptides regulated via Agr virulence induced the release of keratinocyte (KC) IL-1α and IL-36α leading to the induction of IL-17 which was required for the recruitment of neutrophils to the epidermis. In neutrophil-deficient mice, there was invasion of *S. aureus* into the dermis and subcutaneous tissue after epidermal colonization which required Agr virulence. In contrast, Agr virulence was required for pathogen growth in wild-type mice, but not in neutrophil-deficient mice in the intradermal model, suggesting that a main function of Agr virulence is to target neutrophils after invasion. Agr-dependent pathogen genes were induced inside neutrophils leading to the production of cytopathic PSMα and escape of the pathogen to the extracellular space. Thus, *S. aureus* Agr virulence is critical for keratinocyte damage and induction of neutrophil-mediated skin inflammation in the epidermis, while it acts to evade intracellular neutrophil killing and pathogen escape to the extracellular space in the dermis and subcutaneous tissues.

Phenotypes and functional differences: How profibrotic effects of M2 macrophages "Change the game" in lung fibrosis

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a poorly understood, progressively lethal lung disease with no known cure. IPF is characterized by alveolar epithelial cell (AEC) injury, excessive deposition of extracellular matrix proteins, and chronic inflammation. Human and animal studies point to important roles for myeloid cells in IPF disease pathogenesis. There is evidence in the literature to support that the persistent inflammation seen in IPF primarily consists of monocytes and macrophages and that these macrophages are of a more M2-like phenotype. Recent work by Misharin et al. demonstrate that monocyte-derived alveolar macrophages (MoAMs) drive lung fibrosis, but it has not yet been determined if these MoAMs are of a more M2-like phenotype or how secreted factors from M2-like macrophages may alter the fibrotic state of nearby cells in the lung. This study seeks to elucidate answers to these questions and further probe the mechanisms behind M2-driven profibrotic response in the injured lung.

Methods: We used a PrimeFlow RNA assay to characterize specific macrophage populations after 21 days of bleomycin-induced fibrosis in Lyz2Cre^{+} mice. To probe mechanisms of M2-like autocrine signaling, we polarized our bone marrow-derived macrophages (BMDMs) in both a standard procedure (M1: 100ng/ml LPS+50ng/ml IFNy; M2: 10ng/ml IL-4+10ng/ml IL-13) and in the presence of epidermal growth factor receptor (EGFR) inhibitor, Erlotinib (1uM) *in vitro*.

Results: M2-like macrophages are characterized by high expression of HB-EGF, Fizz1, Arginase1, and IL-10. Using a PrimeFlow RNA assay to label HB-EGF mRNA transcripts after 21-day bleomycin treatment and collagenase digest of the lung, we determined that mice receiving bleomycin had significantly more CD11b^{+}CD11c^{+}SiglecF^{int} cells (characterized as MoAMs) than saline-treated mice. These cells were positive for HB-EGF expression, implying that MoAMs are more M2-like in the lung. Given this phenotype, we next wanted to determine if supernatant from M2-like BMDMs caused profibrotic changes in fibroblasts and AECs *in vitro*. After polarizing BMDMs to an M1 or M2 phenotype, we performed western blots and scratch assays with primary lung fibroblasts to investigate fibroblast profibrotic protein expression and fibroblast migration, respectively. Fibroblasts migrate faster and have increased periostin expression when given M2-supernatant compared to M1-supernatant. Using a standard apoptosis assay, we also found that AECs treated with M2 supernatant have much higher levels of apoptosis compared to those treated with M1-supernatant. To understand which components of M2-supernatant might be primarily responsible for these profibrotic effects, we administered the pharmaceutical inhibitor Erlotinib, which blocks EGFR, the primary receptor for HB-EGF. If Erlotinib is administered during BMDM polarization ("M2+Erlotinib"), expression of M2 genes dramatically decrease. Intriguingly, the presence of Erlotinib has not yet been shown to cause any functional alterations in fibroblasts or AECs given M2+Erlotinib supernatant compared to M2 supernatant.

Conclusions: Although current work alludes to the necessity of myeloid-specific HB-EGF in the development of lung fibrosis, we do not yet know the mechanism of action in macrophages or proximal cell types. Ongoing work is aimed at determining the differences between M2 and M2+Erlotinib macrophages via RNAseq, as well as determining the causative agent in M2 supernatants that induces profibrotic effects in fibroblasts and AECs.

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Poster Presentations

#1 - TLR9 in Antibacterial Immunity During Influenza: A Role for Fibrocytes?

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Millions of people worldwide are infected with influenza each year, with tens of thousands of deaths yearly in the United States alone. A significant contributor to mortality from influenza is bacterial super-infection, most commonly from Gram-positive bacteria including *Staphylococcus aureus*. Paradoxically, mice lacking the bacterial DNA sensor TLR9 have reduced morbidity and mortality from methicillin-resistant *S. aureus* (MRSA) super-infection during influenza. Macrophages from influenza-infected $TLR9^{-/-}$ mice have increased production of inducible nitric oxide synthase, as well as improved MRSA phagocytosis and killing. Interestingly, influenza induces TLR9 expression in a wide variety of cells including macrophages and alveolar epithelial cells. While both alveolar macrophages and epithelium upregulate TLR9 approximately five-fold in response to influenza infection, a $CD45^{+}$ subset of cells termed fibrocytes upregulate TLR9 approximately 40-fold. Fibrocytes are hematopoietic cells which are recruited during inflammation, differentiate to a mesenchymal phenotype, and produce cytokines to promote fibroblast migration and wound healing. Supernatants from mixed fibroblast/fibrocyte cultures promote phagocytosis in bone-marrow derived macrophages only when both cell types lack TLR9. Moreover, when WT mice were transplanted with either WT or $TLR9^{-/-}$ bone marrow to generate chimeras, the $TLR9^{-/-}$ BM -> WT chimeric mice did not recapitulate the protection against bacterial super-infection seen in whole-body $TLR9^{-/-}$ mice. Together, these data suggest there is TLR9-dependent crosstalk between structural cells and macrophages that suppresses antibacterial immunity during influenza. The substantial upregulation of TLR9 in fibrocytes in response to influenza suggests that these hematopoietic cells also play an important role in directing antibacterial immunity during influenza, and may constitute a bridge between structural cells and innate immunity to bacteria.

#2 - Mucosal nanoemulsion allergy vaccines induce bystander suppression of reactivity to multiple food allergens

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Atopic diseases are important problems that involve both IgE-mediated immediate hypersensitivity reactions and Th2-driven cellular inflammation. We have previously demonstrated the ability of allergen administered intranasally in a nanoemulsion (NE) mucosal adjuvant to suppress Th2/IgE-mediated allergic responses and protect from allergen challenge in murine food allergy models. Protection was associated with strong suppression of allergen-specific Th2 cellular immunity and increased Th1 cytokines, IL-10 and regulatory T cells. Here we extend these studies to examine the efficacy of NE/allergen vaccination in mice sensitized to multiple foods. Mice were sensitized to both egg and peanut and received NE vaccine formulated with either one or both of these allergens. Mice were then subjected to oral challenges with either egg or peanut to assess reactivity. Immunization with NE formulations containing both egg and peanut markedly reduced reactivity after oral allergen challenge. Interestingly, however, mice that received the vaccine containing only peanut also had reduced reactivity to challenge with egg. Protection from oral allergen challenge was achieved despite the persistence of allergen-specific IgE and was associated with strong suppression of Th2-polarized immune responses. This required the presence of an allergen, however, as immunization with NE alone did not alter reactivity. These results demonstrate that anaphylactic reactions to food allergens can be suppressed using allergen-specific immunotherapy without having to eliminate allergen-specific IgE, and suggests that modulation of Th2 immunity towards one allergen may induce bystander effects that suppress reactivity to other allergens. In addition, these data suggest that the NE/allergen vaccines may lead to a global suppression of allergic responses.

#3 - Intranasal adjuvant induces retinaldehyde dehydrogenase activity in dendritic cells *via* p65 signaling

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Rationale*:* Designing a mucosal adjuvant that induces mucosal as well systemic immunity has been long desired. Mucosal vaccines are more effective in combating pathogens as they induce specific IgA response. However, it is very challenging to develop due to barriers of mucosal environment. In our previous studies we have shown that our intranasal adjuvant (oil-in-water based nanoemulsion-NE) interacts with epithelial cells and induces dendritic cell-specific retinaldehyde dehydrogenase (RALDH) activity in immunized animals. RALDH converts vitamin A into retinoic acid (RA). Thus produced RA orchestrate T cell homing towards the mucosal sites as well as IgA production.
Methods: To examine the efficacy of NE *in-vitro*, we simulated *in-vivo* intranasal immunization in a co-culture model. We treated the epithelial cells with different formulations of NE, followed by co-culturing with dendritic and T cells. Further, animals were intranasally immunized with ova-albumin (Ova) in combination to NE (with and without RA) to study RALDH production by mucosal DCs, T cell gut homing, IgA production and Th1/ Th17 cytokine production.
Results: NE alone induces RALDH activity in co-cultured DCs and induces gut homing marker expression on T cells *in-vitro*. Also, immunization with NE-Ova showed increased RALDH activity in DCs, resulting in increased IgA production as well as T cell gut homing. Furthermore, antibody titers and cytokine data suggested increased Th1/Th17 response while suppressing Th2. *In-vitro* studies showed NE activates RALDH via MyD88 pathway and utilizes p65 signaling in DCs.
Conclusion: In summary, NE activated RALDH activity in DCs via p65 signaling and enhanced mucosal immune responses in gut associated lymphoid tissues. These results suggest potential of NE as immunotherapeutic in mucosa-associated pathogens and diseases.

#4 - WDR26 negatively regulates formyl peptide receptor-1 mediated wound healing in intestinal epithelial cells

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Formyl peptide receptors (FPRs), which serve as pattern recognition receptors (PRRs), are critical regulators of host defense against pathogenic threat in phagocytes and are also expressed in epithelial cells. Activation of FPR signaling in epithelial cells promote intestinal epithelial repair, which is achieved by coordinated migration and proliferation of cells. However, mechanisms that govern upstream FPR1 regulation remain less well defined. In this study, we identified a novel FPR1 interacting protein, WD40 repeat protein 26 (WDR26). WDR26 negatively regulates FPR1-mediate wound closure in intestinal epithelial cells. We identified WDR26 inhibits Rac1 and Cdc42 activation and intracellular reactive oxygen species (ROS) generation. Furthermore, WDR26 is released from FPR1 after stimulation by FPR1 ligand, fMLF. These results suggest that WDR26 interaction with FPR1 functions to inhibit signaling cascade and wound repair in response to fMLF stimulation. Following FPR1 activation with fMLF, WDR26 is released from FPR1, resulting in activation of Cdc42/Rac1 signaling, increased cell migration and wound repair. These findings elucidate a novel regulatory function of WDR26 in FPR1-mediated wound closure in epithelial cells.

#5 - Neutrophil JAM-L and Epithelial CAR Signaling Regulate Transepithelial Migration of Neutrophils

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The intestinal mucosa is lined by a simple columnar epithelium that serves as a dynamic interface, regulating barrier function and homeostasis through signaling at intercellular junctions. The most apical junction, referred to as the tight junction (TJ), is composed of transmembrane proteins including the Coxsackie Adenovirus Receptor (CAR). Our group previously identified Junctional Adhesion Molecule-Like (JAML) expressed in myelomonocytic cells as an endogenous ligand for CAR. We determined that neutrophil (PMN) transepithelial migration in the gut is regulated by JAML-CAR interactions. Ligation of CAR by JAML impedes mucosal wound repair by inhibiting epithelial proliferation, however underlying mechanisms remain unexplored. We explored bidirectional signaling downstream of CAR-JAML interactions. Human PMNs were treated with recombinant CAR and recombinant Adenovirus Knob 5 (Ad5) protein, which binds CAR and inhibits JAML interactions. CAR ligation of PMN JAML increased phosphorylation of ERK1/2 and Akt (T308), which was reduced by Ad5 treatment, indicating specificity of JAML-induced signaling. Conversely, it has been shown that CAR ligation results in decreased phosphorylation of c-Raf and ERK1/2 in epithelial cells. As CAR contains cytoplasmic predicted tyrosine phosphorylation (PY) sites, we investigated whether ligation of CAR results in PY. Immunoblots from epithelial lysates for PY after treatment with Ad5 resulted in enhanced PY. These data reveal that CAR-JAML interactions induce bidirectional signaling in PMN and epithelial cells. We hypothesize that signaling events downstream of PMN JAML and epithelial CAR are key steps that play important roles in regulating PMN interactions and transepithelial migration across the inflamed intestinal epithelium.

#6 - Persistent IL-1β Signaling Aggravates Murine Enteropathogen, *Citrobacter rodentium* Infection in Mice

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The inflammasome cytokine IL-1β is an important mediator of the inflammatory responses against invading pathogens. However, it is not clear whether sustained IL-1β signaling following the loss of its endogenous inhibitor, secretory IL-1 receptor antagonist (sIL-1Ra) could improve mucosal immunity against the murine enteropathogen, *Citrobacter rodentium* (CR). In light of increased prescription of recombinant sIL-1Ra (aka Anakinra) to treat inflammatory bowel disease, we undertook this study. At basal levels, sIL-1Ra-deficient (sIL-1RaKO) mice displayed leukocytosis, and elevated serum and fecal lipocalin 2. Furthermore, bone marrow-derived macrophages and neutrophils from sIL-1RaKO mice generated higher levels of iNOS and nitrite, and ROS and NETs, respectively. These results collectively suggest sIL-1RaKO mice have low-grade chronic inflammation. Based on these results, we hypothesized that sIL-1RaKO mice with persistent IL1-β signaling could efficiently clear CR infection than their WT littermates. Oral challenge of CR ($1x10^9$ CFU/mouse) resulted in luminal colonization, which peaked at day 7 post-infection, in both groups; however, sIL-1RaKO mice displayed a higher CR burden and an exacerbated infection. The aggravated course of infection was further visualized by inoculating sIL-1RaKO mice with bioluminescent CR. Histologic analysis revealed that transmissible colonic hyperplasia was more pronounced in sIL-1RaKO mice. Interestingly, basal ileal Paneth's cell-specific antimicrobial proteins (Angiogenin 4 and Reg3γ) were significantly reduced in sIL-1RaKO mice. Collectively, our results demonstrate that a balanced mucosal IL-1β signaling is required to counter and clear enteropathogen infection.

#7 - A genome-wide screening reveals microbiota regulation of pathogen colonization in the gut

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Diarrheal disease remains a major cause of child mortality and adult morbidity worldwide, especially throughout developing countries. Diarrhea is usually caused by a variety of bacterial, viral and parasitic organisms. Among these, Enteropathogenic *Escherichia coli* (EPEC) and Enterohemorrhagic *Escherichia coli* (EHEC) are major causes of diarrheal disease in children and adults, respectively. *Citrobacter rodentium* has been extensively used as a surrogate model for studying the pathogenesis of human infections with EPEC and EHEC, for which there is no natural animal model of disease. Previously, we have shown that *C. rodentium*-specific IgG is required for pathogen clearance and host survival in adult and neonatal mice, respectively. It has also been described that luminal *C. rodentium* are out competed by the gut microbiota during the eradication phase. However, the mechanisms by which the pathogen overcomes the initial colonization resistance imposed by the microbiota during early infection remain unclear.

To identify genetic determinants that enteric pathogens employ to overcome the colonization resistance imposed by the microbiota, we generated a high-density mutant library in *C. rodentium* through random transposon insertion. We inoculated this library both into conventionally raised mice and germ-free animals and determined the abundance of all individual mutants before and after infection by Illumina sequencing. We found that the pathogen requires amino acid biosynthesis pathways, among other metabolic and virulence components, to colonize conventionally raised mice but not germ-free animals. Expression of amino acid biosynthesis pathways were regulated in response to amino acid levels and the presence of the gut microbiota. Metabolome analyses showed reduced amount of amino acids in the gut of conventionally raised mice compared with germ-free animals. Administration of a high protein diet increased pathogen colonization early during infection. Thus, depletion of amino acids by the microbiota limits pathogen colonization in the gut, and in turn the pathogen activates amino acid biosynthesis to expand in the presence of the microbiota. This study may help the design of new strategies to prevent or treat enteric infections by targeting pathogen metabolic pathways.

#8 - Vascular endothelium–specific STAT3 signaling axis regulates severity of IgE-mediated anaphylactic reactions

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Severe food-induced anaphylaxis is thought to be induced by IgE-mast cell (MC)-derived mediators, including histamine, which induce vasodilatation and fluid extravasation, causing cardiovascular collapse. We have previously revealed a synergistic interaction between IL-4 and histamine through interaction with the vascular endothelial (VE) IL-4Rα chain in the modulation of VE dysfunction and severity of anaphylaxis. We were interested in understanding the molecular pathways behind IL-4 enhancement of histamine- induced VE dysfunction and severe anaphylaxis. The non-hematopoietic Type II IL-4 receptor can signal through STAT3. Moreover, attenuation of STAT3 signaling prevented anaphylaxis. Therefore, we aimed to understand the role of STAT3 signaling in IL-4 exacerbation of histamine-induced vascular leakage.

IL-4C (IL-4+ anti-IL-4 mAb) administration exacerbated histamine-induced hypothermia in WT ($VE^{stat3\ WT}$) mice. VE-specific genetic deletion of STAT3 ($VE^{\Delta stat3}$ mice) abolished the IL-4 amplification. Moreover, histamine increase hematocrit (Hct) percentage in $VE^{stat3\ WT}$ mice. As expected, IL-4C enhanced histamine-induced vascular leakage in $VE^{stat3\ WT}$. Interestingly, $VE^{\Delta stat3}$ mice did not show an increase in Hct in response to histamine. STAT3 VE deletion also prevented IL-4 + histamine response. To understand the effect of IL-4 on VE cells, we performed RNAseq analyses on flow cytometric-isolated lung VE cells ($CD326^{-}$, $CD31^{hi}$, hematopoietic markers-) from WT BALB/c mice administered vehicle or IL4C. Gene Ontology analysis revealed the cluster of genes associated with STAT3 activity. The top pathways are: response to interferon-beta (P= 5.26E-16); defense response (P= 3.89E-07); response to cytokine (P= 8.26E-07); response to virus (P= 1.03E-06); defense response to protozoan (P= 5.86E-06); cellular response to interferon-gamma (P= 1.03E-06). Collectively these studies unveil a novel role for IL-4-STAT3 signaling in the priming of VE cells and hypovolemic shock during severe IgE-mediated reactions.

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#9 - Neutrophil Responses to *Vibrio cholerae* Infection in the Zebrafish Model

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Vibrio cholerae is the bacterium that causes the diarrheal disease cholera, which is spread through the ingestion of contaminated food or water. Cholera endemics occur largely in developing countries that lack proper infrastructure to treat sewage and provide clean water. One environmental reservoir of *V. cholerae* is fish. Diarrheal symptoms similar to those seen in humans are seen in zebrafish, a natural host model, as early as 6 hours after exposure. Our understanding of basic zebrafish immunology is currently rudimentary, and no research has been done to date exploring the immune response of zebrafish to *V. cholerae* infection. Furthermore, the relationship between *V. cholerae* and select antimicrobial proteins has not been established.

Adult, wild-type ZDR zebrafish (*Danio rerio*) were used in all experiments. Bacterial cultures were grown in LB broth at 37°C for 16 to 18 h. Bacterial cells were diluted in sterile 1× PBS to an infectious dose of ~5×10^4 to 5×10^7 CFU/ml. Groups of 4-5 Zebrafish were placed in a 400-ml beaker containing 200 ml of sterile infection water. One mL of bacterial inoculum was added to the beaker with fish. RNA and protein were extracted from intestinal homogenates. A time-course study was performed to assess mRNA expression of neutrophils & neutrophil associated cytokines, as well as select neutrophilic antimicrobial proteins. Bacterial cultures were grown in the presence of purified antimicrobial proteins to assess inhibitory effects.

During the course of *V. cholerae i*nfection, large increases in neutrophils, neutrophil associated cytokines, and neutrophilic antimicrobial proteins were detected. Addition of purified antimicrobial proteins to bacterial culture significantly or completely inhibited growth. Protein variants with altered metal ion binding capacity highlight the importance of select metal ions in *V. cholerae* growth, as well as binding site dynamics.

Our study for the first time describes the neutrophil response in zebrafish to *V. cholerae* infection, as well as establishing relationships between select antimicrobial proteins and *V. cholerae*. These results provide valuable understanding of the natural life cycle of *V. cholerae* and its relationship with zebrafish, and help in understanding differences and similarities between the immune systems of zebrafish and our own.

#10 - Improved diagnosis of food allergy using the activation of LAD2 mast cells

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The diagnosis of food allergy has traditionally relied on clinical history, levels of allergen specific IgE, positive skin prick test and when required, oral food challenge (OFC). The double-blind OFC is considered the gold standard for diagnosis of food allergy, however it involves ingestion of gradually increasing amounts of food under medical supervision. This can yield symptoms ranging from hives to severe anaphylaxis and impart undue stress in both the patient and physician. Therefore, it would be advantageous to develop an *in vitro* assay to predict food allergy without requiring an OFC. The Laboratory of Allergic Diseases (LAD2) is a mast cell line that has proven to be a useful tool in the study of mast cell biology. Initial studies have shown that LAD2 stimulation with ionomycin results in increased intracellular Ca^{+2} levels and release of mediators including TNF-α and β-hexosaminidase. In addition, membrane changes associated with degranulation are observed such as CD107a and CD63 expression on the cell surface. LAD2 cells express the high affinity IgE receptor FcεRI and degranulate upon IgE crosslinking in response to anti-IgE or allergen. Therefore, we are developing an assay for food allergy that involves passive sensitization of LAD2 cells with patient serum, followed by IgE crosslinking with allergen. This approach could provide useful insights into IgE function and predict reaction to OFC better than the current 50% rate seen with IgE quantitation assays.

#11 - Stem Cell Factor Inhibition Enhances Recovery from Colitis

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Epithelial cells that line the intestinal mucosa interface the microbe rich lumen and underlying tissue compartments. Injury to this critical barrier is observed in inflammatory diseases, and has detrimental systemic effects. The 248 AA Stem Cell Factor (SCF^{248}) isoform is generated by immune and structural cells in peripheral tissues, and activates c-Kit signaling in mucosal immune cells that include mast cells, eosinophils and innate lymphoid cells (ILC3, ILC2) to influence the inflammatory response. However, the role of SCF signaling in mediating intestinal inflammation and repair has not been explored. Peak SCF^{248} mRNA was detected in lamina propria derived immune cell populations on day 5 of acute colitis induced by dextran sulfate (DSS). Contribution of SCF in mediating the inflammatory response in colitis was analyzed using novel SCF-specific functionally inhibitory antibodies. Intraperitoneal administration of SCF neutralizing antibody resulted in a decreased disease activity index with improved recovery from colitis compared to mice administered control IgG. Histologic assessment demonstrated significant acceleration of the repair process after DSS colitis by SCF inhibition. Neutralization of SCF was associated with decreased intramucosal pro-inflammatory cytokines IL17a and IFNg. These data suggest that SCF functions as a pro-inflammatory cytokine and blocking its interaction with the c-kit receptor can potentially serve as a therapeutic target to improve recovery from colitis.

#12 - Host-microbe interactions in *Clostridioides difficile* infection during comorbid inflammatory bowel disease

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Background: *Clostridioides* (formerly *Clostridium*) *difficile* has emerged as a noteworthy pathogen in patients with inflammatory bowel disease (IBD)1. IBD is associated with an abnormal gut microbiota and mucosal immune responses. While susceptibility to *C. difficile* colonization and infection (CDI) after antibiotic disruption of the gut microbiota is well characterized, the antibiotic-independent mechanisms of *C. difficile* colonization and disease pathogenesis due to IBD-induced perturbations of the microbiome are not yet understood.
Objective: We sought to determine if IBD is sufficient to render mice susceptible to *C. difficile* colonization and infection in the absence of other perturbations, such as antibiotic treatment.
Methods: C57BL/6 IL-10-/- mice were infected with *Helicobacter hepaticus* to trigger colonic inflammation akin to human IBD. Control mice, not infected with *H. hepaticus*, were pretreated with the antibiotic cefoperazone to render the gut microbiota susceptible to CDI. Mice were gavaged with spores of the toxigenic *C. difficile* strain VPI 10463 and monitored for *C. difficile* colonization and disease. The fecal microbiota at the time of *C. difficile* exposure was profiled by 16S rRNA gene sequencing. Statistical analyses were performed using Mann-Whitney U test or Kruskal-Wallis one-way ANONA with Dunn post-hoc test.
Results: Mice with IBD harbored significantly distinct intestinal microbial communities compared to non-IBD controls at the time of *C. difficile* spore exposure. Mice with IBD were susceptible to *C. difficile* colonization while non-IBD controls were resistant. Mice with IBD were colonized by 10-fold less *C. difficile* compared to cefoperazone pretreated mice (2.34×10^7 CFU vs. 2.71×10^8 CFU respectively, $p = 0.0192$). While cefoparazone-induced CDI resulted in 100% of mice moribund by day 2 post-infection, clinical disease severity was significantly less in mice with comorbid IBD and CDI compared to antibiotic pretreated mice with CDI ($p = 0.0039$).
Conclusion: These studies demonstrate that the IBD-associated microbiome is sufficient for inducing susceptibility to *C. difficile* colonization, and suggest there are important differences in host-microbe interactions in IBD-induced CDI and antibiotic-induced CDI.

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#13 - Interleukin-22-mediated host glycosylation prevents *Clostridioides difficile* infection by modulating the metabolic activity of the gut microbiota

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Clostridioides difficile infection (CDI) is the most prevalent cause of nosocomial infectious diarrhea in hospitalized patients, particularly in those treated with antibiotics. The gut resident microbiota plays a critical role in the prevention of CDI. It has been extensively reported that the resident microbiota directly inhibits *C. difficile* colonization in the intestine through its byproducts, such as secondary bile acids. On the other hand, the involvement of host immunity in the microbiota-conferred colonization resistance remains poorly understood. Herein, we unveil that interleukin (IL)-22, induced by colonization of the gut microbiota, is crucial for the prevention of CDI in human microbiota-associated (HMA) mice. IL-22 signaling in HMA mice regulated host glycosylation, thus fostering the growth of succinate-consuming bacteria *Phascolarctobacterium* spp. within the gut microbiota. *Phascolarctobacterium* spp. reduced the availability of luminal succinate, a crucial metabolite utilized by *C. difficile* for its growth in the intestine. Moreover, IL-22–regulated host glycosylation is likely impaired in patients with ulcerative colitis (UC). The expression of N-glycosylation–related enzymes, *MGAT4A* and *MGAT4B*, was reduced in UC patients and was inversely correlated with that of IL22RA2, a soluble inhibitory IL-22 receptor. Consistently, mice colonized with UC patient–derived microbiotas were susceptible to CDI. Transplantation of healthy human-derived microbiotas or *Phascolarctobacterium* spp. reduced luminal succinate levels and restored colonization resistance in UC-HMA mice. Hence, IL-22-mediated host glycosylation fosters the growth of commensal bacteria that compete with *C. difficil*e for the nutritional niche.

#14 - Regulation of iNKT cells in mucosal tissues by vitamin A

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Invariant NKT (iNKT) cells are CD1d-restricted innate T cells that provide rapid innate T cell responses to glycolipid antigens from host cells and microbes. The numbers of iNKT cells are tightly controlled in mucosal tissues such as the intestines and lungs, but the mechanisms have been largely unclear. We found that vitamin A is a dominant factor that controls the population size of mucosal iNKT cells in mice. This negative regulation is mediated by the induction of the purinergic receptor P2X7 on iNKT cells. The expression of P2X7 is particularly high on intestinal iNKT cells, making iNKT cells highly susceptible to P2X7-mediated cell death. In vitamin A deficiency, iNKT cells fail to express P2X7 and are, therefore, resistant to P2X7-mediated cell death, leading to iNKT cell overpopulation. This phenomenon is most prominent in the intestine. We found that iNKT cells are divided into CD69^{+} sphingosine-1-phosphate receptor 1 (S1P1)$^{-}$ tissue resident and CD69^{-} S1P1^{+} nonresident iNKT cells. The CD69^{+} S1P1^{-} tissue-resident iNKT cells highly express P2X7 and are effectively controlled by the P2X7 pathway. The regulation of iNKT cells by vitamin A by the P2X7 pathway is important to prevent aberrant expansion of effector cytokine-producing iNKT cells. Our findings identify a novel role of vitamin A in regulating iNKT cell homeostasis in mucosal tissues.

#15 - Role of NLRP6 in the regulation of IgA response in the gut

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NOD-like receptor family pyrin domain-containing 6 (NLRP6) has been shown to be involved in microbe recognition and also indicated to play a microbiome-dependent protective role in intestinal pathologies. Given the importance of IgA on the gut-microbe regulation, we sought to test how NLRP6 shapes the antibody response in the gut. Mice deficient for NLRP6 were used in this study. Immune cells from mesenteric lymph nodes (MLN), Payer's patches (PP), cecal follicle (CF), and lamina propria (LP) from either small intestine and colon were isolated in homeostasis. IgA response was determined by Elispot and ELISA. B cell compartment was analyzed by flow. Colon LP and PP from NLRP6$^{-/-}$ mice had decreased numbers of B220+ cells, but no differences in IgA in small intestinal lavage were observed. Deletion of NLRP6 was also associated with significantly decreased IgA in stool and inhibition of IgA class switch recombination (CSR) in vitro. Surprisingly, IgA producing cells were slightly decreased in NLRP6$^{-/-}$ colonic LP, but not significantly, and CSR analysis in the intestinal draining lymphoid organs did not show any differences among wild type and NLRP6$^{-/-}$ mice. On the other hand, IgA+ cells are increased on the B220^{+} population in the colon of NLRP6$^{-/-}$ mice. Our preliminary results suggest that NLRP6 can regulate IgA response in the intestinal lumen without affecting plasma cell responses in the LP. The exact mechanism whereby NLRP6 regulates IgA response in the gut, and how it impacts in the gut-microbiota homeostasis remains unclear.

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#16 - Early-Life Respiratory Viral Infection Leads to Sex-Associated TSLP Driven Chromatin Remodeling and Trained Immunity

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Many studies have linked severe RSV infection during early-life with enhanced immune responses upon secondary exposures, such as during RSV re-infection and childhood asthma. Innate immune cell populations (especially antigen presenting cells) can be "trained" following pathogen exposure that leads to alterations of the immune response later in life. Here we show that long-term systemic alterations are occurring following early-life RSV-infection. Bone marrow-derived dendritic cells (BMDC) isolated from early-life RSV-infected male mice at 4 weeks post-infection retained expression of maturation markers, such as *Cd80/86* and *Ox40l*. Chemokines and cytokines associated with the inflammatory response during RSV infection were also persistently expressed (i.e. *Ccl3, Ccl5, Il6*) along with *Kdm6b* and *Tslp*. The addition of recombinant TSLP to naïve BMDC cultures showed a similar response as BMDC isolated from early-life RSV male mice, verifying a role for TSLP in the persistent BMDC phenotype. Furthermore, knockdown of TSLP signaling using TSLPR-/- male mice abrogated this activated phenotype and led to enhanced anti-viral Th1 responses. When we assessed chromatin structure by ATAC-seq, data indicated differences in the chromatin landscape between WT and TSLPR-/- BMDC, showing more "open" regions of chromatin near anti-viral type-1 genes (*Mid1, Spp1, Cxcl11, Gadd45g*) in TSLPR-/- BMDC. In contrast, more accessibility was observed near genes linked to RSV disease or asthma (*Cxcl1, Cxcl2, Areg, Oxr1*) in the WT BMDC. The altered genes in the WT BMDC can be linked to IRF4 signaling, a transcription factor driven by TSLP and associated with a Th2-type response. RNA-seq data indicated a stronger, more appropriate anti-viral response in TSLPR-/- BMDC following initial RSV infection that may enable proper inflammatory resolution and maturation of TSLPR-/- BMDC. These data further identify that TSLP is involved in persistently altering the immune system in the bone marrow following early-life pulmonary RSV-infection, leading to altered immune responses later in life. Thus, targeting TSLP during early-life may be an effective therapeutic approach.

#17 - Analysis of MRSA Pulmonary Infection in Diabetic Mice

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Diabetes is a major global problem with 425 million people currently diagnosed. It is estimated that this number will grow to over 600 million people by 2045. Although diabetes itself is a non-communicable disease, many diabetic patients are more susceptible to microbial infections and exhibit a higher burden of disease. Previous work from our labs has shown that a failure to heal diabetic wounds corresponds to elevated levels of prostaglandin E2 (PGE2) in the inflammatory macrophages that are recruited to the wound. Furthermore, past studies from our lab have demonstrated that PGE2 signaling can impair the innate immune functions of macrophages. Therefore, we hypothesized that diabetic obese mice would be more susceptible to a respiratory MRSA infection due to impaired function of lung macrophages. Mice were placed on a high fat diet for 12 weeks to induce obesity and insulin resistance. Mice were then infected with methicillin-resistant *Staphylococcus aureus* (MRSA) o.p. Initial experiments suggested a trend to higher CFU burden in lungs of infected diabetic mice. Alveolar macrophages were purified from control and diabetic mice and tested for ability to phagocytize MRSA, but there was no significant difference in the phagocytosis of the bacteria, different from what has been seen previously in wound macrophages. Current studies are examining cytokine differences and trying to determine whether diabetic mice are characterized by elevated levels of macrophage-derived PGE2 in the lung. Our goal is to determine the mechanisms (PGE2-related or not) by which diabetic individuals may be more susceptible to bacterial pneumonia.

#18 - Development of an Experimental Multi-subunit Vaccine to Prevent *Escherichia coli* Urinary Tract Infection

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Urinary tract infections are an important public health scourge affecting more than 11 million women annually. About 20% of these women will contract a second UTI and 30% of those will continue to be plagued by recurrent infections. Uropathogenic *Escherichia coli* (UPEC) causes 80% of these uncomplicated urinary tract infections. UPEC genomes encode numerous virulence factors including iron acquisition systems important for colonization of the iron-limited urinary tract. Four outer membrane iron receptors, Hma, IreA, IutA, and FyuA, have been proven to reduce UPEC colonization of the bladder or kidneys of mice when formulated individually into a vaccine and intranasally administered with adjuvant. Due to the diversity of UPEC strains, single administration of these antigens has the potential to prevent infections from a fraction of UPEC strains, but a multi-subunit vaccine utilizing all four of these receptors is capable of providing protection against infection from 89% of UPEC strains based on the presence of each gene within the genome of sequenced strains. Intranasal administration with 25 µg of each of the four antigens formulated with the adjuvant double mutant heat labile *E. coli* enterotoxin (dmLT) reduced bacterial burden in the urine (9-fold reduction) and bladder (50-fold reduction) when compared to dmLT-only control mice. In the kidneys, a significant reduction in colony forming units (CFU) is observed in vaccinated animals ($P = 0.01$) and the number of mice without detectable CFU is 60% higher in the vaccinated group compared to the control group. Vaccinating with all four antigens results in a serum immune response dominated by anti-Hma antibodies (107 µg/ml) compared to anti-IreA antibodies (18 µg/ml), while anti-FyuA or -IutA antibodies were undetectable. In addition, antigen-specific antibody concentrations in vaccinated mice do not increase 48 hours after transurethral challenge with UPEC strain HM57. Future experiments are focused on improving antigen delivery strategies to overcome the solubility constraints of integral outer-membrane protein antigens and optimizing the immune response to UPEC infection. These data provide promising results that developing a multi-subunit vaccine will increase the breadth of protection against UPEC strains, which is critical in this current age of antibiotic failure.

#19 - Fibrotic Lung Injury Inhibits Clearance of Staphylococcal Lung Infections via Impaired Neutrophil Response

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a disease characterized by progressive collagen deposition in the lung and eventual loss of lung function. IPF is fatal and treatments for the disease are limited and lack efficacy. Much mortality associated with IPF can be attributed to acute exacerbation (AE), a rapid deterioration in lung function, often leading to death within a few months of diagnosis. Recent studies have recognized a role for bacteria within the lung in exacerbating IPF disease course. One study found that respiratory infections resulted in similar mortality rates in hospitalized IPF patient populations compared to AE of no identifiable cause. Other studies have identified the bacterial genus *Staphylococcus* as being significantly associated with poor outcomes in IPF patients, and have isolated methicillin-resistant *Staphylococcus aureus* (MRSA) from sputum of IPF patients undergoing hospitalization for acute respiratory deterioration. Therefore, bacterial pneumonia may be a significant cause of mortality in IPF patient populations. Based on these data, we sought to understand the interplay between fibrotic lung injury and Staphylococcal pneumonia.
Methods: We utilize bleomycin to induce lung injury and fibrosis in mice, followed by infection with MRSA as a model of bacterial pneumonia post-fibrotic lung injury.
Results: We have found that bleomycin-induced lung fibrosis impairs clearance of a MRSA lung infection and increases bacterial dissemination to the spleen compared to infected non-fibrotic mice. In addition, infection with MRSA following bleomycin treatment results in increased mortality compared to that of mice treated with bleomycin alone. As a defect in bacterial clearance is implicated in the elevated mortality in fibrotic infected mice, we sought to understand how pulmonary fibrosis impacts the ability of the innate immune system to respond to infection. We determined that fibrotic mice produce decreased levels of chemokines CXCL1 and CXCL2 following infection, resulting in a subsequent decrease in neutrophil recruitment to the lung. Neutrophils isolated from fibrotic mice also appear to exhibit a defect in MRSA phagocytosis and intracellular killing compared to those isolated from non-fibrotic mice, suggesting that neutrophils that are successfully recruited to the lungs of fibrotic mice do not function optimally. In addition, we have observed decreased production of pro-inflammatory cytokines IL-1β and TNFα in whole lung, indicating that the development of fibrosis broadly dampens immune responses to bacterial lung infections. Ongoing studies are aimed at understanding how the increased bacterial burden in fibrotic mice might contribute to increased lung injury and mortality. These data allow for a greater understanding of how the development of fibrosis alters immune responses to bacterial pneumonia. Future studies may identify ways to prevent or better treat pulmonary infections that exacerbate IPF.
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#20 - Acidic pH reduces *Vibrio cholerae* motility in mucus by weakening lagellar motor torque

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Intestinal mucus is the first line of defense against intestinal pathogens. It acts as a physical barrier between the epithelial tissues and luminal microbes. Enteropathogens, such as *Vibrio cholerae*, must compromise or circumvent the mucus barrier to establish a successful infection. We investigated how motile *V. cholerae* is able to penetrate mucus using single cell tracking in unprocessed porcine intestinal mucus. We found that changes in pH within the range of what has been measured in the human small intestine indirectly affect *V. cholerae* flagellar motor torque, and consequently, mucus penetration. Microrheological measurements indicate that the viscoelasticity of mucus does not change substantially within the physiological pH range and that commercially available mucins do not form gels when rehydrated. Finally, we found that besides the reduction in motor torque, El Tor and Classical biotypes have different responses to acidic pH. For El Tor, acidic pH promotes surface attachment that is mediated by activation of the mannose-sensitive haemagglutinin (MshA) pilus without a measurable change in the total cellular concentration of the secondary messenger cyclic dimeric guanosine monophosphate (c-di-GMP). Overall, our results support that the high torque of *V. cholerae* flagellar motor is critical for mucus penetration and that the pH gradient in the small intestine is likely an important factor in determining the preferred site of infection.

#21 - Alveolar macrophage and dendritic cell phenotype following the resolution of respiratory syncytial virus infection

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Background: Respiratory syncytial virus (RSV) infects virtually all children before two years of age and is a leading cause of bronchiolitis and hospitalization in infants. Severe RSV infection is associated with increased risk of wheeze and asthma during childhood in a pattern that suggests a direct causative relationship. There is emerging evidence that mononuclear phagocytes display long-term phenotypic alterations following inflammatory events, a phenomenon referred to as "trained immunity." We hypothesized that RSV infection causes increased susceptibility to wheeze by altering the phenotype of dendritic cells and alveolar macrophages within the lung.

Methods: We treated mice intratracheally with $1x10^6$ plaque-forming units of RSV, or with media (sham). Three weeks post-infection, we harvested alveolar macrophages from the lungs and stimulated the cells ex vivo with a panel of allergens and Toll-like receptor agonists. For fate-mapping experiments, $CX3CR1^{creert2}xRosa26^{[flox]stop[flox]TdTomato}$ were treated with RSV or media and treated with tamoxifen on days 1, 3, and 5 post infection. TdTomato labeling was assessed via flow cytometry three weeks post infection.

Results and Discussion: Alveolar macrophages harvested from RSV-treated mice displayed upregulation of IL-1b and TNF in response to stimulation with cockroach allergen extract as compared to those from sham-treated mice. We observed an accumulation of TdTomato-labeled CD103 dendritic cells, CD11b+ dendritic cells, and alveolar macrophages in the lungs of RSV-treated mice as compared to sham-treated mice. These results suggest that RSV exposure can generate lasting phenotypic alterations to immune cells within the lung.

#22 - Enhancing influenza vaccine efficacy and breadth through a combined nanoemulsion and RIG-I agonist adjuvant

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Current influenza vaccines elicit strain-specific antibodies due to the immunodominance of epitopes with high cross-strain variability and fail to induce broad cellular immunity—a key component of effective cross-subtype protection. Here, we combine two chemically and biologically distinct adjuvants—an oil-in-water nanoemulsion (NE) and RIG-I agonists derived from defective interfering (DI) RNAs of Sendai and influenza viruses—to develop a better adjuvant through mimicking the pattern of innate activation during natural infection. NE activates TLRs, stimulates immunogenic apoptosis, and enhances cellular antigen uptake, leading to induction of a TH1/TH17 response. DI RNAs activate RIG-I, induce type I IFNs, and elicit TH1 responses. Together, these adjuvants present PAMPs known to activate all three classes of innate receptors (TLR, RLR, NLRP3) critical to influenza immunity. We hypothesized that the combined adjuvant (NE-DI RNA) would induce more robust humoral responses towards less immunodominant conserved epitopes, and cellular responses tailored to influenza. Stimulation of dendritic cells with NE-DI RNA enhanced activation of key innate pathways, and markedly increased IFNβ production. Mice immunized with inactivated A/Puerto Rico/8/1934 (PR/8) with NE-DI RNA demonstrated synergistic enhancement of PR/8-specific IgG, and yielded antibodies with significantly greater neutralization activity compared to either individual adjuvant. HA stalk-specific antibodies were also increased, reflected by broader reactivity towards heterologous and heterosubtypic strains. A unique cellular response with enhanced TH1/TH17 immunity was induced with the NE-DI RNA combinations, demonstrating that the enhanced immunogenicity of the adjuvants was not simply additive. These studies highlight the potential of combination adjuvants for improving the efficacy of influenza vaccination.

#23 - Characterization of mucolytic enzymes of commensal gut bacteria

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Intestinal mucus is a protective lining secreted by goblet cells to prevent contact of lumenal contents, including the microbiota, with the epithelium. A complex glycoprotein, mucin is composed of a protein backbone decorated with a large diversity of O-linked glycosylation (O-glycans). O-glycans contain ~100 unique structures and degradation of these structures requires many different enzymes specific to these linkages. Multiple members of the gut microbiota are known to degrade intact host mucin or its components, such as *Ruminococcus torques* which degrades human Muc2, and *Bacteroides thetaiotaomicron* which degrades released mucin O-glycans. As disruption of the mucus barrier by these bacteria has been shown in our lab to correlate with spontaneous colitis, like that which occurs in Inflammatory Bowel Disease (IBD), identification of bacterial enzymes targeting mucin may reveal novel drug targets for inhibitors to prevent bacterial mucus erosion. We hypothesize that due to the complexity of mucin as a substrate, multiple species within the microbiota act synergistically to completely degrade intact mucin *in vivo*. Further, we hypothesize that mucin-degrading species require systems that sense and degrade specific subsets of mucin-related structures. *B. thetaiotaomicron* is a common human gut commensal that degrades mucin O-glycans using enzymes encoded in Polysaccharide Utilization Loci (PULs). We have identified a PUL highly induced by keratan sulfate, a poly-N-acetyllactosamine backbone with sulfation, which is present in mucin O-glycans. Fluorescent immunostaining informed cellular localization of enzymes in this PUL, which are located at either the cell surface or in the periplasm. Further, using Thin Layer Chromatography (TLC) and High Performance Anion Exchange Chromatography (HPAEC), we have characterized the activities of four glycoside hydrolase (GH) enzymes in this PUL, including the identification of a GH with novel endo-mucinase activity for its family. Together, this data has allowed us to construct a working model of this PUL and its sequential breakdown mechanism of keratan sulfate. Future studies will focus on the regulation of this PUL by its hybrid two-component system (HTCS), such as identifying the signal sugar that binds and activates the HTCS to upregulate expression of the PUL. While *B. thetaiotaomicron* can utilize O-glycans released from the peptide backbone of mucin, we seek to characterize mucolytic enzymes in species capable of degrading more intact mucin, which likely release the O-glycans that become available to other species for further breakdown. *Ruminococcus torques* was previously identified as a mucin-degrader and has increased prevalence in patients with IBD. While it is known that *R. torques* can degrade intact mucin, the enzymes it uses to target these structures remain unknown. We have demonstrated growth of *R. torques* on glycoprotein derived from porcine rectal mucin and observed the temporal release of sugars by TLC. Future studies will include RNA-seq to identify genes upregulated during growth on porcine rectal glycoprotein to identify putative enzymes to be cloned and recombinantly expressed to characterize activities on mucin-related structures *in vitro*. Further, we will evaluate the ability of *R. torques* to release O-glycans for utilization by species like *B. thetaiotaomicron* in co-culture experiments.

#24 - IgA induced immune response in lungs

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Mucosal tolerance is the capacity of the immune system to adapt to innocuous environmental antigens, food, and commensal bacteria. Immunoglobulin A (IgA) may be one of the key strategies to generate immune protection in a non-inflammatory manner. Despite extensive literature in the field, mechanisms of how environmental antigens are rendered non-immunogenic through mucosal tolerance remain elusive. The aim of the present study is to set up a mouse model to investigate the role of IgA in the tolerance to mucosal antigens. IgA secreted by MOPC 315 plasmacytoma that recognizes TNP was given intratracheally (i.t) along with TNP coupled Ovalbumin. Mice desensitized with IgA along with Ova-TNP (IgA+Ova-TNP) and challenged with Ova-TNP did not show a systemic effect but resulted in a decrease in lung pathology and reduced IL-17 as compared to Ova-TNP alone. In a separate experiment multiple doses of IgA were utilized to determine if chronic IgA mediated stimuli were necessary to induce tolerance to the mucosal antigen exposure. Multiple doses of IgA induced increased number of FoxP3+ cells along with increased mRNA expression of Tgf-b1 and IL-10. However, it also increased mucus genes Muc5Ac and Gob5; and Il-13 significantly and led to enhanced mucus formation and inflammation in the airways. Thus, while there appeared to be an induced response to IgA-Ova-TNP that was characterized by increased regulatory responses (Tregs and anti-inflammatory cytokines) there was also an increase in pathogenesis to the IgA-mediated stimuli.

#25 - Nanoparticle Delivery of Antigen Attenuates Anaphylactic Response in a Murine Model of Peanut Allergy

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The current methods to manage food allergies are limited primarily to avoidance of the allergen or use of oral immunotherapy (OIT), which is poorly understood and exhibits considerable variability in both the magnitude and duration of efficacy. In addition to reducing mortality, well-tolerated methods to induce a robust desensitization to food allergens would represent a tremendous success for the fields of allergy and immunology and would significantly improve patient quality of life. Here, we developed poly(lactide-co-glycolide) nanoparticles for intravenous delivery of relevant antigen and tested their ability to attenuate anaphylactic response to challenge in an alum-induced model of peanut allergy. Importantly, we demonstrated reduction of allergen-associated anaphylactic reactions in both prophylactic and therapeutic contexts and identified critical parameters to ensure safety of these types of interventional therapies. Interestingly, we observed that induction of allergen unresponsiveness within mesenteric lymph nodes was associated with significantly reduced anaphylaxis while unresponsiveness of splenic cell populations alone was not predictive of attenuation of symptoms after challenge. This may suggest that successful strategies to prevent anaphylaxis will require reprogramming of both systemic and local immunity. This research represents a translatable technology capable of safely and effectively inhibiting allergic response while providing critical biological insights related to methods to promote allergen desensitization.

#26 - Desmosomal Cadherins Desmoglein-2 and Desmocollin-2 Regulate Intestinal Epithelial Barrier Function and Mucosal Repair

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The intestinal epithelial barrier plays a pivotal role in controlling mucosal homeostasis. In a number of pathological states such as inflammatory bowel disease, epithelial damage with disruption of the mucosal barrier results in a compromised intestinal mucosal homeostasis. To avoid chronic mucosal damage, efficient wound repair is critical in re-establishing epithelial barrier properties and homeostasis. Epithelial barrier properties are achieved by intercellular junction proteins in the apical junctional complex and desmosomes. Intestinal epithelial cells express desmosomal cadherins, desmoglein 2 (Dsg-2) and desmocollin 2 (Dsc-2). To analyze contribution of these cadherins in controlling epithelial adhesion and homeostasis we generated mice with inducible intestinal epithelial specific (VillinCre-ER^{T2}) deletion of Dsg-2 or Dsc-2. Inducible downregulation of Dsg-2 or Dsc-2 resulted in compromised epithelial barrier function as assessed by paracellular FITC dextran flux using an intestinal loop model. Additionally, perturbation of these cadherins increased susceptibility to dextran sodium sulfate induced acute colitis. In addition to controlling epithelial barrier function, intercellular junction proteins have been reported to regulate signaling events involved in mediating repair. We therefore determined if desmosomal cadherins control intestinal mucosal repair. Knock-down of either Dsg-2 or Dsc-2 delayed colonic mucosal wound repair in a murine biopsy induced colonic injury model.

#27 - Developing and optimizing an *ex-vivo* alveolar macrophage cell line to dissect the mechanisms of protection during pulmonary disease

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Pulmonary infection of mice with *Mtb* is characterized primarily by colonization of permissive alveolar macrophages and a delayed adaptive immune response. We are interested in understanding host pathways that contribute to protective immunity during Mtb infection in the lungs. We previously showed that mice lacking NADPH Phagocyte Oxidase (Phox) have a defect in disease tolerance driven by the hyperactivation of the NLRP3 inflammasome. Surprisingly, mice lacking both Phox and Caspase1/11, were unusually susceptible, dying within 25 days of low dose aerosol Mtb infection. Given that mice lacking either Phox or Caspase1/11, have modest defects in survival our data suggests a strong genetic interaction between these pathways. While we are currently examining the mechanisms that result in this hypersensitivity, preliminary data suggests dysregulation of bacterial control and inflammation in these knockout animals. Given the early susceptibility of Phox/Casp1/11 mice, we predict a major role of alveolar macrophages in the susceptibility of these animals. Unfortunately, limitations in acquiring and maintaining alveolar macrophages restrict our ability to study genetic interactions in this context. To address this limitation, we have optimized functional genetic approaches in an ex-vivo macrophage model that closely resembles alveolar macrophages and not BMDMs. Here, we show that these cells, termed Max Planck Institute (MPI) cells, show similar surface expression and cytokine responses to infection and exogenous stimuli compared to alveolar macrophages, and are amenable to CRISPR-mediated genetic editing. In combination with our in vivo model, this genetically tractable ex vivo system will allow us to dissect the susceptibility of Phox/Caspase1/11 mice during *Mtb* infection allowing us to understand the mechanisms that contribute to protection against TB disease.

#28 - Anoctamin 1 (ANO1) regulates esophageal epithelial proliferation in Experimental Eosinophilic Esophagitis

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Background: Eosinophilic Esophagitis (EoE), a chronic inflammatory disease of the esophagus, is characterized by esophageal eosinophilia and epithelial remodeling, including basal zone hyperplasia (BZH) and dilated intercellular spaces (DIS). We have identified Anoctamin 1 (ANO1), a calcium activated chloride channel protein in esophageal epithelial proliferation. Herein, we employed murine models of EoE to probe the relationship between ANO1 and esophageal epithelial proliferation and EoE severity.

Methods: WT BALB/c mice were intraperitoneally injected with peanut extract (PN, 100 µg / 1mg alum), received intranasal challenges with PN (50 µg / 50 µL PBS), and oral gavaged with ground PN (2mg / 200 µL PBS). *Krt5-rtTA* mice backcrossed on the *tetO-IL-13* background (*Krt5-rtTA tetO-IL-13*) received Dox (2 mg / ml plus 50 mg / ml sucrose) to activate esophageal IL-13 expression. EoE disease pathology (eosinophils per high power field (HPF) and BZH) was examined. Immunofluorescence (IF) analysis of murine esophagus was used to study KI-67 and ANO1 expression.

Results: PN challenge induced an esophageal eosinophilia in PN-sensitized as compared to control mice (Eos / HPF; 1.9 ± 0.8 vs. 7.3 ± 4.0; control vs. PN-sensitized; $n = 9$ per group; $p < 0.001$). IF staining revealed an increase in KI-67+ esophageal epithelial cells and robust ANO1 expression in PN-sensitized mice (KI-67+ cells / µM; 16.4 ± 1.8 vs. 21.6 ± 6.8; control vs. PN-sensitized; $n = 9$ per group; $p < 0.05$). Similarly, in the transgenic model, we observed increased eosinophil counts (Eos / HPF; 5.5 ± 3.5 vs. 14.0 ± 2.6; untreated vs. Dox-treated; n = 2 and 3 mice per group), KI-67+ esophageal epithelial cells (KI-67+ cells / µM; 0.08 ± 0.07 vs. 0.27 ± 0.18; untreated vs. Dox-treated; $n = 3$ per group) and esophageal epithelial ANO1 expression in Dox-treated versus untreated *Krt5-rtTA tetO-IL-13* mice.

Conclusions: Our data reveals a relationship between esophageal epithelial proliferation and ANO1 expression in experimental EoE.

#29 - Resolvin E1 is a pro-repair mediator that promotes intestinal epithelial wound healing

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Resolution of inflammation and epithelial repair are active processes mediated by protein/peptides and lipids known as specialized pro-resolving mediators. Resolvin E1 (RvE1), a pro-resolving bioactive lipid mediator derived from omega-3 fatty acid has been described to decreased the inflammatory response and promote the establishment of a restitution phase in different tissues. Using a mouse colonoscopy based biopsy wound model we found that RvE1 is secreted in response to colonic injury. Complimentary *in vitro* experiments showed how RvE1 treatment of model intestinal epithelial cell (IEC) lines promotes wound repair by increasing epithelial cell proliferation and migration. Analysis of the signaling pathways revealed activation of CREB, mTOR and Src pathways, which promote epithelial cell proliferation, migration and ultimately wound repair. To harness this mechanism of repair, we performed intramucosal injection of synthetic nanoparticles containing RvE1 in injured murine colon. RvE1 nanoparticles increase wound closure compared to naked RvE1 and empty nanoparticles. In summary, the above findings provide important insight on mechanisms of intestinal mucosal wound repair driven by RvE1 and determine its potential as a therapeutic agent aimed at facilitating intestinal epithelial wound closure and barrier recovery at the intestine.

(from: https://www.socmucimm.org/about/history/)
-This summary of the history of SMI was prepared by Dr. Charles Elson for the 7th International Congress of Mucosal Immunology held in Prague, Czech Republic, 1992.

The idea for a formal organization of mucosal immunologists was generated in 1985 during the annual scientific meeting of the American Association of Immunologists (AAI).

This meeting is held together with a number of other societies which are collectively known as FASEB (Federation of American Societies of Experimental Biology). Because of its 15-20,000 attendees, the meeting is held in a cavernous hall or convention center.

In 1985, the mucosal immunology posters were dispersed in dozens of different locations, because mucosal immunology was not listed as a distinct scientific category in the program. On this particular day, a number of us interested in mucosal immunology kept meeting one another at the same posters. After reviewing the poster, we would scatter in different directions, only to meet once again at the next mucosal poster somewhere else in the vast hall.

Many members of SMI who have attended this meeting in the past will recognize this shuffle. “We should have our own sessions” was the refrain repeated among us at each encounter. In order to do that it was obvious that we would need to organize into a formal group. This was the seed that germinated into our current Society.

Just as all investigators stand on the shoulders of their predecessors, I believe that the SMI is the fruition and logical extension of the multiple International Congresses of Mucosal Immunology that have been held over the past two decades, starting with the Congress organized by Drs. Jiri Mestecky and Jerry McGhee in Birmingham in 1973. These Congresses have pulled together investigators from all over the world which has generated a sense of community among them. They have clarified the major scientific problems remaining to be addressed and have generally fostered the progressive growth of the field. These meetings built the foundation on which SMI now stands. It seems only appropriate that the first major undertaking of SMI was the organization of the Seventh International Congress of Mucosal Immunology.

Following that day in 1985, an organizing committee was formed at the Niagara Falls Congress, consisting of Drs. John Bienenstock, Jiri Mestecky, Pearay Ogra, Anne Ferguson, Robert Clancy, Warren Strober and myself as chairman. The international composition of the committee reflected the early recognition that, although the idea for it may have sprung from frustration at an American meeting, any Society in this field would have to be international in its focus and membership.

Over the following year articles and bylaws were written, the Society legally incorporated in the State of Virginia and non-profit, tax-free status was applied for and received in the United States. The members of the organizing committee served as the initial Governing Board, and elected Dr. Jiri Mestecky as acting President. Two cycles of elections have subsequently been held and all current officers have been elected by the membership at large.

The purposes of the Society are to advance research, literary and educational aspects of the scientific field of mucosal immunology; to organize and coordinate national and international research congresses, seminars, workshops in mucosal immunology; to interest and promote new investigators into the field; and to stimulate interactions among members of diverse disciplinary interests and expertise within mucosal immunology who are dispersed around the world.

These are worthy goals which will benefit all investigators active in this field. The rapid increase in membership in just a few years indicates that a large number of investigators support these aims, which is an important step toward advancing our field. Significant accomplishments have already been achieved. The Society is now a guest at the FASEB meeting with separate mucosal immunology sessions and input into the organization. Mucosal immunologists no longer have to chase all over the hall in running shoes! Much more remains to be done, but considering the collective talents of the membership, the future looks bright.

Mucosal Immunology is the official publication of the Society for Mucosal Immunology and is published by Nature Publishing Group. It aims to provide a forum for both basic and clinical scientists to discuss all aspects of immunity and inflammation involving mucosal tissues.

Its 2016 Impact Factor was 7.478. The Mucosal Immunology journal reflects the interests of scientists studying gastrointestinal, pulmonary, nasopharyngeal, oral, ocular and genitourinary immunology through the publication of original research articles, scholarly reviews and timely commentaries, editorials and letters. The content features basic, translational and clinical studies. Mucosal Immunology also aims to provide a primary method of communication for the SMI governing board and its members through the publication of society news and meeting and conference announcements.

ABOUT THE

(from: https://www.socmucimm.org/about/about-smi/)

The Society for Mucosal Immunology (SMI) was formed in 1987 to advance research and education related to the field of mucosal immunology. Today the society represents a diverse group of more than 900 scientists and clinicians from all over the world.

Since 1990 mucosal immunology has expanded from what many considered a niche discipline to one of the hottest areas in immunobiology today.

Mucosal immunologists focus on the sites at which most antigens enter—the mucosal surfaces of the gastrointestinal, respiratory and urogenital tracts, which are the body's first line of defense against an array of pathogenic microbes. They study of the disease states that result when the mucosal immune system's ability to distinguish pathogens from innocuous antigens fails (i.e. inflammatory bowel diseases, food allergies, gluten intolerance).

Harnessing the mucosal immune system's unique features to prevent or treat disease is another rapidly growing area of interest that includes the development and refinement of orally and nasally administered vaccines, adjuvants and immunotherapeutics.

SMI and its members have been responsible for the organization of 18 international congresses in the United States, Europe, Asia and Australia. To help achieve its mission, SMI has developed and maintained collaborative partnerships with other important immunological organizations, including the Crohn's & Colitis Foundation of America (CCFA), American Association of Immunologists (AAI), the Federation of Clinical Immunology Societies (FOCIS), the European Mucosal Immunology Group (EMIG), the Japanese Society for Mucosal Immunology (JSMI) and the Australian Society for Immunology (ASI).

SMI provides forums for the exchange of research, policy formulation relevant to the profession and research work environments, and learning opportunities for professionals in one of the most prominent and emerging scientific disciplines.

Mission

SMI supports research and education and fosters communication and collaboration in the field of mucosal immunology.

Vision

SMI is the preeminent international community of researchers working together to advance the field of mucosal immunology and improve health worldwide.

Local Chapter 2020 - Michigan

March 6, 2020, Ann Arbor, MI
Hosted by: The University of Michigan Medical School

The Mucosal Immunology – Michigan Local Chapter will bring together researchers from the University of Michigan ,

Michigan State University , Wayne State University, and the University of Toledo , to discuss current topics in immunology at mucosal surfaces. This symposium is sponsored by the Society for Mucosal Immunology and invites all investigators to share their work, but specifically aims to promote the work of junior investigators. In addition to presentations from invited speakers, additional talks will be chosen from submitted abstracts.

Organizers of the 2020 Local Chapter Michigan Conference: **Catherine Ptaschinski, PhD, Jennifer Brazil, PhD, Roberta Caruso, MD, PhD, Taeko Noah, PhD and Veronica Azcutia, PhD**

THE 3RD ANNUAL MUCOSAL IMMUNOLOGY CONGRESS AND SYMPOSIUM (MICS2020) will be held July 20-23 at the Westin in Denver, CO.

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