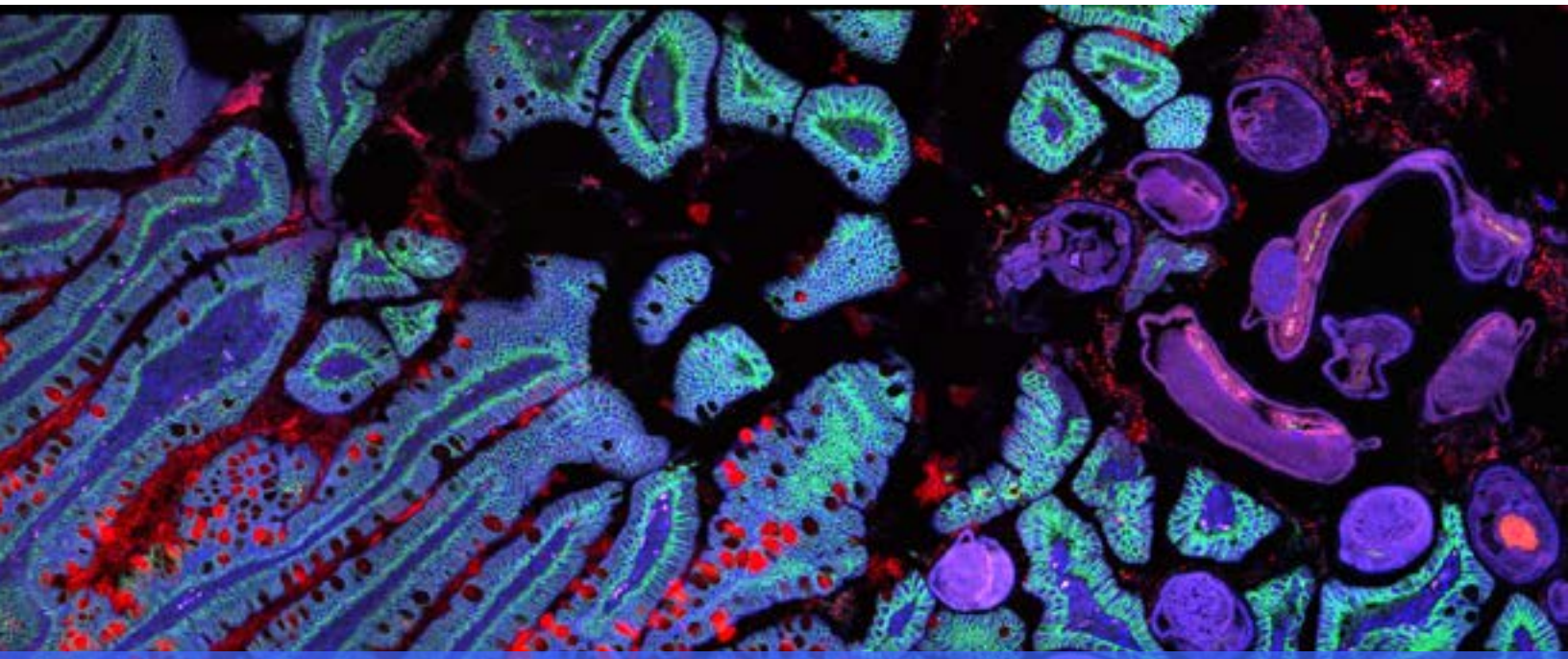
FASI | FOOD ALLERGY SCIENCE INITIATIVE

ANNUAL SYMPOSIUM



MUCOSAL IMMUNITY

FRIDAY, JUNE 3 2022

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WELCOME

Welcome to the Annual FASI Symposium – Mucosal Immunity. At FASI, our motto is collaboration, not competition. In that spirit, we are thrilled to have you here as we share ideas and advance our mission. We believe that these conversations will spark new ideas and encourage scientists from diverse fields to come together to develop more effective treatments and, one day, a cure for food allergies. The power of collaboration has already led to scientific discoveries that have significantly impacted our understanding of food allergies, including the role of interactions between food, the gut, the brain, and the nervous system, and the millions of bacteria that comprise the digestive system's microbiome.

After such a challenging year, we are grateful to be able to gather in person and virtually from around the globe. We hope you find the sessions inspiring and know our time together will lead to future breakthroughs.

Ruslan Medzhitov, Ph.D.
FASI Chief Scientific Officer
Professor of Immunobiology, Yale School of Medicine

Chris Olsen, M.D.
FASI Co-Founder and CEO

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WHY FASI

Food allergies are a worldwide public health threat with no signs of slowing down. Some 85 million Americans live with food allergies and intolerances in the U.S. alone, of which 32 million have life-threatening allergies. In addition, 10% of adults have food allergies, with nearly half developing them as an adult.

What makes the food allergy epidemic even more concerning is the dearth of knowledge about what causes them in the first place. We need solutions. Yet efforts to unravel this challenging disorder have been underfunded. Until FASI, there was no concerted effort to understand the biology underlying food allergy. This quest to understand the biology of food allergy makes FASI different from other organizations. At FASI, we believe that those suffering from life-threatening food allergies deserve better. We believe there is a cure for food allergies and that foundational research is the path to that cure.

Incubated at the Broad Institute, FASI spun out into a separate 501c(3) in early 2021. As an independent organization, FASI has assembled a collaborative group of preeminent scientific experts from some of the most widely respected institutions to increase our understanding of food allergies. Over the past five years, this effort has expanded from six to 21 labs, involving over 100 scientists. Our initial investment of $30M in foundational science and technology has transformed how we think about the development and science of food allergies.
Since its inception, FASI has achieved significant milestones critical to advancing therapeutic development. A brief list includes mapping out all the cells in the GI tract, the enteric (GI) nervous system, sensing cells in the gut involved in both nutrient and noxious detection, quality control mechanisms of food, bitter taste receptor exploration, and mapping out the areas in the brain responsible for remembering the allergen. All of this was accomplished by our team of great investigators.

FASI investigators are advancing the field, and there is much more work to be done. We look forward to working with the food allergy research community to find a cure.

To learn more about FASI or any questions you may have, please contact Chris Olsen at ChrisOlsen@foodallergyscience.org or John Walter at jwalter@foodallergyscience.org.

OUR SCIENTIFIC TEAM

Food allergies are a worldwide public health threat with no signs of slowing down. In the U.S. alone, a food-allergic adult or child is sent to the emergency room every three minutes. Some 85 million Americans live with food allergies and intolerances, of which 32 million have life-threatening allergies. What makes the food allergy epidemic even more concerning is the dearth of knowledge about what causes them in the first place. At FASI, we believe there is a cure for food allergies, and that foundational research is the path to that cure. FASI was founded on this principle. Led by renowned immunologist Ruslan Medzhitov, PhD, FASI is the first collaborative effort to create a field of study around food allergy, bringing together an exceptional team of experts, detailed below, from diverse scientific backgrounds in our quest for a cure.

"As a team of scientific investigators, we are committed to change the landscape of treatment options for those suffering from food allergies."

Ruslan Medzhitov, Ph.D.

Ruslan Medzhitov, Ph.D. is the Chief Scientific Officer at the Food Allergy Science Initiative, Sterling Professor of Immunobiology at Yale University School of Medicine, and a Howard Hughes Medical Institute investigator.

Dr. Medzhitov is probably best known for the foundational discoveries he's made concerning the roles that the immune system's Toll-like receptors play in controlling adaptive immunity. Along with these seminal findings, he has also made invaluable scientific contributions to other research areas, such as tissue biology, host-pathogen interactions, and mechanisms of inflammation.

The current research in his laboratory is focused on allergy, inflammation, infection biology, cell signaling, and transcriptional control of cell fate decisions. One of the biggest puzzles in current immunology is how and why allergens induce immune responses. The team is pioneering critical research to solve this puzzle. Dr. Medzhitov believes that allergies are part of an essential defense mechanism that protects us from noxious substances – the body's food quality control system. The major symptoms of allergic reactions – tears, sneezing, coughing – help expel unwanted agents from the body, while the excessive activity of these defenses causes allergic diseases.

Dr. Medzhitov is the recipient of many awards, including the Searle Scholarship, the William B. Coley Award from the Cancer Research Institute, the Blavatnik Award for Young Scientists, the Lurie Prize in Biomedical Sciences, the Shaw Prize in Life Science and Medicine, the Charles W. Bohmfalk Teaching Prize at the Yale University School of Medicine, and honorary doctorates from the University of Munich and Utrecht University. He is a member of the National Academy of Sciences, the National Academy of Medicine, the Connecticut Academy of Science and Engineering, and the European Molecular Biology Organization. He is also a fellow of the American Academy of Microbiology.

In addition to his scientific research, he serves on the scientific advisory boards of research institutions in Europe and several biotech companies. He is the co-author of a textbook on evolutionary medicine.

Dr. Medzhitov earned a B.S. at Tashkent State University and a Ph.D. in Biochemistry from Moscow State University. He was a postdoctoral associate in the laboratory of Charles Janeway at the Yale University School of Medicine and joined the faculty in 1999.

Isaac M. Chiu, Ph.D.

Isaac M. Chiu, Ph.D. is an associate professor in the department of immunology at Harvard Medical School. His research focuses on interactions between the nervous and immune systems at major barrier tissues, including the skin and gastrointestinal tract. He has found that nociceptive sensory neurons directly sense microbes and their molecular mediators to regulate pain. These neurons then signal to innate immune cells via neuropeptides to regulate their recruitment and activation. He is interested in defining neuro-immune signaling that drives allergic reactions, including itch and inflammation.

Dr. Chiu did his Ph.D. work in immunology and postdoctoral work in the neurobiology of pain. His lab combines interdisciplinary approaches from sensory neurobiology, immunology and microbiology to study neuro-immune crosstalk at major barrier sites including the gut and skin. The goal of Isaac Chiu's research at FASI is to uncover neuro-immune interactions and communications with the brain. His project will focus on understanding the role of DRG nociceptor neurons and neuro-immune signaling in food allergies. The aim of his research is to determine whether we can target specific DRG neurons to determine their role in allergic sensitization, determine if DRG neurons signal to immune cells and epithelial cells in the gut, and determine how cellular and molecular signals from DRG neurons contribute to food allergies.

Ivan de Araujo, D.Phil.

Ivan de Araujo, D.Phil. is a professor in the department of neuroscience at the Icahn School of Medicine at Mount Sinai. His research focuses on identifying and characterizing the largescale neural networks that link the body to the brain, with an emphasis on the gut-brain axis.

The goal of Ivan de Araujo's research with FASI is to understand allergen-sensing pathways in the gut, and how these signals access the central nervous system. His project will focus on neuronal mechanisms of altered gastric motility in food allergy. In order to investigate this, he will use neurobiological tools to map and manipulate the activity of gut innervating neurons upon arrival of allergens to the intestine. The aim is to trace the circuitry linking allergen-sensing cells to motility controlling neurons in the gut. These investigations may lead to novel gut-brain models of allergen sensing, and potential ways to peripherally switch allergic signals off.

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Marcelo O. Dietrich, M.D., Ph.D.

Marcelo O. Dietrich, M.D., Ph.D. is an Associate Professor of Comparative Medicine and of Neuroscience at Yale School of Medicine. He studies the development of homeostatic systems, focusing on neuronal circuits involved in feeding and energy balance regulation and has pioneered computational methods to quantify animal behaviors and physiological processes. In collaboration with other investigators, he also studies the participation of peripheral tissues in communications with the brain in physiological and pathological processes, including how the brain coordinates responses to allergens in the food.

Marcelo Dietrich's research at FASI focuses on food allergy and behavior by uncovering the neuro-immune mechanisms underlying gut-brain communication in allergies. The aims of this research include defining the role of immune cells and inflammatory mediators in avoidance behavior and allergic reactions, characterizing somatosensory pathways linking allergen sensing to behavioral and physiological responses, and determining the brain circuits that control parasympathetic and behavioral responses to food allergens.

Stephanie Eisenbarth, M.D., Ph.D.

Stephanie Eisenbarth, M.D., Ph.D. is Chief of Allergy and Immunology, and Director of the new Center for Human Immunobiology at Northwestern University's Feinberg School of Medicine. Her laboratory focuses on defining the cellular and molecular mechanisms that regulate antibody responses. The development of these antibodies relies on the interactions between three immune cells – dendritic cells (DCs), T cells and B cells. Her lab studies how these three cell types communicate to shape different types of antibody responses, some protective and some harmful. Using mouse models and human samples her lab has identified novel and unexpected cell subsets and functions. In the allergy field, the lab recently discovered a new T follicular helper cell that drives IgE responses to allergens. This has important implications for potentially altering and tracking the inappropriate immune response to food allergens. In parallel, her lab is working to identify how the gut mucosal immune system promotes the production of potentially protective IgA to food allergens instead of pathogenic IgE.

Esther Borges Florsheim, Ph.D.

Esther Borges Florsheim, Ph.D. is an assistant professor in the Biodesign Center for Immuno-therapy, Vaccines and Virotherapy at Arizona State University. Her research aims to understand how immunological processes affect normal physiology, with a focus on mast cell biology, neuro-immune pathways, and the impact of allergies on behavior.

Dr. Florsheim's research with FASI applies the development of novel models to study neuro-immune interactions and provide the foundation to identify mechanisms that drive allergic inflammation in the gastrointestinal mucosa. This involves a multidisciplinary approach bridging the fields of physiology, behavior, neuroscience, and immunology, and will provide insights into three major areas: mast cell biology, neuro-immune connections, and brain circuits involved in the behavioral responses of avoidance and aversion.

Elinor Karlsson, Ph.D.

Elinor Karlsson, Ph.D. is an assistant professor in bio-informatics and integrative biology at the University of Massachusetts Medical School and the director of the Vertebrate Genomics Group at the Broad Institute of MIT and Harvard. Her research looks at our own evolutionary history to understand how the human genome works, and how this knowledge can lead to advances in medicine. She uses high throughput genomic tools with the goal of identifying genes, pathways, and functional variants underlying polygenic traits, including susceptibility to infectious diseases, such as cholera and viral hemorrhagic fevers, as well as psychiatric disorders.

Vijay Kuchroo, D.V.M., Ph.D.

Vijay Kuchroo, D.V.M., Ph.D. is a member of the Broad Institute, the Samuel L. Wasserstrom professor of neurology at Harvard Medical School, co-director of the Center for Infection and Immunity at the Brigham Research Institutes, and director of the Evergrande Center for Immunologic Diseases at Harvard Medical School and Brigham and Women's Hospital. His major research interests include studies on autoimmunity and anti-tumor immunity. He first described the TIM family of genes and was co-discoverer of highly pathogenic IL-17 producing Th17 cells.

Vijay Kuchroo's research at FASI focuses on Innate Lymphoid Cells-2 and neuropeptide interaction in induction and regulation of food allergies. The aim of his research is to determine the mechanisms by which NMU/NMUR1 interactions convert homeostatic ILCs into proinflammatory ILCs and study how CGRP regulates ILC2 function and development of food allergies.

Stephen D. Liberles, Ph.D.

Stephen D. Liberles, Ph.D. is a professor in the cell biology department at Harvard Medical School and a Howard Hughes Medical Institute investigator. His research focuses on the molecular neuroscience of sensory systems, including olfaction, pheromone-sensing, taste, and internal organ senses of the vagus nerve. His work has significantly advanced the understanding of vagus nerve cell types that control autonomic physiology, identified non-classical families of olfactory receptors, and revealed molecular mechanisms underlying sensation within internal organs.

The goal of Stephen Liberles's research is to understand allergen-sensing pathways in the gut by investigating the role of enteric neurons and the gut-brain axis in allergic disease. The aims of this research are to develop a genetic toolbox for study of enteroendocrine cells and control enteroendocrine cells through chemical genetics.

Christopher Love, Ph.D.

Christopher Love, Ph.D. is an associate member of the Broad Institute and an associate member of the Ragon Institute of MGH, MIT, and Harvard. The Love Laboratory at MIT aims to advance science and technologies to reach patients on a global scale. Some successes of his lab include; single-cell analysis, metastatic cancer sequencing, and on-demand biomanufacturing. His research interests include; micro/ nanofabrication and surface chemistries, cellular immunology and infectious diseases, immunotherapy/ vaccines, and clinical diagnostics.

Christopher Love's Laboratory at MIT is involved in research with FASI. Their research with FASI focuses on single-cell profiling of T cells from peanut-allergic patients and EoE subjects. Their research has found that there are distinct clonally related subsets of cells with food allergy-associated T cell phenotypes. They have observed that gene expression modules associated with general T cell activation are more associated with clinical responses to OIT than specific subsets of T cells or their modulation during treatment. They have also found by pathway analysis that the Th2 cells in EoE have an upregulation of genes related to eicosanoid synthesis, suggesting that production of the eosinophil chemoattractant prostaglandin-D2 is a feature of Th2 cells in EoE. Gene signatures in both mast cells and eosinophils also suggest a positive feedback loop among these cell types that maintains the proinflammatory environment. The five-year objectives of this laboratory include two aims; characterization of peanut-reactive T cells, and cellular network mapping in EoE.

Daniel Mucida, Ph.D.

Daniel Mucida, Ph.D. is a professor of immunology, vir-ology, and microbiology at the Rockefeller University, and a Howard Hughes Medical Institute Investigator. His lab focuses on the characterization of tolerance and resistance pathways in the intestinal tissue and on the consequences when these processes are compromised. He and his team hypothesize that distinct signals within niches of the intestinal tissue dictate the pro- versus anti-inflammatory function of immune cells.

Daniel Mucida's research with FASI focuses on visualizing immune-tissue cell interactions in the gut. The aims of his research are to define immune-tissue cell interactions in the intestinal epithelium during allergic responses, elucidate neuro-immune interactions in the intestinal tissue, and characterize the modulation of local systemic immunity towards luminal antigens.

Hans C. Oettgen, M.D., Ph.D.

Hans C. Oettgen, M.D., Ph.D. is a professor of pediatrics at Harvard Medical School and is the associate chief in the Division of Immunology at Boston Children's Hospital. The Oettgen laboratory focuses on IgE, the antibody that mediates allergic reactions, and its influence on mast cells. These are the cells that produce chemicals involved in the inflammatory response. His team's research focuses on the effects of IgE growth and function of mast cells in the intestine. They are also examining how IgE and mast cells regulate immune sensitization in the intestine in the setting of food allergy.

Hans Oettgen's research with FASI suggests that IgG and FcγR2b act to counter the Th2 adjuvant function of IgE and mast cells and that strategies that harness this effect might benefit food-allergic patients. For their Year 5 FASI work Hans's laboratory is looking into whether the protective effect of IgG antibodies in vivo,

suppressing Th2 and enhancing Treg responses, is mediated by their negative signals, delivered via FcγR2b specifically in mast cells.

José Ordovás-Montañés, Ph.D.

José Ordovás-Montañés, Ph.D. is a research faculty member at Boston Children's Hospital, an assistant professor at HMS, and principal investigator at HSCI. He is a New York Stem Cell Foundation Robertson Investigator. His research focuses on how inflammation drives memory formation in barrier tissues, in order to program and re-program them in disease. He is particularly interested in identifying and testing human disease-relevant cell states.

The Ordovás-Montañés laboratory is committed to applying single-cell approaches to help understand the cellular memory networks which promote food allergy. Their research with FASI focuses on applying their tools, such as single-cell RNA-sequencing, to measure sensitization and challenge within barrier tissues at high cellular resolution. The goal of their FASI project is to catalyze food allergy research in their emergent laboratory and collaborate with FASI colleagues to address ongoing challenges regarding the understanding of how food allergies collectively impact the cell types and subsets of the intestine in pre-clinical models and human studies. This project will provide a critical resource for the FASI community, with data shared rapidly amongst colleagues.

Sarita Patil, M.D.

Sarita Patil, M.D. is an assistant professor at Harvard Medical School and an assistant in medicine at Massachusetts General Hospital. She is a physician-scientist and member of the MGH Food Allergy Center specializing in the treatment of patients with food allergies. Her laboratory, in the Center for Immunology and Inflammatory Diseases, focuses on understanding antibody and B cell responses in both the initiation and treatment of allergic diseases, with a particular focus on food allergies. Her interests include; food allergies, eosinophilic esophagitis, eosinophilic gastrointestinal disease, and chronic hives.

Sarita Patil's research with FASI focuses on understanding how peanut-specific IgG antibodies develop and contribute to clinical protection. Her laboratory pioneered the application of allergen specific florescent multimers to affinity select allergen-specific B cells in peanut allergic individuals. Using these techniques, she will identify precursors of the Ara h 2 specific BCR repertoires that correlate with tolerance in OIT and with peanut sensitization compared to allergy to study the evolution of allergen-specific antibodies in peanut allergy. She will also use these methods to evaluate the contribution of allergen-specific B cells to the antibody repertoire in peanut allergy. Using these tools, her research will inform and influence the development of next-generation therapeutics for food allergies.

Seth Rakoff-Nahoum, M.D., Ph.D.

Seth Rakoff-Nahoum, M.D., Ph.D. is an assistant professor of pediatrics at Harvard Medical School and an associate physician in pediatrics in the Division of Infectious Diseases at Boston Children's Hospital.

Seth Rakoff-Nahoum's research with FASI focuses on pediatric gastroenterology and the biological mechanisms involved in regulation in small intestinal protein transfer. Evidence suggests that protein transfer is not a passive process, and therefore may occur for a specific physiological purpose. In order to investigate this Nahoum's laboratory will develop model systems that would allow them to look at the mechanisms of transfer and to study its regulation. His laboratory will examine the fate of transferred dietary proteins. They will explore the properties of neonatal small intestine that enable more efficient protein transfer and because efficiency of transfer is downregulated with age, they will examine which factors control this process. Lastly, they will examine what factors (endogenous or exogenous) determine the outcome and how the outcome can be manipulated.

Wayne Shreffler, M.D., Ph.D.

Wayne Shreffler, M.D., Ph.D. is the director of the Food Allergy Center at Massachusetts General Hospital, the division chief of pediatric allergy and immunology at Mass General Hospital of Children, a principal investigator at the Center for Immunology and Inflammatory Diseases, and an associate professor of pediatrics at Harvard Medical School.

His research and multi-disciplinary clinical program focus on characterization of the immune response to food allergens and the early stages of allergen-induced immune reaction. In collaboration with Christopher Love at MIT, he conducts research for FASI focused on single cell profiling of T cells from peanut-allergic patients and individuals suffering from the allergic disease eosinophilic esophagitis.

Caroline Sokol, M.D., Ph.D.

Caroline Sokol, M.D., Ph.D. is an assistant professor of medicine at Harvard Medical School and a physician in the Allergy and Clinical Immunology Unit at Massachusetts General Hospital. Her laboratory investigates how allergens are recognized by the innate immune system, the role of allergens in acute and chronic itch, and how innate and adaptive immune interactions lead to the initiation of the allergic immune response. They use mouse models and human samples to track and define the interactions between immune cells, sensory neurons and allergens. Combining techniques from neurobiology and cellular immunology, they have defined novel neuroimmune pathways required for immune responses to allergens.

Caroline Sokol's research with FASI focuses on the role of cutaneous neuroimmune interactions in allergen-induced itch and the initiation of allergic immune responses to cutaneous allergens. In their first project they are defining the innate-like T cells that control sensory neuron activation by allergens, i.e., the itch response, as well as the mechanism by which this occurs. In their second project they are elucidating how sensory neurons develop memories of prior allergen exposure, and how this leads to changes in the immune response to subsequent allergen exposure.

Andrew Wang, M.D., Ph.D., A.B.

Andrew Wang, M.D., Ph.D., A.B. is an assistant professor in the Departments of Internal Medicine and Immunobiology at Yale School of Medicine. His lab is interested in understanding how the env-ironment impacts inflammatory disease expression to discover pathways that can be therapeutically targeted to treat diseases ranging from sepsis, allergy and autoimmunity to psychiatric diseases like depression. They use mouse models, cell culture, and human samples and apply techniques spanning the disciplines of metabolism, immunobiology, and behavioral biology.

Andrew Wang's laboratories research with FASI focuses on understanding how xenobiotics act as allergenic adjuvants. His laboratory hypothesizes that modern xenobiotics may be acting as adjuvants sufficient for allergic sensitization. The specific aims of this research include identifying the mechanism by which xenobiotics act as allergic adjuvants and performing a retrospective single-center case-control study in children.

Jing-Ke Weng, Ph.D.

Jing-Ke Weng, Ph.D. is an associate professor of biology at MIT and a member of the Whitehead Institute for Bio-medical Research. His research aims to probe the origin and evolution of plant metabolism, as well as how plants exploit discrete small molecules to interact with their surrounding environments.

Dr. Weng also uses plant natural products with specific medicinal properties as chemical probes to query human disorders like metabolic syndromes and protein-misfolding diseases. Weng is particularly interested in elucidating the molecular bases of traditional herbal medicines and developing nature-inspired new medicines to fight various human diseases. Dr. Weng's work at FASI focuses on elucidating the molecular mechanisms by which certain chemicals present in food are detected by the human gastrointestinal tract to modulate metabolism and/or elicit different immune responses.

Duane Wesemann, M.D., Ph.D.

Duane Wesemann, M.D., Ph.D. is an associate professor of medicine at Harvard Medical School. His laboratory uses mouse genetics, human studies, cellular biology, single cell transcript-omics, and computation to elucidate underlying features and elasticity of antibody recognition capacity. They study the dynamic regulation, functional significance, and evolutionary implications/ origins of the anticipatory naïve antibody repertoire and its somatically evolving counterpart in germinal centers. Findings from these projects have paradigm-shifting potential for fundamental immunology and are relevant to infectious disease, vaccinology, allergy, and autoimmunity.

Duane Wesemann's research with FASI focuses on understanding how IgE is regulated. His laboratory generated mouse models, which showed that IgE is likely manufactured in large part at or near sites where CSR to IgE occurs – thus linking IgE production to stimulus locale. They are examining hypotheses in three projects. First, determining the mechanisms of IgE expression dynamics on IgE B cell function, second, elucidating mechanisms underlying IgE distribution from point of origin to effector sites, and third identifying the distribution of IgE-expressing cells in bone marrow plasma cell pools.

Ramnik Xavier, M.D., Ph.D.

Ramnik Xavier, M.D., Ph.D. a core institute member of the Broad Institute of MIT and Harvard, Kurt Isselbacher professor of medicine at Harvard Medical School, Co-Director of MIT's Center for Microbiome Informatics and Therapeutics, the director of MGH's Center for Computational and Integrative Biology, and the director of the Klarman Cell Observatory at the Broad Institute. As a clinical gastroenterologist and molecular biologist, he studies the molecular mechanisms involved in innate and adaptive immunity as well as the genetic variants associated with IBD and autoimmunity. His lab has led landmark studies characterizing microbiome composition in health and disease.

Ramnik Xavier's research with FASI also explores the role of the enteric nervous system of the small bowel in allergic disease. The aims of this research include generating an ENS cellular map of the small intestine. To this end, he has co-developed new experimental technologies to facilitate dissection of ENS function and characterize the ENS at a molecular level. His work generating reference maps of the colon enteric nervous system of adult mice and humans at single-cell resolution serves as a foundational reference for GI biologists, allergists and immunologists.

Xavier completed his residency in internal medicine and fellowship in gastroenterology at Massachusetts General Hospital and was chief of Gastroenterology at MGH.

AGENDA

8:00 – 8:45 AM	**Registration & Breakfast**
8:45 – 9:00 AM	**Opening Remarks** Ruslan Medzhitov, PhD, Yale School of Medicine
9:00 – 10:30 AM	**Session 1: Diet, Microbiome, and Immunity**
9:00 – 9:30 AM	**Microbiome Control of Host Immunity** Yasmine Belkaid, PhD, National Institutes of Health
9:30 – 10:00 AM	**Maternal Regulation of Neonatal Food Tolerance** Michiko Oyoshi, PhD, Massachusetts General Hospital
10:00 – 10:30 AM	**Microbiota-directed Complementary Foods for Treating Childhood Undernutrition** Jeffrey Gordon, MD, Washington University School of Medicine
10:30 – 10:45 AM	**Break**
10:45 – 12:15 PM	**Session 2: Gut Mucosa Biology**
10:45 – 11:15 AM	**Immune Crosstalk in the Upper Digestive System Through Shared Lymph Node Drainage** Daria Esterhazy, PhD, University of Chicago
11:15 – 11:45 AM	**The Evolution of Mucosal Immune Systems in Vertebrates** Irene Salinas, PhD, University of New Mexico
11:45 – 12:15 PM	**A Repertoire of Goblet Cells Contribute to Form a Functional and Protective Mucus Layer** Malin Johansson, PhD, University of Gothenburg
12:15 – 1:15 PM	**Lunch**

1:15 – 2:30 PM	**Session 3: FASI Research Spotlight**
1:15 – 1:30 PM	**T Cell Control of Neuronal Allergen Sensing** Cameron Flayer, PhD, Harvard Medical School
1:30 – 1:45 PM	**Enteric Neurons in Type 2 Inflammation and Host Defense** Rocky Barilla, BS, Harvard Medical School
1:45 – 2:00 PM	**A Nociceptor-Goblet Cell Communication Axis Promotes Gut Barrier Protection** Daping Yang, PhD, Harvard Medical School
2:00 – 2:15 PM	**NRF2 Activators are Type II Adjuvants** Anna Eisenstein, MD PhD, Yale School of Medicine
2:15 – 2:30 PM	**Break**
2:30 – 4:00 PM	**Session 4: Type 2 Immune Responses**
2:30 – 3:00 PM	**Immune Basis of Food Protein-Induced Enterocolitis Syndrome (FPIES)** Cecilia Berin, PhD, Icahn School of Medicine at Mount Sinai
3:00 – 3:30 PM	**Perforin 2 Pokes Its Way into Type 2 Immunity** De'Broski Herbert, PhD, University of Pennsylvania
3:30 – 4:00 PM	**Myeloid Cell Activation During Type 2 Immunity** William Gause, PhD, Rutgers University
4:00 – 4:15 PM	**Closing Remarks** Ruslan Medzhitov, PhD, Yale School of Medicine
4:30 – 6:00 PM	**Poster Session & Reception**

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SPEAKERS

Rocky Barilla, BS
PhD Student, Harvard Medical School

Yasmine Belkaid, Ph.D.
Director NIAID Microbiome Program; Co-Director NIH Center for Human Immunology Chief Metaorganism Immunology Section; Chief Laboratory of Host Immunity and Microbiome Laboratory; NIAID, NIH

Cecilia Berin, Ph.D.
Professor of Pediatrics and Deputy Director of the Jaffe Food Allergy Institute, Icahn School of Medicine at Mount Sinai

Anna Eisenstein, M.D., Ph.D.
Postdoctoral Research Fellow, Instructor of Dermatology, Yale School of Medicine

Daria Esterhazy, Ph.D.
Assistant Professor of Pathology, University of Chicago

Cameron Flayer, Ph.D.
Postdoctoral Research Fellow, Harvard Medical School

William Gause, Ph.D.
Director, RBHS Institute for Infectious and Inflammatory Diseases (i3D), Rutgers University

Jeffrey Gordon, M.D.
Dr. Robert J. Glaser Distinguished University Professor; Director, The Edison Family Center for Genome Sciences & Systems Biology, Washington University School of Medicine

De'Broski R. Herbert Ph.D.
Presidential Associate Professor of Immunology, University of Pennsylvania School of Veterinary Medicine

Malin Johansson, Ph.D.
Professor, Dept. Medical Biochemistry and Cell Biology, Inst. Biomedicine, University of Gothenburg

Michiko Oyoshi, Ph.D.
Assistant Professor, Massachusetts General Hospital

Irene Salinas, Ph.D.
Associate Professor, Department of Biology, University of New Mexico

Daping Yang, PhD
Postdoctoral Research Fellow, Harvard Medical School

ABSTRACTS

1. Tissue remodeling by an opportunistic pathogen triggers allergic inflammation

Karen Agaronyan[1], Lokesh Sharma[2], Bharat Vaidyanathan[3,5], Keith Glenn[2], Shuang Yu[3], Charles Annicelli[3], Talia D. Wiggen[4], Mitchell R. Penningroth[4], Ryan C. Hunter[4], Charles S. Dela Cruz[2], Ruslan Medzhitov[1,6]

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Abstract
Different effector arms of the immune system are optimized to protect from different classes of pathogens. Defense from bacterial infections requires the type-1 immune system, whereas defense from parasitic worms relies on the type-2 immune system which also causes allergic responses. In some cases, pathogens manipulate the host immune system to promote the wrong type of effector response – a phenomenon known as immune deviation. Typically, immune deviation helps pathogens to avoid destructive immune responses. Here we report on a type of immune deviation whereby an opportunistic pathogen, *Pseudomonas aeruginosa*, induces the type-2 immune response resulting in mucin production that is used as an energy source by the pathogen. To demonstrate that, we used animal *in vitro* lung organ cultures and *in vivo* lung infection and allergy inflammation models, as well as human sputum samples and microbiome datasets. Specifically, we found that *P. aeruginosa*-secreted toxin, LasB, processed and activated epithelial amphiregulin to induce type-2 inflammation and mucin production. This 'niche remodeling' by *P. aeruginosa* promoted colonization, and as a by-product, allergic sensitization. Our study thus reveals a type of bacterial immune deviation that promotes infection by increasing nutrient supply. It also uncovers a mechanism of allergic sensitization by a bacterial virulence factor.

Funding
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2. A Mast Cell-Epithelial Axis in Allergen Avoidance

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Abstract
Allergic sensitization constitutes a defense against environmental toxins, which includes the development of specific food aversions. Using a two-bottle preference test paradigm after allergic sensitization, food allergy models, and transgenic mice, we investigated the immunological underpinnings of food allergen avoidance behavior. We found allergic sensitization elicits robust avoidance behavior in mice, the magnitude of which varies between background strains and correlates with IgE titers and intestinal mast-cell numbers. Using knockout mice and genetically targeted cell-ablation approaches, we defined a critical role for IgE and FceR1 expressing cells in this behavior. We found intestinal mast cell express high levels of aLOX5, the rate-limiting enzyme in leukotriene synthesis, and that intestinal aLOX5 transcripts are elevated in food allergy models in a manner largely dependent on IgE and FceR1 expressing cells. Acute pharmacological inhibition of aLOX5, but not 5HT3, PAR2, or NK1R, reduced aversive behavior in allergic mice. Leukotrienes are known to act on nociceptors, resulting in pain and itch. Unexpectedly, we found no effect of TRPV1+ neuron ablation by resiniferatoxin on this aversion, suggesting other pathways are involved. GDF15 is a stress-induced cytokine that acts on the area postrema to mediate nausea-like behavior. We found GDF15 is induced in experimental food allergy in an IgE, FceR1 expressing cell and aLOX5 dependent fashion. Using qPCR and FISH, we found food allergen induced GDF15 is primarily produced by colonic epithelial cells flanking the crypt, many of which formed direct contact with mast cells. Acute pharmacologic blockade of GDF15 using a neutralizing antibody reduced late-phase allergen aversion. Thus, we find an unexpected role of mast cell-epithelial interactions in driving aversive behavior to food allergens and suggest mast-cell derived leukotrienes may be an important mediator of these interactions.

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3. Plant Chemistry in Allergy and Oral Tolerance

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Abstract
The components of food essential for allergy or oral tolerance initiation are incompletely understood. Importantly, while T cell maturation relies on antigen recognition, some evidence suggests that antigens are not sufficiently immunogenic to initiate allergy independently. Our research aims to identify the contribution of food matrix components (i.e., proteins, lipids, small molecules co-encountered with dietary antigens) to the development of immune responses. We plan to expose mice to antigens in different matrix contexts through co-formulation or generation of transgenic antigen-expressing plants. As an alternative antigen-containing matrix, Golden Nugget and MicroTom tomatoes were engineered to express selected antigens (e.g., ovalbumin [OVA], Ara h 1) through agrobacterium-mediated transformation. Comparing immune responses to antigens expressed in an engineered matrix, or other co-formulated matrices, to the native matrix (e.g., Ara h 1 expressing tomato vs Ara h 1 mixed with soybean vs peanut) will allow us to determine the degree of intrinsic allergenicity and contribution of molecular context. Our allergy models will use oral and skin exposure routes and monitor antibody formation and body temperature drop as allergy indicators. For tolerance measurements, we will leverage adoptive transfer models of OVA-specific T cells that enable cell-level monitoring of T cell phenotype (e.g., Foxp3 induction). Parallel efforts aim to identify antigens that underlie tolerance to diet.

Funding
ONO Pharmaceutical Company

4. High-Fat Diet Causes Rapid Loss of Intestinal Group 3 Innate Lymphoid Cells Through Microbiota-Driven Inflammation

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Abstract
Innate lymphoid cells (ILCs) are increasingly appreciated to play a critical role in tissue homeostasis, immunity, and tolerance. At steady state, intestinal ILC3 are the main source of IL-22, ensuring epithelial barrier integrity, containing the microbiota, and protecting against pathogenic bacteria. In addition, ILC3 support intestinal tolerance to commensal bacteria and dietary antigens. ILC3 can also sense changes in dietary nutrients, such as vitamin A and aryl hydrocarbon receptor ligands (AHR-L) that affect their development, function and ultimately intestinal health. ILC3 have been described to be able to acquire high amounts of extracellular fat but the effect of dietary fat on ILC3 homeostasis is unknown. Here we show that ILC3 are severely depleted from the small and large intestine of obese mice fed high-fat diet (HFD) but not in leptin deficient obese mice. Notably, consumption of HFD for only 24 hours is sufficient to trigger ILC3 cell death. Total loss of ILC3 is reached after one week of HFD without significant weight gain or impaired glucose tolerance. However, we found that this short-term consumption of HFD increases intestinal permeability and host susceptibility to *Citrobacter rodentium* infection. Unexpectedly, we found that ILC3 were maintained in germ-free (GF) mice fed HFD. However, ILC3 were depleted when HFD-fed GF mice were inoculated with either living, heat-killed bacteria or with lipopolysaccharides, which associated with intestinal permeability and inflammation. Gene expression profiling of ILC3 from short term HFD-fed mice revealed that LPS phenocopied the differential effect of microbiota on dampening peroxisome proliferator-activated receptor (PPAR) signaling, and activating TNFα target genes involved in cell activation, exhaustion, oxidative stress and lipotoxicity. In vitro, lipid laden ILC3 are susceptible to TNFα induced cytotoxicity in a dose dependent manner. In vivo, TNFα blockade, the use of antioxidant, or TLR4 deficiency was sufficient to protect ILC3 from HFD-induced lipotoxicity. Specifically, restricted depletion of TLR4 on Cx3cr1^{+} mononuclear phagocytes curtailed their HFD-induced early expansion, TNFα production and protected from ILC3 loss. Collectively, our findings reveal differential regulation of ILC3 homeostasis by the crosstalk of dietary fat with microbiota-mediated inflammation, ranging from activation to cell death, preventing early repair of intestinal permeability, and increasing susceptibility to opportunistic pathogens independently of obesity and metabolic syndrome.

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5. Intravital Imaging of Enteric Neurons

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Abstract

Background: The activity pattern of enteric neurons determines how the gastrointestinal tract responds to external stimuli, specifically whether digestive (pro-motility, secretory) or defensive (paretic) responses should be initiated. Enteric neurons are therefore critical for altering motility and glandular secretion upon detection of food allergens in gut. However, observing enteric neuron activity in living organisms is currently unavailable.

Methods: We are developing new methodologies to monitor the activity of enteric neurons *in vivo* during physiological conditions. We take advantage of mouse models to cell-specifically express genetically encoded calcium indicators (GCamp) in select populations of gut neurons. *Cre*-inducible GCamp expression is achieved either via genetic crossings or site-specific injections of viral vectors. A glass window is implanted over the tissue of interest, which is fixed onto the window. In lightly anesthetized animals, a confocal or 2-photon microscope lens is placed over the window for *in vivo* monitoring of calcium transients from genetically defined enteric neurons.

Results: Interim data reveal that enteric neurons respond to physiological stimuli *in vivo* with specific activity patterns. We are focusing on enteric neurons that control motility and/or secretory patterns in stomach and intestine in response to the release of gut peptides. We found that nitric oxide (Nos+) gastric neurons robustly and specifically respond to rises in ileal GLP-1 levels, in agreement with the finding that cell-specific activation of Nos+ neurons is sufficient to cause gastroparesis. We are currently determining whether food allergens cause secretion of gut peptides that control motility, and which enteric populations might specifically be involved in allergen responses.

Conclusions: Monitoring enteric activity *in vivo* may identify which population(s) of gut neurons are specifically involved in allergen-induced alterations in gut transit and glandular secretion, thereby revealing novel potential drug targets.

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6. NRF2 Activators Are Type 2 Adjuvants

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Abstract

The incidence of food allergies is rising at a rate that cannot be explained by genetic factors alone, implicating an environmental factor as a cause for the development of allergies. We hypothesized that commonly used environmental xenobiotics, such as over-the-counter medications and food preservatives, that are now more prevalent in our everyday lives, may account for this rise in allergic disease by acting as type 2 adjuvants. To test this, we sensitized mice to ovalbumin by concurrent administration of commonly used medications and food preservatives. In an anaphylaxis model, we found that sensitization with ovalbumin and certain nonsteroidal anti-inflammatory drugs or food preservatives led to similar levels of Ova-specific IgE and IgG1 as compared to mice sensitized with ovalbumin and alum. Strikingly, the mice sensitized with these xenobiotics also underwent anaphylaxis physiology upon challenge with allergen. Moreover, we found that these chemicals were all able to activate the xenobiotic receptor, Nuclear factor-erythroid factor 2-related factor 2 (Nrf2) and that traditional Nrf2 activators could also act as a type 2 adjuvant in allergic sensitization. This research suggests that Nrf2 activation may be one common mechanism of action of allergic adjuvants and implicates the increased use of certain manmade drugs and chemicals in the rising prevalence of food allergies with broad implications for clinical practice and public policy.

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7. To Each Their Own: Distinct Pathways to Type 2 Immunity for Disparate Allergen Classes

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Abstract
Unlike many pathogens that are detected directly by the innate immune system, protease allergens such as papain are initially detected by TRPV1$^+$ sensory neurons in the skin. TRPV1$^+$ neurons directly detect protease allergens and release substance P, which induce the migration of CD301b$^+$ dendritic cells (DCs) into the draining lymph node (dLN) to initiate T-helper 2 (Th2) cell differentiation. However, this mode of detection may not be universal as allergens are highly heterogeneous. We found that bee venom allergens such as phospholipase A2 (bvPLA2) activate neurons to specifically release CGRP instead of substance P. To elucidate the role of neuronal sensing in the allergic sensitization to venoms, we administered purified bvPLA2 intradermally in mice to study the DC and CD4$^+$ T cell responses in the dLNs. Although CGRP had no effect on DC migration, bvPLA2 induced the activation and migration of the Th2-skewing CD301b$^+$ dermal cDC2 subset similarly to that seen in response to the substance P-releasing allergen, papain. However, bvPLA2 immunization led to poor antigen uptake by the CD301b$^+$ DCs. This corresponded to decreased CD4$^+$ T cell activation and Th2 differentiation in comparison to papain immunization, despite equal numbers of activated CD301b$^+$ DCs in the dLN. To determine whether such defects in DC antigen uptake and CD4$^+$ T cell activation were mediated by allergen-induced neuronal signaling or neuropeptide release, we examined the response to bvPLA2 in mice depleted of TRPV1$^+$ neurons. Surprisingly, TRPV1$^+$ neuronal depletion rendered mice highly susceptible to death after a non-lethal dose of bvPLA2 injection. We conclude that (1) bvPLA2 immunization leads to robust DC activation and migration but defective antigen uptake; (2) said defect may be responsible for impaired antigen presentation and Th2-skewing of naïve CD4 T cells; and (3) TRPV1$^+$ neurons play a protective role against the toxicity of bvPLA2.

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8. A γδ T Cell-IL-3 Axis Controls the Responsiveness of Sensory Neurons to Protease Allergens

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Abstract
Allergens activate sensory neurons, causing the sensation of itch and promoting neuropeptide release to initiate the allergic immune response. Yet, given similar levels of environmental allergen exposure, it is unclear what defines individual heterogeneity in the development of allergic itch and immune responses. In the setting of atopic dermatitis (AD), an allergic disease of the skin, Th2 cells producing IL-4 and IL-13 enhance itch by priming sensory neurons, lowering their threshold for activation by pruritogens. We hypothesized that in the allergen-naïve state, an innate immune cell sets the “allergen activation threshold” in sensory neurons, priming neuro-immune reactivity upon the first exposure to an allergen. We found that mice lacking gd T cells (*Tcrd*$^{-/-}$) exhibited defects in protease allergen-induced itch derived from diverse sources. Flow cytometry and bulk and single-cell transcriptomic analysis revealed that mouse skin is home to three subsets of gd T cells: dermal gd T cells, Vg5$^+$ dendritic epidermal gd T cells (DETCs), and a novel *Skint1*-independent subset that we termed Vg5$^-$ epidermal gd T cells. Supernatant from aCD3/CD28-stimulated Vg5$^-$ epidermal gd T cells was sufficient to enhance allergic itch in wild-type mice, indicating that this subset released a factor responsible for sensory neuronal responsiveness to allergens. Vg5$^-$ epidermal gd T cells uniquely produced IL-3, which was required for allergic itch and restored itch in *Tcrd*$^{-/-}$ mice. Ratiometric calcium imaging showed that IL-3 induced the influx of Ca^{2+} into dorsal root ganglia neurons and primed their responsiveness to allergen. Furthermore, *Tcrd*$^{-/-}$ mice showed a defect in allergen-induced dendritic cell migration and Th2 differentiation, linking deficient itch to impaired allergic immune responses. We propose that Vg5$^-$ epidermal gd T cells are an endogenous immune rheostat that tunes the responsiveness of sensory neurons to protease allergens. Targeting the gd T cell-IL-3 axis may be a novel strategy to treat AD and other itch conditions.

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9. From Skin to Gut: How Distant Barrier Disruptions Shape Local Immune Responses

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Abstract
Clinical data and mouse models have suggested a connection between skin barrier disruption, allergen exposure at the skin, and the development of allergic sensitization in the intestine. Mouse studies specifically have shown that tape-stripping and allergen exposure at the skin lead to elevated immune cell frequencies in the small intestine alongside increases in systemic IgE levels. Such studies, however, have only explored cell types and genes previously associated with allergy development. To uncover additional cell types, subsets, and states that may be involved in the spread of allergic sensitization from skin to gut, we conducted single-cell RNA-sequencing on samples of murine small intestine. Mice were either unmanipulated, tape-stripped and exposed to saline at the skin, or tape-stripped and exposed to ovalbumin at the skin, and successful sensitization was confirmed via serum IgE measurements. On day nineteen, we dissociated the jejunums of the mice and prepared sequencing libraries. We used computational methods to reduce background RNA levels and demultiplex samples. In total, 64,498 cells from nine mice were analyzed – three mice per group, and two tiers of clustering were performed using Seurat. The first tier demonstrated recovery of a range of immune, epithelial, and stromal cell types; the second tier scrutinized B, plasma, T, epithelial, and myeloid cells at higher resolution. Our data suggest that the connection between skin barrier disruption, allergen exposure, and gut sensitization may involve changes in enterocyte, B cell, plasma cell, and plasmacytoid dendritic cell frequencies, immune cell gut-homing, and antibody production in the intestine. Future experiments will utilize flow cytometry and histology to confirm our findings and block gut-homing to evaluate its impact on the spread of allergic sensitization. Further understanding how skin barrier disruption enables gut allergic sensitization could inform novel methods of food allergy prevention that strategically protect barrier tissues.

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10. Induction of Natural IgE by Glucocorticoids

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Abstract
Allergy is one of the most prevalent chronic diseases, affecting hundreds of millions of people worldwide. In allergy, environmental allergens such as pollen or peanut induce B cells to undergo class switch recombination and produce Immunoglobulin E (IgE) antibodies. IgE is a key molecule that mediates allergic responses by coating mast cell surfaces and inducing mast cell degranulation upon binding a specific allergen. IgE can also be spontaneously produced in the absence of exogenous allergens, yet the biogenesis and functions of such "natural" IgE still remains largely unknown. Here, we discovered that glucocorticoids, steroid stress hormones, enhance IgE isotype class switching in B cells both *in vivo* and *in vitro*. This IgE class switching is directly promoted by B cell-intrinsic glucocorticoid receptor signaling that synergizes with the IL-4/STAT6 pathway. In addition, we found that rare B cells residing in the mesenteric lymph nodes are responsible for the production of glucocorticoid-inducible IgE. Furthermore, we showed that locally produced glucocorticoids in the gut may induce IgE during gut perturbations like dysbiosis. Notably, mice preemptively treated with glucocorticoids were protected from subsequent IgE-mediated pathogenic anaphylaxis in vivo. Thus, our results suggest that glucocorticoids, classically considered to be immunosuppressive, hold an immunostimulatory role in B cells instead, which could be a new therapeutic target to alleviate allergic responses.

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11. Adaptations in the Intestinal Immune System to Activation of the Gut-Innervating Neurons

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Abstract
The gastrointestinal tract (GI) is overseen and controlled by both the immune system and the nervous system. Recent advances in understanding the role of the interplay between both systems have uncovered a pivotal role of the nervous system in modulating immune responses at homeostasis and during inflammation. The goal of this proposal is to identify how different neuron types modulate activity of gut immune cells. To address this, we exploited a chemogenetic approach to activate specific neuron cell subtypes. Artificial DREADD (Designer Receptors Exclusively Activated by Designer Drugs) proteins were delivered with a neuron-specific AAV vector, which allows Cre recombinase dependent expression in defined neurons (sensory, inhibitory/excitatory enteric neurons, etc.) in a panel of eight Cre-driven mice. The DREADD can then be activated by administration of a specific synthetic ligand (CNO). The effects of this controlled neuronal activities on immunocytes will be evaluated by immunophenotyping (cytometry) and transcriptome analysis (bulk and single-cell RNAseq). Data from the above Cre lines have been collected. These methods produce robust labeling of neuronal populations in the enteric nervous system, dorsal root ganglia, and the nodose/jugular vagal ganglia. Preliminary data suggest different subtypes of neurons can differentially regulate the gut immune profile. For example, inhibitory Nos1+ neuronal activation increased the percentage of Rorg+ conventional CD4+ T cells in the ileum. In contrast, excitatory ChAT+ activation caused a decrease of neutrophils in the ileum. The preliminary data from this study suggests that different subtypes of neurons can uniquely modulate immune cell populations in the ileum, cecum, and colon. Future work will determine the mechanisms behind the neuronal modulation of immune cells.

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12. Regulatory T Cells in Intestinal Homeostasis and Allergic Sensitization

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Abstract
Tolerance to food antigen is maintained by regulatory T cells (Tregs) in the small intestine; however, the mechanisms by which Tregs induce a state of tolerance and prevent food allergy are incompletely understood. To better understand the cells specifically responsible for maintaining tolerance, we deleted IRF4 in Tregs using Irf4 fl/fl Foxp3 Cre+/Y mice, resulting in impaired Treg function at mucosal sites. These mice showed marked baseline small intestine pathology and impaired oral tolerance to novel food antigen. To better understand the role of IRF4+ Tregs in the small intestine, we conducted RNA sequencing and identified Wnt4 as a unique marker of small intestine Tregs and a promising mediator of Treg tolerogenic function. Notably, Wnt4 was the only Wnt ligand expressed by small intestine Tregs and its expression was abolished in Tregs from Irf4 fl/fl Foxp3 Cre+/Y mice. To investigate the role of Treg-derived Wnt4, we generated mice whose Tregs are incapable of Wnt secretion (Wls fl/fl Foxp3 Cre+/Y). At baseline and in a model of subcutaneous allergic sensitization, these mice appeared normal. However, in an oral allergy model, these mice showed persistent serum antigen-specific IgE suggesting that Tregs control the magnitude of type 2 immune responses in a tissue-specific manner. This work supports a role for the Treg/Irf4/Wnt4 axis in intestinal homeostasis, the disruption of which enhances allergic sensitization.

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13. Skin Damage Signals Mediate Allergic Sensitization to Spatially Unlinked Antigen

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Abstract
The increasing prevalence of food allergy has been postulated to be a result of environmental modernization that has skewed the immune-activating context to promote allergic sensitization. However, the molecular characteristics of allergic adjuvants and the “rules” of how allergic sensitization occurs are still incompletely understood and rely on biology gleaned from “type 1” antimicrobial responses. Recently, much research has focused on how allergy develops when food allergens enter damaged/inflamed skin which provides the allergic adjuvant signal. However, this assumes that allergic responses follow the “type 1” rule that the antigen and adjuvant must be spatiotemporally linked. Unexpectedly, we discovered that allergic sensitization can occur when the adjuvant signal, generated from skin damage, is anatomically distal to the antigen in any portal of entry, including the gut. The mechanisms by which a spatially distant adjuvant signal and a protein antigen converge to induce immunologic memory have been uncharacterized. We hypothesized that an endocrine signal must be present to contextualize the “remote” antigen and discovered that the alarmin IL33, which is endogenously released into circulation after skin injury, is sufficient to induce allergic sensitization regardless of the portal of entry of antigen. Thus, we have for the first time established a highly molecularly defined model system in which to dissect the rules of allergic sensitization.

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14. Nociceptor Neurons Direct Goblet Cells via a CGRP-RAMP1 Axis to Drive Mucus Production and Promote Gut Barrier Protection

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Abstract
Neuroepithelial crosstalk is critical for gut physiology. However, the mechanisms by which sensory neurons communicate with epithelial cells to mediate gut barrier protection at homeostasis and during inflammation are not well understood. Here, we find that Nav1.8^{+}CGRP^{+} nociceptor neurons are juxtaposed with and signal to intestinal goblet cells to drive mucus secretion and gut barrier protection. Via mucin immunostaining, gut explant culture and 16S rDNA sequencing, we found that nociceptor ablation led to decreased mucus levels and dysbiosis, while chemogenetic nociceptor activation or capsaicin treatment induced mucus growth. Single cell RNA sequencing and RNAscope analysis indicated that both mouse and human goblet cells expressed RAMP1, receptor for the neuropeptide CGRP. Nociceptors signal via the CGRP-RAMP1 pathway to induce rapid goblet cell emptying and mucus secretion as shown by AB/PAS staining of colonic goblet cells. Notably, by dorsal root ganglia cell culture in vitro, we found that commensal microbes activated nociceptors to control homeostatic CGRP release. Further analysis showed that, in the absence of nociceptors or epithelial RAMP1, mice showed increased epithelial stress and susceptibility to DSS induced colitis. Conversely, chemogenetic nociceptor activation or CGRP administration protected against colitis. In conclusion, our findings demonstrate a neuron-goblet cell axis that orchestrates gut mucosal barrier protection.

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15. Within-Host Evolution of a Gut Pathobiont Facilitates Bacterial Translocation

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Abstract
Gut commensals with the capacity to translocate across the intestinal barrier can drive the development of diverse immune-mediated diseases. However, the key factors that dictate bacterial translocation remain unclear. Recent studies have revealed that gut microbiota strains can adapt and evolve throughout the lifetime of the host, raising the possibility that changes in individual commensals themselves over time may impact their propensity to elicit inflammatory disease. Here we show that within-host evolution of the model gut pathobiont *Enterococcus gallinarum* facilitates bacterial translocation and initiation of inflammation. Using a combination of *in vivo* experimental evolution and comparative genomics, we found that *E. gallinarum* diverges into independent lineages adapted to colonise either luminal or mucosal niches in the gut. Compared to ancestral and luminal *E. gallinarum*, mucosally-adapted strains evade detection and clearance by the immune system, exhibit increased translocation to and survival within the mesenteric lymph nodes and liver, and induce increased intestinal and hepatic inflammation. Mechanistically, these changes in bacterial behaviour are associated with non-synonymous mutations or indels in defined regulatory genes in *E. gallinarum*, altered microbial gene expression programs, and remodelled cell wall structures. *Lactobacillus reuteri* also exhibited broadly similar patterns of divergent evolution and enhanced immune evasion in a monocolonisation-based model of within-host evolution. Overall, these studies define within-host evolution as a critical regulator of commensal pathogenicity that provides a unique source of stochasticity in the development and progression of microbiota-driven disease.

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16. IgE Biology

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Abstract
Each IgH isotype (e.g IgM, IgG and IgE) can produce secreted (s) or membrane-bound (m) Ig via alternative RNA splicing. mIg forms the antigen binding portion of the B cell receptor (BCR). IgE B cells are unique in that mIgE is infrequently made, leading to very dilute BCR on the surface of IgE B cells, which may contribute to the unique features of IgE cells. We previously found that IgH isotypes exhibit a stable hierarchy of mIg levels where mIgM>mIgG1>mIgE regardless of cell stage suggesting that splicing regulation of the IgE locus may be an upstream regulator of IgE cell fate by limiting IgE BCR density. To explore how splicing-related regulation of mIgE mRNA influences IgE cell behavior, we generated IgEspAd mice, that have the polyadenylation (pA) site for the sIgE deleted and IgG1spAd control mice that have pA for sIgG1 deletion. The goal is to increase IgE BCR to determine how BCR density may influence B cell fate. IgEspAd B cells undergo normal class switching to IgE in vitro and exhibit a higher density of surface mIgE. Secreted IgE was not detected. Allergic inflammation induced by respiratory challenge with Alternaria Alternata extract in congenically marked IgEspAd and WT mixed bone marrow chimeras led to equal accumulation of germinal center IgE B cells in mediastinal lymph nodes, however, IgEspAd IgE cells exhibited less apoptosis. Strikingly, IgEspAd cells were far less likely than WT to accumulate IgE plasmablast (PB). These results were confirmed via scRNA sequencing of IgEspA heterozygous mice that produce either mutant or WT IgH, which were bioinformatically resolved. Cells expressing IgE exhibited 1:1 ratio in GC while mostly from the WT IgH locus in PB. These data suggest that availability of the mIgE splice variant influences B cell fate. In another project, we are exploring how distribution of IgE from point of origin to effector sites is regulated. WT mice immunized at the point of origin once induced asymmetry IgE distribution and twice with challenge resulting differential IgE anaphylaxis from two facilities indicating naïve B cells might play a key role and be shaped by microbiota.

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17. Molecular Dissection of Area Postrema and Its Role in Nausea

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Abstract
Nausea is an unpleasant sensation of visceral malaise often accompanied by vomiting. Nausea responses are evolutionarily beneficial behaviors to avoid or expel toxins, but they can also be maladaptive, as therapeutics for cancer and diabetes induce nausea as a major side effect. Current anti-emetic drugs have only limited efficacy, so, new strategies for nausea are needed and may be enabled by a mechanistic understanding of how the sensation of nausea arises, which is largely unknown. Known as the “chemoreceptor trigger zone” for nausea, the area postrema was identified by classical lesion studies as a brain structure that mediates nausea responses. Unlike other parts of the nervous system, neurons in the area postrema occupy a unique anatomical location outside the blood-brain-barrier, allowing them to be regulated by humoral factors. Using single-nucleus RNA-sequencing combined with genetic approaches for cell-selective activation, ablation, and gene knockout and rescue, we have recently identified a population of the area postrema excitatory neurons that, in response to emetic cues, induce nausea-associated aversive behaviors in mice. Clinically relevant receptors were identified in these aversion promoting neurons, such as receptors for glucagon-like peptide 1 (GLP1) and cytokine GDF15. GLP1 receptor activation in the area postrema was required and necessary for aversion, which was likely responsible for the aversive side effects during diabetes treatments. These neurons project to the midbrain aversive learning pathway, providing a circuit-based mechanism for the observed responses. Finally, we also identified area postrema inhibitory neurons that projected locally and elicited inhibitory currents in the nausea-promoting area postrema excitatory neurons. Targeted activation of the area postrema inhibitory neurons counteracted nausea-associated poison responses evoked by area postrema excitatory neurons. Altogether, we have uncovered the basic organization of area postrema nausea circuitry and provided a framework towards understanding, predicting, and therapeutically controlling nausea.

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18. The Biology and Physiological Roles of IgE

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Abstract
IgE is an antibody isotype most commonly associated with allergy and anti-parasite responses, which fall under Type II immunity. However, while the mechanism by which the IgE-FcεRI axis causes allergy and anaphylaxis is clear, the requirement of IgE in other Type II responses is less so. The persistence of IgE in mammal immune systems for 150 million years suggests that IgE must exert some protective function. To this end, roles for IgE have been posited in other immune contexts as well, including antiviral and antitumor responses. However, we currently lack the understanding of the mechanisms underlying IgE biology in order to explore these roles.
To tackle this question, we used a combination of a fluorescent tracer and flow cytometry staining. The former consisted of an APC fluorophore covalently linked to an ovalbumin-specific recombinant IgE antibody. Passive infusion of this tracer demonstrated that neither endogenous IgE nor FcεRI expression regulate the serum half-life of IgE. Flow cytometry staining revealed that IgE genetic knockout mice have increased basophil numbers in the bone marrow, but decreased liver mast cells as compared to WT C57BL6 mice. In addition, passively infused IgE antibody was undetectable in the peritoneum and meninges of IgE knockout mice, suggesting a preferential localization of IgE. From our data we propose two complementary models. In the first, the liver, as a major recipient of mesenteric and cardiac output, serves as a sink for serum IgE. Conversely, the bone marrow may control basophil development or circulation in response to the presence of IgE. Overall, these results highlight previously unidentified roles for IgE in regulating FcεRI-expressing cells, as well as pointing to the need to study other interaction partners for IgE, such as FcεRII.

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