Program and abstracts of papers presented at the

13th Annual Salk Institute/Fondation IPSEN/*Science*

Symposium on Biological Complexity:

MICROBES WITHIN THE HOST
IN HEALTH AND DISEASE

January 22 – 24, 2019

SALK INSTITUTE FOR BIOLOGICAL STUDIES, LA JOLLA, CALIFORNIA, USA

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13th Annual Salk/Fondation IPSEN/Science Symposium on Biological Complexity: Microbes Within the Host in Health and Disease
January 22-24, 2019

Date	Start Time	End Time	Details/Speaker	
Tuesday, 1/22				
	8:00am	9:00am	Registration Check-In	
	8:00am	9:00am	Breakfast for registered attendees	
	9:00am		Welcome and Opening Remarks by Organizing Chair, Janelle Ayres	
	9:15am		Keynote Address by Margaret McFall-Ngai	
	10:15am	12:00pm	Session 1: The Gut-Brain Axis, chaired by Janelle Ayres	
			Sarkis Mazmanian	
			Rosa Krajmalnik-Brown	
			Gil Sharon*	
			John Cryan	
	12:00pm	1:30pm	Break for Lunch (on own for lunch)	
	1:30pm	4:00pm	Session 2: Immunometabolism, chaired by Susan Prescott	
			Susan Prescott	
			Janelle Ayres	
			Luke O'Neill	
			Justin McCarville*	
			Ruth Ley	
	4:00pm		Brenner Lecture by Jeffrey Gordon	
	5:00pm	6:00pm	Brenner Lecture Reception	
Wednesday, 1/23				
	8:00am	9:00am	Breakfast for registered attendees	
	9:00am	12:00pm	Session 3: Cancer and Cancer Therapies, chaired by Laurence Zitvogel	
			Laurence Zitvogel	
			Cindy Sears	
			Ting Fu*	
			Omer Yilmaz	
			Thomas Gajewski	
			Mathieu Groussin*	
	12:00pm	2:00pm	Break for Lunch (on own for lunch)	
	2:00pm	5:00pm	Session 4: Host-pathogen Interactions, chaired by Denise Monack	
			Denise Monack	
			Andreas Bäumler	
			Brian Tsu*	
			Thad Stappenbeck	
			Manuela Raffatellu	
			Fabian Rivera-Chavez*	
	5:00pm	6:30pm	Reception & Poster Session 1	
Thursday, 1/24				
	8:00am	9:00am	Breakfast for registered attendees	
	9:00am	12:00pm	Session 5: Immune - Microbiome Interactions, chaired by Lora Hooper	
			Lora Hooper	
			Greg Barton	

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			Araceli Perez-Lopez*	
			Andrew Macpherson	
			Yasmin Belkaid	
			Katia Troha*	
	12:00pm	2:00pm	Break for Lunch (on own for lunch)	
	2:00pm	5:00pm	Session 6: Microbial Communities in Health and Disease, chaired by Marty Blaser	
			Marty Blaser, Chair	
			Susan Lynch	
			Daniel McDonald*	
			Dan Littman	
			Michael Fischbach	
	5:00pm	6:30pm	Reception & Poster Session 2	
			*Short Talk	
			**Last Update 1.14.19	

Speaker Abstracts

Evolutionary 'Experiments' in Symbiosis: Insights into the Integration of Microbes into Host Biology

Margaret McFall-Ngai
Pacific Biosciences Research Center, University of Hawai'i at Mānoa,
Honolulu, HI 96822

The extant thirty to forty animal phyla arose in the microbe-rich Paleozoic oceans. This microbial biotic pressure appears to have shaped all aspects of the biology of animals, from their ability to occupy certain ecological niches to the trajectory of their genome evolution. The diversity of associations presents the opportunity to use the patterns of symbiosis to reveal the basic, evolutionarily conserved 'rules' of animal-microbe interactions. This presentation will highlight recent research efforts on the form and function of various animal model systems of symbiosis that are providing insights into the mechanisms underlying the establishment and maintenance of animal partnerships with the microbial world.

Development of Microbiota-Directed Complementary Foods for Treatment of Childhood Undernutrition

Jeffrey Gordon
The Edison Family Center for Genome Sciences & Systems Biology,
Washington University School of Medicine in St. Louis, MO

Talk Title To Be Announced

Sarkis K. Mazmanian
Division of Biology and Biological Engineering,
California Institute of Technology, Pasadena, CA 91125

The intestinal microbiome influence neurodevelopment, modulate behavior, and contribute to neurological disorders. However, a functional link between gut bacteria and neurodegenerative diseases remain largely unexplored. Synucleinopathies are characterized by aggregation of the protein α-synuclein (αSyn), often resulting in motor dysfunction as exemplified by Parkinson's disease (PD). Using mice that overexpress αSyn, we report herein that gut microbiota are required for motor deficits, microglia activation, and αSyn pathology. Antibiotic treatment ameliorates, while microbial re-colonization promotes, pathophysiology in adult animals, suggesting postnatal signaling between the gut and the brain modulates disease. Remarkably, colonization of αSyn-overexpressing mice with microbiota from PD patients enhances physical impairments compared to microbiota transplants from healthy human donors. Furthermore, we have identified a specific species of bacteria that promotes intestinal and brain aggregation of αSyn, and motor deficits, via production of a bacterial amyloid in the gut. These findings reveal that gut bacteria regulate movement disorders in mice, and suggest that certain compositions of the human microbiome represent a risk factor for PD.

Microbiota Transfer Therapy Sustainably Improves Gastrointestinal and Autism Symptoms by Changing Gut Bacterial Community Structure and Function

Rosa Krajmalnik-Brown[1], Dae-Wook Kang[1], James B. Adams[2], Alexander Khoruts[3,4,5], Juan Maldonado[1], Sharon McDonough-Means[6], Elena L. Pollard[2], Michael J. Sadowsky[4,7], and J. Gregory Caporaso[8,9]
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Recent studies have shown a link between dysbiotic gut microbiota and autism. Fecal microbiota transplant (FMT) is a promising therapy to repair dysbiotic gut microbiota. We pioneered an open-label clinical trial of microbiota transfer therapy (modified FMT) for autism. We monitored gastrointestinal and behavioral symptoms, gut bacterial communities and fecal metabolites on 18 autism-diagnosed children receiving this microbiota transfer therapy. Participants responded to treatment with rapid improvements in both gastrointestinal and behavioral symptoms, which sustained after treatment. At the end of the study, we observed about 80 percent reduction of gastrointestinal symptoms and 20-25 percent improvement in autism behaviors. Fecal microbiota transplant significantly altered each recipient's microbiome toward the donor's, indicating some successful engraftment. The treatment resulted in increased bacterial diversity and a transition toward gut microbiota composition of 20 age/gender matched neurotypical children. We detected 669 metabolites in fecal samples and 620 in blood samples, and found that children with ASD had significant changes in fecal and blood metabolite profiles after fecal microbiota transplant. We are exploring the relationship between metabolites, pathways and microbes changing during treatment. The microbiota transfer therapy performed in this trial is a promising approach for sustainably altering the gut bacterial communities and their metabolites, and improving gastrointestinal and behavioral symptoms associated with autism spectrum disorders.

Microbiomes of Autism Spectrum Disorder Patients Induce Behavioral Deficits in Mice

Gil Sharon, Nikki J Cruz, DaeWook Kang, Michael Gandal, Bo Wang, Young-Mo Kim, Lisa Bramer, Nancy Isern, David Hoyt, Cecilia Noecker, Elhanan Borenstein, Carlos Lois, Janet Jansson, Tom Metz, Daniel H Geschwind, Rosa Krajmalnik-Brown and Sarkis K. Mazmanian
Division of Biology and Biological Engineering,
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Autism spectrum disorders (ASD) manifest with deficits in social communication and repetitive, stereotyped behaviors. The gut microbiome differs between typically developing (TD) and ASD individuals, however it is unclear whether or not it contributes to etiology. To directly test the role of the gut microbiome in ASD, we colonized wild-type germ-free mice with fecal samples from TD and ASD children and tested their offspring for ASD-relevant phenotypes. We show that exposing mice to some ASD microbiomes is sufficient to induce behavioral deficits. We observed extensive effects on alternative splicing in genes known to contribute to ASD. Moreover, we observed changes in the availability of various metabolites in the colon. We further show that the administration of putatively protective metabolites, elevated in TD-colonized mice, to an idiopathic mouse model of autism from gestation can attenuate behavioral deficits and decrease excitation in L5 pyramidal neurons in the prefrontal cortex of these mice. Conversely, the administration of some “pathogenic” metabolites, that were elevated in ASD mice, induced deficits in locomotion. These results suggest that the altered metabolism by the gut microbiome contributes to behavioral deficits in ASD.

Talk Title To Be Announced

John F. Cryan
Department of Anatomy and Neuroscience,
University College Cork, Ireland T12 K8AF

The Microbiome and Mutualism: Integrated Ecological Approaches to Dysbiotic Drift for Personal and Planetary Health

Susan Prescott
Perth Children's Hospital, University of Western Australia

Microbiome science provides new evidence of vital relationships between biodiversity and health at every level. New perspectives of ecological interdependence connect personal and planetary health; the human health crisis cannot be separated from the social, political and economic 'ecosystems' otherwise driving 'dysbiosis' (life in distress) at every level. Changes in macroscale ecology—of food systems, lifestyle behaviors, socioeconomic disadvantage and environmental degradation—all impact the microbial systems which sit at the foundations of all ecosystems.

Human health depends on mutualistic relationships with microbes. Changes in the function and composition of the human-associated microbiome has been implicated in the mounting global burden of non-communicable diseases (NCDs), exacerbating inflammation and metabolic dysregulation through multiple pathways across the lifespan. This underscores the need for ecological approaches aimed at restoring symbiosis, balance and mutualism. While there is promise with supplement-based strategies (e.g. probiotics, prebiotics), it is essential to focus on upstream factors implicated in dysbiosis; including the health of wider environments, lifestyle, nature relatedness, and the social policies and practices which can facilitate or inhibit "dysbiotic drift".

This underscores the necessity for ambitious integrative approaches which not only define these interconnections, but capitalize on them to create novel, collaborative and mutualistic solutions to our vast interdependent global challenges. Moreover, it calls to revive Jonas Salk's biophilosophy—a call to action for collaboration between biologists, humanists, and scholars of all disciplines the possibility of a new epoch in which mutualism will be considered imperative.

Talk Title To Be Announced

Janelle S. Ayres
Nomis Center for Immunobiology and Microbial Pathogenesis,
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Krebs Cycle Reprogrammed for Cytokines

Luke A. J. O'Neill
School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland

Metabolic changes triggered by innate immune receptors have become a particular focus for researchers interested in immunity and inflammation. This area has direct relevance to inflammatory diseases such as rheumatoid arthritis since the rheumatoid joint is known to undergo a range of metabolic alterations and enzymes in glycolysis are well known autoantigens. Furthermore metabolites from the Krebs Cycle such as succinate have been found to be elevated in rheumatoid synovial fluid and act via the receptor SUCRN1 on macrophages to boost IL-1beta production. LPS-activated macrophages undergo metabolic reprogramming with a major increase in glycolysis, which is required for ATP production and also the generation of biosynthetic intermediates. Changes in the Krebs cycle also occur such that intermediates such as citrate are withdrawn for lipid biosynthesis. We have found a key role for citrate in the induction of a range of inflammatory genes and a complex mechanism involving malonylation of GAPDH leading to enhanced translation of a range of mRNA including those encoding TNF and COX2 has been revealed. We have also found a role for the Krebs cycle intermediate succinate in activated macrophages. Succinate induces HIF-1alpha and it's target genes, which include that encoding IL-1beta, can act on the aforementioned succinate receptor SUCRN1 on cells (which can synergise with TLRs) also can be oxidised by Succinate Dehydrogenase which because of the high mitochondrial membrane potential leads to reverse electron transport (RET) via Complex I in the mitochondria. This drives ROS production with inflammatory consequences. Succinate might therefore act as important signal for inflammation. We have also found that inhibition of SDH leads to IL-10 production, indicating that this enzyme is a key arbiter of cytokine production. Finally, another metabolite generated from citrate, itaconate, has profound anti-inflammatory effects, acting via Nrf2 to limit the induction of cytokines such as IL-1beta and Type I interferons. These insights are providing a new view of metabolism in immunity and inflammation and might indicate new therapeutic approaches.

Elucidating the Metabolic Response to *Legionella pneumophila* Infection in Young and Aged Mice

Justin L. McCarville[1], Thekla Cordes[2], Abigail Miller[1], Christian Metallo[2], and Janelle S. Ayres[1]
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Salk Institute for Biological Studies, La Jolla, CA 92037
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La Jolla, CA 92093

Legionella pneumophila (Lp) is a bacterial pathogen that colonizes the lung by replicating in alveolar macrophages. Recent *in vitro* work has determined that Lp infection shifts macrophage metabolism to a glycolytic state which impairs clearance of the pathogen. The influence of Lp infection on *in vivo* systemic host metabolism, however, is unknown. The elderly are more susceptible to Lp infection than younger individuals, possibly due to defects in glucose metabolism, since 80% of the elderly (>65 years) display hyperglycemia and glucose intolerance. This project aimed to characterize young and aged hosts metabolic responses to Lp infection and impacts on disease outcomes. We found that intranasal infection of young (7-week-old) mice with Lp led to muscle and fat-specific weight loss, decreased core body temperature, and anorexia. At 48 hrs post-infection, young mice developed hypoglycemia, increased glucose tolerance, and underwent active lipolysis of white adipose tissue. Despite elevated obesity and hyperglycemia, aged mice (73-week-old) showed similar levels of hypoglycemia and glucose tolerance to young mice at 48 hrs post-infection, though, aged mice lost percent body mass at a slower rate and underwent less lipolysis of white adipose tissue than young mice. Analysis of serum prior to, during, and post-infection with Lp revealed age and infection-specific changes in circulating metabolites, with alterations in abundances of tricarboxylic acid cycle intermediates, amino acids, and numerous free-fatty acids. These results demonstrate that Lp infection alters host glucose metabolism, independent of age. However, changes in other metabolic parameters in response to Lp, such as lipid metabolism, are age-dependent. This suggests that deviations in host metabolism that occur with aging alter metabolism in response to pathogen challenge and may ultimately change the course and severity of an infection.

Bacteroidetes in the Gut are Linked Through Sphingolipid Production to Insulin Resistance

Ruth Ley[1], Elizabeth L. Johnson[1,2], Stacey Heaver[1], Benjamin I. Kim[3], Alexis Bretin[4], Jillian L. Waters[1], Andrew L. Goodman[5], Andrew Gewirtz[4], and Tilla S. Worgall[3]
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New Haven, CT 06520

The human gut microbiome is dominated by members of the phylum Bacteroidetes. These are also the only gut microbiota known to produce sphingolipids (SLs), a class of lipids with various and important roles in host biology. To date, bacterial SL has been implicated in microbe-host interactions solely in the context of immunity. Here, we describe a role for bacterial SL in the regulation of host metabolism. We show that bacterial SL from the gut can regulate levels of host liver ceramides, a class of SLs linked to insulin resistance. In-vitro experiments have indicated that SLs of bacterial origin are uptaken by epithelial cells and enter mammalian SL-processing pathways. In mouse models, administration of Bacteroides thetaiotaomicron was associated with reduced hepatic de-novo synthesis, higher hepatic ceramide levels, and reduced insulin sensitivity, in comparison with administration of a strain deficient in the capacity for SL synthesis. These findings establish bacterial SLs as a novel class of molecules produced by specific gut bacteria and capable of perturbing host metabolism. Our results add to the evidence that products of Bacteroides species and their relatives may be contributing to insulin resistance phentypes.

The Unsuspected Role of Gut Microbiota in Cancer Therapies

Laurence Zitvogel
INSERM, Gustave Roussy Cancer Center, University Paris Saclay

We recently highlighted the crucial role of gut microbiota in eliciting innate and adaptive immune responses beneficial for the host in the context of effective therapies against cancer. Chemotherapeutic agents facilitate the gut permeability and selective translocation of Gram positive bacteria in secondary lymphoid organs. There, anti-commensal pathogenic TH17 T cell responses are primed, facilitating the accumulation of TH1 helper T cells in tumor beds post-chemotherapy as well as tumor regression (Viaud S, *Science* 2013). These findings have been extended to platinum salts as well as to a combination of anti-IL-10R mAb+CpG (Iida et al. Science Nov 2013). The immune checkpoint blockers (ICB) anti-CTLA4 Ab and anti-PD1/PD-L1 Ab are first-in class compounds approved for reinstating cancer immunosurveillance and prolonging survival in metastatic patients. Zitvogel's group showed that antibiotics compromise the efficacy of these ICB in mice and patients and unveiled the immunomodulatory role of distinct commensals from the intestinal ecosystem in mobilizing anticancer immunosurveillance. Vétizou et al. showed that the antitumor effects of CTLA4 blockade, largely dependent upon Toll like receptor (TLR)2/TLR4 receptors, markedly rely on the regulatory commensal *Bacteroides fragilis (*in coordination with *Burkholderia cenocepacia, Science Nov 2015).* Next, the demonstration of the deleterious role of antibiotics in the clinical efficacy of PD-1 blockade in lung, kidney and bladder cancer patients was brought up, highlighting the role of *Akkermansia muciniphila* as the main player in the immunomodulatory effects of pembrolizumab or nivolumab (Routy et al. Science 2017 Nov2). The mechanisms by which *A. muciniphila* restores gut dysbiosis will be discussed, involving CCR9 and IL-12. From these findings, we infer that oncomicrobiotics and/or fecal microbial transplantation could be considered as adjuvants to the current oncological armamentarium in dysbiotic cancer bearers.

Talk Title To Be Announced

Cynthia L. Sears
Johns Hopkins Medical Institute, Baltimore, MD 21287

The microbiome and specific microbes have increasingly been implicated in the initiation and progression of human colon cancer. This talk will discuss data on microbial–specific and microbiome community contributions, in particular, mucus-invasive biofilms, to human colon cancer. Germ-free and specific pathogen-free mouse models will be described that display the tumorigenic potential of human colon biofilms. Preliminary epidemiologic data from a community-based colonoscopy project to detect human colon biofilms will be discussed. Antibiotic exposure routinely disrupts the microbiome and has been proposed to impact the success of cancer immunotherapy. Data on the association of antibiotic exposure and colon neoplasia development will be presented. Collectively, the data support the role of both specific bacteria and bacterial communities in the pathogenesis of human colon cancer.

Novel Microbial Conjugated Bile Acids in Colon Cancer

Ting Fu[1], Zhenjiang Xu[2], Tao Huan[3], Fritz Cayabyab[1], Pieter Dorrestein[4], Annette Atkins[1], Ruth Yu[1], Michael Downes[1], Rob Knight[2], and Ronald Evans[1,5]
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Colon cancer (CRC) remains a significant public health concern and is the third-leading cause of cancer-associated deaths in the US. While the underlying causes are poorly understood, genetic and environmental factors are known to contribute to disease progression. We recently identified the bile acid (BA)-FXR axis as a point of convergence for genetic and dietary risk factors in CRC. As early sensors of dietary status, BAs are critical for host physiology, in part through their activation of Farnesoid X Receptor (FXR). However, disease associated changes in the gut microbiome and the consequential effects on cancer progression have not been clarified. In this study, we determined the effects of genetic (APCmin mice) and dietary (high fat diet) factors on the composition and transcriptional profiles of the microbiome. While diet appears a major determinant of the microbiome, the host physiology appears to be shaped more by genetics. In particular, we find that the levels/composition of serum primary BAs correlate with the host status, whereas the secondary BAs in the cecum affect the microbiome. In addition, we identify select host genes that correlate with the microbiome changes. Strikingly, we identified novel microbially modified BAs in the cecum but not in serum samples. These novel BAs, enriched in APCmin mice and under HFD, are positively associated with bacteria previously linked to TMAO production. Intriguingly, some are potent activators for FXR and cell surface bile acid receptor, TGR5. These studies support the importance of the gut microbiome and its modification of the BA pool in disease progression, and suggest that detailed characterization of the changes will hasten the development of novel biomarkers and therapeutics for CRC.

Dietary Suppression of MHC-II Expression in Intestinal Stem Cells Enhances Intestinal Tumorigenesis

Omer Yilmaz
Koch Institute for Integrative Cancer Research,
Massachusetts Institute of Technology, Cambridge, MA 02139

Little is known about how interactions between diet, immune recognition, and intestinal stem cells (ISCs) impact the early steps of intestinal tumorigenesis. Here, we find that a high fat diet (HFD) reduces the expression of the major histocompatibility complex II (MHC-II) genes in ISCs. This decline in ISC MHC-II expression in a HFD correlates with a reduction in intestinal bacterial diversity and is recapitulated in antibiotic treated and germ-free mice. Although MHC-II expression on ISCs is dispensable for stem cell function *in vitro*, as assayed in organoid cultures, upon loss of the tumor suppressor gene *Apc* in a HFD, MHC-II$^{low/-}$ ISCs harbor greater *in vivo* tumor-initiating capacity than their MHC-II$^{high/+}$ counterparts, thus implicating a role for epithelial MHC-II in suppressing tumorigenesis. Finally, ISC-specific genetic ablation of MHC-II in engineered *Apc*-mediated intestinal tumor models increases tumor burden in a cell autonomous manner. These findings highlight how a HFD alters the immune recognition properties of ISCs through the regulation of MHC-II expression to contribute to intestinal tumorigenesis.

Talk Title To Be Announced

Thomas Gajewski
The Microbiome Center, University of Chicago, IL 60637

Phylogeny, Abundance, Cell Wall Architecture and Host Lifestyle Drive Extensive Gene Transfers in Each Person's Gut Microbiome

Mathieu Groussin[1,2,3,4], Mathilde Poyet[1,2,3,4], Ainara Sistiaga[4,5], Sean Kearney[1], Roger Summons[5], Ramnik Xavier[2,3,4], and Eric Alm[1,2,3,4]
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Previous studies have shown the importance of horizontal gene transfer (HGT) in shaping human gut bacteria. However, whether genomes acquire new genes daily, or whether millennia separate transfer events, remains unknown despite its importance on gut microbiome function and human health. Here, we generate thousands of gut bacterial genomes across hundreds of species, from different human populations worldwide, to show that the timescale of these HGTs is short, and that they frequently occur in each person's gut microbiome. We reveal that within people HGT is a function of species abundance and bacterial cell wall architecture, independently of phylogeny. We further find that bacterial species that engage in HGTs within people are the same across different people. Finally, we show that the gut bacteria of individuals with industrialized lifestyle exchange genes more frequently than in non-industrialized populations, suggesting that host lifestyle also drives HGTs. As compared to the functions recently exchanged in bacteria sampled across people, functions exchanged within urban US people are enriched in antibiotic resistance. Our study sheds new light on the factors influencing how gut bacteria acquire new functions during our lifetime. It also shows the importance of sampling and characterizing the gut microbiome biodiversity of underrepresented traditional populations. I will finish my talk presenting our new initiative, the Global Microbiome Conservancy, a non-profit initiative that aims to preserve the full biodiversity of the human gut microbiome in a biobank of bacterial isolates and genomes to advance human health.

Salmonella-Microbiome Interactions in the Gut and their Impact on Transmission

Denise M. Monack
Department of Microbiology and Immunology, Stanford School of Medicine
Stanford University, Stanford, CA 94305

Disease transmission is a multifaceted process mediated by the interactions between the pathogen and host. *Salmonella enterica* serovars are important human enteric pathogens that cause disease ranging from self-limiting gastroenteritis to persistent systemic infections, such as typhoid fever. Transmission occurs via the fecal-oral route, and epidemiological analyses have revealed heterogeneity in host transmission capabilities. The underlying mechanisms that lead to this heterogeneity is an area of intense study. We will explore the dynamic interactions between *Salmonella* and the high-density polymicrobial community, the microbiota, in the distal gut. We will explore mechanisms by which the intestinal microbiota provides protection against this invading pathogen by limiting pathogen expansion and transmission into the environment. Previously described mechanisms of microbiota-mediated colonization resistance have been identified by observing loss of colonization resistance after community perturbations such as antibiotic treatment or diet changes. I will describe a new microbiota-mediated mechanism of colonization resistance against *S. Typhimurium*. By comparing high-complexity communities with different levels of colonization resistance, we demonstrate that *Bacteroides* species mediate colonization resistance against *S.* Typhimurium *in vivo* through the production of the short-chain fatty acid propionate. We show that propionate directly inhibits *S. Typhimurium* growth *in vitro* by disrupting intracellular pH homeostasis. We provide the first mechanistic understanding of the role of individualized microbial communities in host-to-host variability of pathogen transmission.

Dietary Copper Shifts the Gut Microbiota to Reconfigure Pathogen Growth

Denise N. Bronner[1], Erin E. Olsan[1], Connor R. Tiffany[1], Eleonora Tassinari[2], Gaëtan Thilliez[2], Diane Tu[3], Austin Byndloss[1], Yael Litvak[1], Henry Nguyen[1], Andrew W. L. Rogers[1], Eric Velazquez[1], Mariana X. Byndloss[1], Carlito Lebrilla[3], Robert A. Kingsley[2], and Andreas J. Bäumler[1]
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The composition of the gut microbiota varies with the diet, but little is known about how shifts in the microbiota composition affect functionality, such as the ability to confer colonization resistance against pathogens. After non-therapeutic antibiotic use was banned in the European Union in 2006, dietary copper supplementation became a common practice for improving growth performance in livestock. The use of dietary copper supplementation coincided with the spread of an epidemic copper-resistant Salmonella clone that acquired the sopE virulence gene on multiple occasions via phage-mediated horizontal transfer. Here we show that the sopE gene changes the composition of the fecal leukocyte population in mice, thereby rendering Salmonella more resistant to niche protection conferred by Clostridia, but more sensitive to niche protection conferred by Bacteroidia. Notably, dietary copper supplementation depletes Bacteroidia from the murine gut microbiota, thereby generating an environment in which the sopE gene confers a growth advantage. Our results suggest that a shift in the gut microbiota composition induced by dietary copper alters colonization resistance, which provides a plausible explanation for the acquisition of the sopE virulence gene by an epidemic Salmonella clone currently circulating in Europe.

Whole Genome Evolutionary Fingerprinting Suggests Novel Primate Gene Functions in Evolutionary Arms Races

Brian Tsu[1], Benjamin Murrell[2], Matthew Daugherty[1], and Joel Wertheim[2]
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Previous positive selection analyses have helped to elucidate differences in primate adaptation. However, many of these studies have been focused on small gene families and genes known or suspected to play a role in the host innate immune system. With the genome sequencing of numerous primate species, we are now capable of comparing the evolutionary profiles of primate genes across the whole genome. Using these evolutionary profiles as guides, we identify new gene functions that are rapidly evolving and insight on the biological pressures that affect them.

Talk Title To Be Announced

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Talk Title To Be Announced

Manuela Raffatellu
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Cholera Toxin Promotes Pathogen Acquisition of Host-derived Nutrients

Fabian Rivera-Chávez and John J. Mekalanos
Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA 02115

Vibrio cholerae is the causative agent of cholera, a potentially lethal enteric bacterial infection. Cholera toxin (CT) is required for *V. cholerae* to cause severe disease and is also thought to promote transmission of the organism in that victims can shed many liters of diarrheal fluid that typically contains in excess of 10^{11} organisms. However, how the pathogen is able to reach such high concentrations in the intestine during infection remains poorly understood. Here we show that CT-mediated disease enhances pathogen growth and induces a distinct *V. cholerae* transcriptome signature that is indicative an iron-depleted gut niche. We show that *V. cholerae* heme and vibriobactin utilization genes confer a growth advantage to the pathogen only when CT is produced. Furthermore, CT-induced capillary congestion pathology in the terminal ileum correlated with an increased bioavailability of luminal heme. CT-induced disease in the ileum also led to increased luminal concentrations of long-chain fatty acids (LCFAs) and L-lactate metabolites, as well as upregulation of *V. cholerae* iron-sulfur cluster-containing TCA cycle enzyme genes. Genetic analysis of *V. cholerae* suggested that heme and LCFA uptake-dependent growth of *V. cholerae* occurs during infection but only in a strain capable of producing CT *in vivo*. We conclude that CT-induced disease creates an iron-depleted metabolic niche in the gut that selectively promotes the explosive growth of this pathogen through acquisition of host-derived heme and fatty acids as nutrients.

How Interactions between the Microbiota and the Circadian Clock Control Host Metabolism

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Circadian rhythmicity is a defining feature of mammalian metabolism that synchronizes metabolic processes to day-night light cycles. We have found that the intestinal microbiota programs diurnal metabolic rhythms in the mouse small intestine through histone deacetylase 3 (HDAC3). The microbiota induces expression of intestinal epithelial HDAC3, which is recruited rhythmically to chromatin and produces synchronized diurnal oscillations in histone acetylation, metabolic gene expression, and nutrient uptake. HDAC3 also functions non-canonically to coactivate estrogen related receptor alpha (ERRalpha), inducing rhythmic transcription of the lipid transporter gene Cd36 and promoting lipid absorption and diet- and jet lag-induced obesity. These findings reveal that HDAC3 integrates microbial and circadian cues to regulate diurnal metabolic rhythms, and identify a key mechanism by which the microbiota controls host metabolism.

Talk Title To Be Announced

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CRTAM Shapes the Microbiota and Enhances the Severity of Gut Infection

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Gut lymphocytes and the microbiota establish a reciprocal relationship that tunes the host immune response. For example, the gut microbiota is required for T helper (Th)-17 cell differentiation; Th17 cytokines, in turn, stimulate the intestinal epithelium to produce antimicrobial proteins that control the gut microbiota. Residency and maintenance of T cells in the gut is controlled by Class I-restricted T cell-associated molecule (CRTAM). We thus evaluated whether CRTAM influences the gut microbiota composition. We found that CRTAM expression caused a shift in several members of the gut microbiota under homeostatic conditions. As the gut microbiota and T cells contribute to host defense against intestinal pathogens, we tested whether CRTAM plays a role during infection with the enteric pathogen *Salmonella*. We found lower levels of *Salmonella* colonization in the gut of Crtam-/- mice, along with lower expression of the Th17 cytokines *Il17* and *Il22* in the cecum. *Salmonella*-infected *Crtam*$^{-/-}$ mice exhibited fewer gut T cells and IL-17-producing cells. To investigate whether the altered gut microbiota composition of *Crtam*$^{-/-}$ mice influenced the host response to infection, we transplanted the fecal microbiota from WT or *Crtam*$^{-/-}$ littermate mice into germ-free mice, and then infected the mice with *Salmonella*. Relative to mice transplanted with "WT" fecal microbiota, mice transplanted with "*Crtam*$^{-/-}$" microbiota exhibited lower numbers of T cells and IL-17-producing cells in the gut upon infection. Our results thus suggest an interplay between CRTAM expression and the gut microbiota, which ultimately impacts the mucosal immune system and the host response to enteric pathogens.

Distinct Mucosal or Systemic Responses to Non-pathogenic Intestinal Microbes Build the Baseline B Cell Repertoire and Shape its Functional Responsiveness

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Microbiota colonisation causes profound B cell stimulation and immunoglobulin induction. Using transient microbial exposures in germ-free mice we have studied initial microbiota-dependent B cell repertoire development. Distinct oligoclonal responses to all isotypes after intestinal mucosal exposure differ from those after intravenous systemic exposure or germ-free controls. The selective IgA repertoire after intestinal dose escalation becomes progressively restricted and clonally related, whereas the systemic IgG repertoire can be expanded to a range of microbial cytoplasmic as well as cell-surface antigens. The hierarchical repertoires during B cell development are shaped by microbial exposure at memory and plasma cell stages, with clonal overlaps generated between earlier transitional states. Whereas sequential systemic exposure to different taxa expands the IgG repertoire and multiplies specific responses, sequential mucosal exposure produces limited overlapping repertoires and attrition of initial IgA binding specificities. This shows a contrast between a flexible response to systemic exposure consistent with the need to avoid fatal sepsis and a restricted response to mucosal exposure reflecting the generic nature of mucosal microbial mutualism.

Control of Skin Immunity and Repair by the Microbiota

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Barrier tissues are primary targets of environmental stressors and home to the largest number of antigen-experienced lymphocytes in the body, including commensal-specific T cells. We found that skin-resident commensal-specific T cells express a unique program allowing them to control tissue physiology ranging from antimicrobial defense to tissue repair. Notably, commensal specific T cell harbor a paradoxical program characterized by a type-17 program associated with a poised type-2 state. Thus, in the context of injury and exposure to inflammatory mediators, these cells rapidly release type-2 cytokines, thereby acquiring contextual functions. Aberrant type-2 responses to the microbiota can also be unleashed in the context of local defects in immunoregulation. Thus, commensal-specific T cells co-opt tissue residency and cell-intrinsic flexibility as a means to promote both local immunity and tissue adaptation to injury.

Nephrocytes Mediate Immune Tolerance to Microbiota by Removing Peptidoglycan from Systemic Circulation

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Maintenance of immune tolerance to microbiota is essential to organismal homeostasis. Here, we report that the *Drosophila* nephrocyte, a cell type with molecular, anatomical, and functional similarity to the glomerular podocyte of the vertebrate kidney, promotes immune tolerance to Gram-positive microbiota. Flies deficient for *Klf15*, a transcription factor required for nephrocyte development and function, are viable but lack nephrocytes. *Klf15* mutants display constitutively elevated Toll pathway activity, and as a consequence, are both shorter-lived and more resistant to microbial infection. Our analysis revealed that nephrocytes uptake Lys-type peptidoglycan from systemic circulation and degrade it inside lysosomes. Without nephrocyte function, microbiota-derived peptidoglycan accumulates in circulation, triggering Toll pathway activation even in the absence of infection. These results unveil a role for hemolymph (insect blood) filtration in the maintenance of immune tolerance to microbiota and also identify a potential root cause for the chronic immune activation observed in animals suffering from impaired blood filtration.

The Role of Antibiotic Exposures in Model Systems of Pandemic Chronic 'Non-communicable' Diseases

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Antibiotics are being used in multiple manners for varied indications in health care. Because of a nearly universal perception that their benefits far outweigh their disease risks, the scale of usage is massive in most parts of the world. During the antibiotic era (beginning in many countries in the 1940's), there have been substantial increases in the rates of a number of metabolic (including obesity, diabetes) and immunological (asthma, allergies, IBD) diseases. To assess whether antibiotic exposures may be contributing to these phenomena, we have developed experimental model systems in mice to assess causal roles. We have found that early life antibiotic exposure affects both metabolic and immunologic development and phenotypes. The antibiotic-perturbed microbiome was both necessary for the development of immunological properties, and sufficient in three different models, indicating that the effects were due to the altered microbiome, and were not side-effects of the antibiotic chemical activities. In one model system, inoculating germ-free pregnant mice that had genetic susceptibility (IL10-/-) for colonic inflammation with an antibiotic-perturbed microbiota resulted in enhanced tissue injury in their pups. That neither the pups nor their mothers received any antibiotic indicates that the disease signal resided entirely in the perturbed microbiota, and that it could cross generations. These findings in model systems support a causal role of antibiotic exposures in the current pandemics of metabolic and immunologic diseases, and are consistent with cumulative (communicable) intergenerational effects.

Talk Title To Be Announced

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The Microsetta Initiative: Creating a Map of the Global Gut Microbiome

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Populations differ in host genetics, diet, lifestyle and environment. Within that variation, unattributed factors drive variation in the gut microbiome, a result that has been replicated by multiple independent research groups. However, the data from these studies are often not directly comparable for technical reasons. Here, we bring together data from The Microsetta Initiative, which spans multiple populations, and integrate these data in a meta-analysis with other population datasets that also used the Earth Microbiome Project protocols. The combined analysis spans over 30,000 samples. We tested for technical vs. biological determinants of microbiome structure across populations, and constructed a hierarchy of population similarity. Specifically, 16S rRNA V4 amplicon data from The Microsetta Initiative and other studies using common molecular protocols were integrated using Qiita. With previously recorded sample collection and sample storage information, we tested whether population effects are readily driven by sample handling practices or true biological signal. Finally, using the subset of countries represented by The Microsetta Initiative, we determined which health and lifestyle effects tracked by a participant questionnaire translate between populations. These data recapitulate and extend Yatsunenko et al. (2012, Nature), showing that European, Australian, and North American populations are more similar to one other than each is to South American or sub-Saharan African populations. Unexpectedly, individuals from Bangladesh, the Philippines and Iran exhibit an intermediate relationship between Western and non-Western groupings. Defining how populations relate is a critical step in translating microbiome results. This backdrop carries medical implications, as it is presently unknown whether such massive differences in microbial community composition among populations have the potential to modulate microbiome therapies.

Talk Title To Be Announced

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Talk Title To Be Announced

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Poster Abstracts

Human Gut Microbiome Mediates Colonization Resistance to *Vibrio cholerae*

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The objective of our research is to characterize how the native microbial community of the human gastrointestinal tract, the gut microbiome, affects the ability of the host to resist colonization by pathogenic bacteria. Specifically, my research focuses interactions between the gut microbiome and the diarrheal pathogen *Vibrio cholerae*, which causes the devastating diarrheal disease cholera that affects millions of people across the globe and causes over 100,000 deaths yearly. Using a variety of *in vitro* and *in vivo* models, we are testing our hypothesis that microbial community configuration and differences in that configuration can drive resistance to infection by *V. cholerae*. To examine the role of human gut microbes in colonization resistance, we have modified the common suckling mouse model of infection followed by gavage of cultured human bacteria. We constructed a "susceptible" microbiome composed of microbes commonly seen in malnutrition or diarrhea-associated human microbiomes, and a "resistant" microbiome composed of common taxa in normal gut commensal communities. We then evaluated the effects of these different model human gut microbiomes on *V. cholerae* colonization. Mice containing the model susceptible microbiome were indeed highly susceptible to oral *V. cholerae* infection while mice bearing the resistant model microbiome were 100-fold more resistant. Co-inoculation with susceptible and resistant microbial mixes recapitulated the resistant phenotype. Our experimental pipeline establishes the basis for a mechanistic dissection of pathogen-microbiome interactions during colonization, and the consequences of these interactions for disease susceptibility and dissemination. Our results suggest that these interactions may have important effects for both colonization and post-colonization behavior by *V. cholerae*. We hope that these studies can identify next generation human gut probiotics or prebiotics that can act as prophylactics for *V. cholerae* infection.

Altered Circadian Rhythm Cycling of Microbes and Metabolites in a Murine Model of Obstructive Sleep Apnea

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Approximately half of patients with cardiovascular disease (CVD) have obstructive sleep apnea, characterized by intermittent hypoxia and hypercapnia (IHC), which significantly increases its morbidity. Both dysbiosis and circadian rhythm disruption are known to contribute to the pathogenesis of CVD. Atherosclerosis-prone 10-week-old male ApoE-/- mice on an atherogenic diet were housed in computer-controlled atmosphere chamber system under IHC or normal air conditions. After 6 days in the chambers, fecal samples were collected every 4 hours for 24hrs. The gut microbiome and metabolome were then characterized using 16S rRNA amplicon sequencing and untargeted liquid chromatography-tandem mass spectrometry, respectively. There are marked compositional changes in the cycling of both microbial and chemical species in the gut. The Lachnospiraceae family of Class Clostridia, known to modify bile acids, generally had robust cycling under normal conditions ($p<0.05$) but dampened cycling in IHC conditions. Interestingly, atherosclerosis-associated *Ruminococcus* sp. was not shown to have any cyclicity during normal conditions but did cycle during IHC conditions resulting in higher relative abundances during both day phase ($p=0.0002$) and night phase ($p=0.01$). *Akkermansia* sp., which is thought to be protective against CVD, revealed robust cyclicity under both conditions but significantly decreased levels at night for mice exposed to IHC conditions ($p=0.0004$). Furthermore, IHC mice had a significantly increased production of both primary ($p=0.01$) and secondary bile acids ($p=0.005$) during the night phase. Mice under normal air conditions had no difference in primary or secondary bile acid production during either day or night phase. Thus, IHC induces significant changes in microbe-host circadian dynamics that are associated with pro-atherosclerotic shifts, including bile acids, which could potentially affect host metabolism through FXR and TGR5 signaling mechanisms.

Promoting Healing-Macrophage Plasticity and Systems Innovation in Veteran Wound Care

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As of 2013, there were nearly 30 million Americans with diabetes; that's one in ten adults, but by 2050 estimates from the American Diabetes Association indicate that one in three adults (~350 million Americans) will be diabetic. At current rates of amputation (~25%), this population will have to absorb the social and economic burden of over 85 million diabetic amputees. While normal wound healing proceeds through a well-described iterative process, in non-healing wounds this stalls at the transition from inflammation to tissue repair. Our hypothesis is that macrophage functional plasticity is key to this transition and directly facilitates shifting the wound environment from pro-inflammatory to anti-inflammatory, proactively antimicrobial to fostering tissue repair. Our understanding of macrophages has altered from being garbage collectors to keystone species in the functional ecology of wound-healing, as well as such diverse fields as embryonic development and oncology. In our lab, an ex vivo macrophage culture model with six macrophage functional phenotypes not only provides the most comprehensive model system for understanding the contribution of metabolism to macrophage functional plasticity, but also enables our lab to probe how this functionality changes with environmental disruptions such as pathogenic biofilm co-culture, dysregulated systemic metabolic disease, and the spectrum of host diabetic status. By integrating metabolomics into our systemic characterization of these phenotypes, we have developed quantifiable metrics that not only define metabolic and functional phenotype, but also provide insight into the cellular functions fundamental to macrophage plasticity. We will present on how metabolomics is fundamentally changing our understanding of immunomodulation in diverse macrophage populations and how that deeper understanding is informing our long-term goals of developing novel diagnostic and therapeutic approaches to wound healing.

Host Genetic Effects on Gut Microbiome Composition and Metabolites in 1,200 Outbred Rats, and Associations with Drug-abuse Behaviors and Metabolic Traits

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Understanding how genetics and gut microbiome interact to affect physiology and behavior poses a formidable challenge. We leveraged an ongoing study of thousands of outbred Heterogeneous Stock (NIH-HS) rats to tackle this issue. More than 7 million genetic variants segregate in the NIH-HS population, giving rise to variation in rat behaviour, metabolism and gut microbiota composition. The NIDA center for GWAS in outbred rats (http://ratgenes.org/) has characterised the genetic makeup of each rat and collected numerous addiction-related and metabolic phenotypes. For this study we characterised gut microbiome composition and gut metabolites in a subset of 1,200 rats using 16S sequencing, and LC-MS-MS. Rats were housed in two different housing colonies and, as expected, we observed general differences in gut microbiome composition between the two colonies. For each colony separately we quantified the overall contribution of host genetics to differences in gut microbiome (heritability), and found heritabilities of 0-20% for most taxa. Genome-wide association study (GWAS) of individual taxa revealed several genome-wide significant loci where candidate genes suggested insulin production and signalling are key mechanisms underlying host genetic effects on microbiome composition. While an effect of the microbiome on insulin metabolism has been reported before, our unprecedented findings suggest that the opposite direction of effect is also important. Follow-up experiments are underway to further investigate effects of insulin on gut microbiome.16S and metabolomics data were generated through the Center for Microbiome Innovation Seed Grant Program.

Antibiotic-induced Microbiome Depletion Alters Metabolic Homeostasis by Affecting Gut Signaling and Colonic Metabolism

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Antibiotic-induced microbiome depletion (AIMD) has been used frequently to study the role of the gutmicrobiome in pathological conditions. However, unlike germ-free mice, the effects of AIMD on host metabolismremain incompletely understood. Here we show the effects of AIMD to elucidate its effects on gut homeostasis,luminal signaling, and metabolism. We demonstrate that AIMD, which decreases luminal Firmicutes andBacteroidetes species, decreases baseline serum glucose levels, reduces glucose surge in a tolerance test, andimproves insulin sensitivity without altering adiposity. These changes occur in the setting of decreased luminalshort-chain fatty acids (SCFAs), especially butyrate, and the secondary bile acid pool, which affects whole-bodybile acid metabolism. In mice, AIMD alters cecal gene expression and gut glucagon-like peptide 1 signaling.Extensive tissue remodeling and decreased availability of SCFAs shift colonocyte metabolism toward glucoseutilization. We suggest that AIMD alters glucose homeostasis by potentially shifting colonocyte energy utilizationfrom SCFAs to glucose.

Shotgun Metagenomics of Fecal Samples Reveals Subgroups in Psoriasis Patients with Distinct Microbial and Host Signatures

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Psoriasis is an immune-mediated, inflammatory skin disease that affects 1-3% of the world's population. Psoriasis is characterized by hyperproliferation of keratinocytes driven by an overactive Th17 response. Besides cutaneous manifestations, psoriasis patients are at higher risk of developing psoriatic arthritis, cardiovascular disease, type II diabetes, and inflammatory bowel disease, suggesting a systemic component of psoriasis pathogenesis. The underlying mechanism of how psoriasis is comorbid with many systemic diseases is not yet well understood. Gut microbial dysbiosis has been linked to many of these psoriasis comorbidities and functional studies have demonstrated the role of the microbiome in some of these diseases. To understand the potential contribution of gut microbiome to pathogenesis of psoriasis and its comorbidities, we performed shotgun sequencing on fecal samples from 33 psoriasis patients and 15 healthy controls to profile both microbial taxonomical composition and functional capacity. In addition, we collected transcriptomic profiles from sigmoid colonic biopsies to provide information about host-microbe interactions. Although the overall structure of microbiome and transcriptome is very similar between psoriasis patients and healthy controls, we identified a group of microbial gene families that are differentially abundant in psoriasis gut. Interestingly, the abundance of these gene families group psoriasis patients in our cohort into three distinct enterotypes. Enterotype 1 consists of some psoriasis patients and most of the healthy controls (15 psoriasis and 14 healthy subjects) while both enterotype 2 (8 psoriasis) and enterotype 3 (8 psoriasis and 1 healthy subjects) are dominated by psoriasis patients. Differential analysis revealed microbial signatures and host transcriptomic signatures that are associated with each enterotype. Enterotype 1 has higher *Phascolarctobacterium succinatutens* and *Turicibacter sanguinis*. Enterotype 2 is characterized by more abundant *Bacteroides xylanisolvens* and less abundant *Streptococcus thermophilus.* Enterotype 3 has low abundance in *Ruminococcaceae bacterium* D16 compared to the other two groups. Enterotype 2 has lower capacity in arginine and polyamine biosynthesis compared to the other two groups. Intriguingly, subjects with different enterotypes have distinct transcriptomic profiles suggesting potential host-microbe interactions. Overall, our study showed that there are distinct subgroups within psoriasis patients based on the functional capacities of their fecal microbiome. We identified distinct host gene expression associated with each subgroup, suggesting that these different enterotypes may help shape the host environment, or vice versa.

Insulin Resistance Protects against *Citrobacter rodentium* Infection

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Diabetes is a metabolic disorder caused by the body's inability to synthesize (type 1) or respond to (type 2) insulin, a hormone produced by the pancreas to regulate blood glucose levels. Diabetes decreases lifespan and is associated with a number of complications including cardiovascular disease, neuropathy and nephropathy. Research shows that insulin resistance, a metabolic state which precedes diabetes, can be modulated by inflammation and the gut microbiota. Interestingly, insulin resistance is an evolutionarily conserved response among animals and it has been proposed that short-lived insulin resistance may be advantageous during times of starvation, trauma, and infection, likely by diverting limited nutrients for tissue repair and survival. To investigate the physiological role of insulin resistance during bacterial infection, we employed the *Citrobacter rodentium*-mouse infection model, which is commonly used to study gastrointestinal diseases. We employed both dietary and pharmacological methods to transiently induce insulin resistance in mice. Our findings show that insulin resistance protects mice from intestinal inflammation caused by *C. rodentium*. Importantly treatment with metformin, a drug that improves insulin sensitivity, worsens infection. Surprisingly, insulin resistance alters *C. rodentium* virulence gene expression, allowing mice to persistently carry *C. rodentium* in the gut and drive *C. rodentium* to an attenuated state. These mice are also protected against subsequent infections by *C. rodentium*, even when insulin sensitivity returns. Further research will be required to elucidate the mechanisms by which insulin resistance protects or harms the host during infection with *C. rodentium* and other pathogens. Together these findings support the notion that insulin resistance is a cooperative defense mechanism to attenuate pathogens and prevent infection-related damage. Therefore, understanding the role of host metabolism in host-microbe interactions will be helpful in devising novel strategies to counteract microbial infections and to engineer microbes to alleviate human metabolic syndromes.

Biogenesis and Structure of a Type VI Secretion Baseplate

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Bacterial antagonistic relationships are considered as being a critical factor in defining the dense ecosystem of the intestinal microbiota. Such mechanisms rely on a broad repertoire of secretion machineries dedicated to toxins export. Among these molecular weapons, the type VI secretion system (T6SS) is a contractile nano-machine found in 25% of Gram (-) bacteria that targets both prokaryotic and eukaryotic cells. T6SS assembles a cytoplasmic bacteriophage-related-tail structure anchored to the cell envelope by a membrane complex. The tail is composed of an inner tube wrapped by a sheath whose contraction is thought to translocate the tube and tip proteins, and puncture the prey's cell wall. The tail is built from an assembly platform, the baseplate, that connects the tail to the membrane complex, hence serves as an evolutionary adaptor. Here we define the biogenesis pathway of the T6SS baseplate and highlight the requirement of a stable intermediate complex, the wedge, to initiate baseplate assembly. We also report for the first time the cryo-electron microscopy (cryo-EM) structure of the T6SS wedge protein complex from the pathogenic bacteria entero-aggregative *Escherichia coli* (EAEC) and unveil molecular details of the whole T6SS baseplate architecture. Altogether, this study revealed detailed insights into the biogenesis and function of a key factor involved in microbial competition and paved the way toward molecular scale manipulation of such devices to serve therapeutic applications in Human health.

Light Controls Dimorphic Transitions in *Histoplasma capsulatum*

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Histoplasma capsulatum (Hc), a dimorphic fungal pathogen, is the most common cause of fungal respiratory infections in immunocompetent hosts. In the soil, Hc grows in a filamentous form. Once inhaled, the filaments and associated spores convert to a yeast form that expresses virulence genes and causes disease. Host temperature is the key signal that triggers this switch in Hc. In the laboratory, cells grow in the filamentous form at room temperature (RT) and in the yeast form at 37^{o}C. In previous studies, we identified four key regulators, Ryp1,2,3,4, that are required for yeast-phase growth at 37^{o}C. Ryp1 and Ryp4 are transcriptional regulators that are conserved in the fungal kingdom. Ryp2 and Ryp3 are both members of the Velvet protein family, which regulates development and secondary metabolism in many fungi. Interestingly, in *A. nidulans*, Velvet family proteins can form a complex with various photosensors to regulate sexual and asexual development in response to light. In Hc, the role of light in regulating cell morphology and virulence has not been fully explored. In this study, our goal is to investigate its role in the regulation of phenotypic traits in Hc. In our preliminary studies, we found that conidiation was dramatically affected by light. Additionally, we found that the yeast-to-filament transition of Hc is light-regulated and the morphological changes were accompanied by changes in transcript levels of *RYP* genes. We found that *RYP2* and *RYP4* transcript levels were lower in light compared to dark conditions at RT, suggesting that accumulation of these transcripts is light-responsive. Overexpression of *RYP2* or *RYP4* in light conditions was sufficient to prevent the yeast-to-filament transition at RT. Taken together, our results suggest that dark promotes (or light inhibits) yeast-phase growth via regulation of Ryp transcript accumulation, and that light is an important environmental signal for the physiology of Hc.

Watching and Modeling Horizontal Gene Transfer in Microbial Communities

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Horizontal gene transfer (HGT) plays a pervasive role in microbial evolution. HGT also plays a villain in medical settings, because it enables bacteria to swap and collect antibiotic resistance genes. Most multi-drug resistant bacteria develop via HGT, rather than by evolving resistance to dozens of antibiotics *de novo*, and an increasing number of these "superbugs" can shrug off every drug in our arsenal. One mechanism of HGT is natural competence, in which bacteria pull in and incorporate DNA from the external environment. However, the population-level dynamics of natural competence in microbial communities, as well as the impact of parameters, such as DNA degradation in the extracellular space, have remained unclear. Using a model community of *Acinetobacter baylyi* and *E. coli*, we showed that Acinetobacter could dramatically increase acquisition of *E. coli* genes by killing nearby *E. coli* cells. We explored the dynamics using live cell microscopy in microfluidic chips, in which we observed multiple HGT events, as well as through mathematical modeling, using a novel method to measure biophysically meaningful competence parameters. The model accurately captured the population dynamics of killing and HGT in developing biofilms in a variety of conditions. Perhaps surprisingly, DNA released in situ through cell lysis was no less available for uptake than *in vitro* purified DNA. Additionally, predator cells gained privileged access to DNA released from lysed prey cells, even in the presence of DNase. Finally, we are exploring these killing and HGT dynamics within a model animal microbiome, in particular, the guts of living *C. elegans*.

Opioid Treatment Drives a Shift of Enteric Bacteria, *Actinobacteria* and *Verrucomicrobia*, *In vivo* and Strengthens the Intestinal Barrier *In vitro*

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Clinical use of opioids is associated with severe GI symptoms, potentially linked to alterations in the composition and distribution of the microbiota (dysbiosis) and changes in the intestinal epithelial barrier. After opioid administration mice show a dysregulated immune response, increased intestinal barrier permeability, and bacterial translocation. The effects of opioids on human fecal microbiota and the intestinal barrier are not well understood. Using Deep Gene Sequencing, we characterized the fecal microbiota of individuals on methadone treatment. Our findings show no global differences in the alpha and beta diversity between control and methadone treatment. However, we identified two microbial phyla with altered relative abundance in the methadone group: increased *Actinobacteria* and decreased *Verrucomicrobia*, which in the healthy gut are important for intestinal barrier homeostasis and mucus degradation, respectively. To study the direct effects of opioids on the intestinal barrier, we developed an *in vitro* model using human intestinal epithelial cells that establish an intact, semi-permeable monolayer. Measuring Transepithelial Electrical Resistance we observed that mu-Opioid Receptor (OR)- and kappa-OR-agonist treatment strengthened the intestinal barrier in a dose and time-dependent manner, while delta-OR agonist treatment had no effect. Due to our laboratory's observations that delta-OR agonists regulate human T lymphocyte activation, we used an established epithelial/T cell co-culture model to demonstrate that delta-OR agonist treatment strengthens the intestinal barrier by acting indirectly on T lymphocytes. Together these results indicate that exposure to exogenous opioids modulates the intestinal microbiome and epithelial barrier function in a cooperative manner.

Naturally Occurring Pathogens in Naked Mole Rats

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Tissue stress and damage are a consequence of a diverse array of diseases including infections, cancer, metabolic disease and aging. Low oxygen, metabolites and energy supply and build up of toxins are common stressors for tissues during disease. The immune system regulates both the fight against a threat, the protection against the collateral damage and the return to homeostasis. It is also becoming increasingly clear that the microbiome and its interactions with the host also play a key role in defending homeostasis during disease. Understanding the physiological mechanisms that regulate these processes is central to promote health in diseased patients. Naked mole rats (NMR) have evolved to tolerate extreme tissue environments. NMRs are eusocial mammals that live in large colonies with a strict reproductive division of labor. NMRs are also subterranean and thus lack circadian rhythms and vitamin D. In their burrows, the temperature is 30ºC year-round and thus NMRs have no fur and a poor ability to defend themselves from cold. NMRs live in low O2 and high CO2 conditions and are able to survive O2 absence for up to 30min. Moreover, NMRs live up to 30 years without a significant decay in fitness and they are resistant to cancer. We have established a NMR facility and have identified naturally occurring NMR pathogens in an individual that died of sepsis and a NMR parasite in several individuals with subclinical symptoms to study how the immune system and the microbiome interact to withstand extreme environments.

The Woodrat-gut Microbiota as an Experimental System for Understanding Microbial Detoxification of Dietary Compounds

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The microbial communities inhabiting the alimentary tracts of mammals, particularly those of herbivores, are estimated to be one of the densest microbial reservoirs on Earth. The significance of these gut microbes in influencing the physiology, ecology and evolution of their hosts is only beginning to be realized. To understand the microbiome of herbivores with a focus on nutritional ecology, while evaluating the roles of host evolution and environment in sculpting microbial diversity, we have developed an experimental system consisting of the microbial communities of several species of herbivorous woodrats (genus *Neotoma)* that naturally feed on a variety of dietary toxins. We designed this system to investigate the long-standing, but experimentally neglected hypothesis that ingestion of toxic diets by herbivores is facilitated by the gut microbiota. Like several other rodent species, the woodrat stomach has a sacculated, nongastric foregut portion. We have documented a dense and diverse community of microbes in the woodrat foregut, with several genera potentially capable of degrading dietary toxins and/or playing a role in stimulating hepatic detoxification enzymes of the host. The biodiversity of these gut microbes appears to be a function of host evolution, ecological experience and diet, such that dietary toxins increase microbial diversity in hosts with experience to these toxins while novel toxins depress microbial diversity. Moreover, dietary toxins appear to have a greater impact than evolutionary history on microbial communities. These microbial communities are critical to the ingestion of a toxic diet as reducing the microbial community with antibiotics impairs the host's ability to feed on dietary toxins. Furthermore, the detoxification capacity of gut microbes can be transferred from *Neotoma* both intra and interspecifically to naïve animals that lack ecological and evolutionary history with these toxins. In addition to advancing our knowledge of complex host-microbes interactions, this system holds promise for identifying microbes that could be useful in the treatment of diseases in humans and domestic animals.

Antiviral Consequences of an Evolutionary Arm Race between the Family of ISGs: IFITs and RNA Viruses

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Host-virus conflicts have recurrently driven mammalian evolution, resulting in complex, multi-faceted immune defenses to prevent virus infection. Some of the most highly upregulated genes IFN-stimulated genes (ISGs) in response to viral infection are the IFIT family of genes, which are also some of the most rapidly evolving human genes. IFITs are known to inhibit many different human viruses, likely through specific binding of viral RNA and prevention of viral mRNA translation. However, the molecular and biochemical mechanisms by which individual or complexes of IFITs distinguish “self vs. non-self” RNA remains largely uncharacterized. We propose that IFITs can directly interact with each other and with RNAs in-vivo leading to a broad but specific antiviral response. However, because IFITs may form heteromers, their binding specificities are expected to be complex. We predict that IFIT-IFIT interactions have evolved specific regulatory functions against certain viral RNAs by recognizing viral RNA modifications or RNA structure.

Functional Screening of Bacterial Products Identifies Species that Promote DNA-replication in Human Cells

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The bacterial mechanisms involved in colorectal cancer (CRC) are still poorly understood, mainly because it remains difficult to separate cause from consequence. The primary means through which microbes communicate with the host and influence physiology is via secreted metabolites (small molecules and peptides). In order to investigate the bacteria-to-host communication, we have designed a functional screening method that evaluates the effect of bacterial products on human cell cycle activity (DNA-replication, cell proliferation and cell death). The results show that intestinal tumor mucosal adherent bacteria *E. faecalis*, *E. faecium* and *S. agalactiae* all promote significant increases in DNA-replication in both primary human cells and colonocytes of non-cancerous origin. These findings illustrate successful application of our screening method and demonstrate a functional overlap in bacterial products from different species, and their potential for modifying human cell responses.

Novel Microbial Conjugated Bile Acids in Colon Cancer

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Colon cancer (CRC) remains a significant public health concern and is the third-leading cause of cancer-associated deaths in the US. While the underlying causes are poorly understood, genetic and environmental factors are known to contribute to disease progression. We recently identified the bile acid (BA)-FXR axis as a point of convergence for genetic and dietary risk factors in CRC. As early sensors of dietary status, BAs are critical for host physiology, in part through their activation of Farnesoid X Receptor (FXR). However, disease associated changes in the gut microbiome and the consequential effects on cancer progression have not been clarified. In this study, we determined the effects of genetic (APCmin mice) and dietary (high fat diet) factors on the composition and transcriptional profiles of the microbiome. While diet appears a major determinant of the microbiome, the host physiology appears to be shaped more by genetics. In particular, we find that the levels/composition of serum primary BAs correlate with the host status, whereas the secondary BAs in the cecum affect the microbiome. In addition, we identify select host genes that correlate with the microbiome changes. Strikingly, we identified novel microbially modified BAs in the cecum but not in serum samples. These novel BAs, enriched in APCmin mice and under HFD, are positively associated with bacteria previously linked to TMAO production. Intriguingly, some are potent activators for FXR and cell surface bile acid receptor, TGR5. These studies support the importance of the gut microbiome and its modification of the BA pool in disease progression, and suggest that detailed characterization of the changes will hasten the development of novel biomarkers and therapeutics for CRC.

Phylogeny, Abundance, Cell Wall Architecture and Host Lifestyle Drive Extensive Gene Transfers in Each Person's Gut Microbiome

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Previous studies have shown the importance of horizontal gene transfer (HGT) in shaping human gut bacteria. However, whether genomes acquire new genes daily, or whether millennia separate transfer events, remains unknown despite its importance on gut microbiome function and human health. Here, we generate thousands of gut bacterial genomes across hundreds of species, from different human populations worldwide, to show that the timescale of these HGTs is short, and that they frequently occur in each person's gut microbiome. We reveal that within people HGT is a function of species abundance and bacterial cell wall architecture, independently of phylogeny. We further find that bacterial species that engage in HGTs within people are the same across different people. Finally, we show that the gut bacteria of individuals with industrialized lifestyle exchange genes more frequently than in non-industrialized populations, suggesting that host lifestyle also drives HGTs. As compared to the functions recently exchanged in bacteria sampled across people, functions exchanged within urban US people are enriched in antibiotic resistance. Our study sheds new light on the factors influencing how gut bacteria acquire new functions during our lifetime. It also shows the importance of sampling and characterizing the gut microbiome biodiversity of underrepresented traditional populations. I will finish my talk presenting our new initiative, the Global Microbiome Conservancy, a non-profit initiative that aims to preserve the full biodiversity of the human gut microbiome in a biobank of bacterial isolates and genomes to advance human health.

Available Carbon Source, Lactate, Promotes the Resistance of *Helicobacter pylori* Against Complement

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Helicobacter pylori (*H. pylori*) is gram-negative bacterium, colonizing the stomach of nearly half the world's population. *H. pylori* can live asymptomatically, acting like a member of the microbiota, but is also recognized as one of the leading causes of stomach inflammation and ulcers, and a risk factor for stomach cancer. *H. pylori* can live within the mammalian stomach for their whole life, without activating a robust immune response. *H. pylori* has evolved sophisticated strategies for its long-term colonization. One of the successful strategies to thrive in the austere stomach environment is to utilize host metabolic products to modify the bacterial environment. For example, *H. pylori* protects itself from low stomach pH by converting host-derived urea to ammonia. *H. pylori* also can defend itself from NO stress by utilizing arginine. However, it is not fully understood how *H. pylori* resists host complement, one of the common strategies the host applies to eliminate invading pathogens at mucosal surfaces.Complement, as part of host innate immune system, is commonly observed in serum and the stomach mucus layer. *H. pylori* is killed by complement *in vitro*, and it seems likely that *H. pylori* must evade complement to initiate or maintain colonization. *H. pylori* uses chemotactic motility for colonization, and recent work has shown that it seeks out lactate using chemotaxis. We thus examined the benefits conferred by lactate. We report here that lactate can enhance *H. pylori*'s ability to survive in the presence of complement. This protection requires lactate uptake via lactate permease (LctP); *H. pylori* survives complement exposure less well if lctP is deleted. Furthermore, we noticed that lactate is able to protect all tested *H. pylori* strains from complement killing. To better understand the role of lactate in the promotion of complement resistance, we pre-treated *H. pylori* with physiological concentration of lactate for long term (overnight supplementation) or short time exposure (1 hour) before human serum exposure. We found that short time pre-treatment can efficiently to trigger complement resistance, but the protection is short lasting. In contrast, long exposure to lactate resulted in extended complement resistance. To gain insight into mechanisms of complement resistance, an *H. pylori* transposon library was screened for mutants with high levels of complement resistance. To date, ten complement resistant mutants were identified, that mapped to four genes. These genes encode cell surface structures, and the gene's roles are being verified by clean mutations. Overall, our data suggest a model in which host lactate is taken up by *H. pylori* and used to promote complement resistance, possibly by upregulating key surface exposed proteins with a temporal pattern. Our investigation will contribute to a better understanding of how *H. pylori* survives the challenges of mucosal colonization in the stomach environment to result in chronic colonization.

Epigenetic Deregulation Renders Loss of Intrinsic Anti-Viral Activity of Cancer Cell

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ICP0 is an essential viral protein of Herpes Simplex Virus 1 for counteracting against cellular intrinsic anti-viral immunity. Loss of ICP0 extremely reduces plaque forming efficiency of HSV-1 in most human cells that are highly permissive to wild type HSV-1. However, a few specific cancer cells are highly permissive to both of wild type and the ICP0 mutant viruses due to unknown mechanism. Here we try to find a transcriptional signature of cancer cells permissive to the ICP0 mutant HSV-1, and also demonstrate a potential driving factor that renders the permissiveness. To systematically search permissive cancer cells over resistant cancer cells, we developed a fluorescent virus by CRISPR knock-in of mScarlet red fluorescent protein-expressing cassette into the viral genome. Using the mScarlet HSV-1 in incucyte system, more than 30 cancer cell lines were screened to be stratified as permissive or resistant cells in terms of the ICP0 mutant HSV-1. Then, cell lines were analyzed by RNA-seq analysis for finding transcriptional signature of the permissiveness. Although there was rarely found any consensus of transcriptional signature related to conventional genetic pathways, a few pericentromeric satellite RNA and MAGE genes were consistently up-regulated in permissive cell lines compared to resistant cell lines. Coincidently, upregulation of pericentromeric satellite and MAGE genes are both related to loss of DNA methylation at their loci arising through specific developmental stages or in diseases, and some permissive cancer cells were already known to have hypomethylated genome compared to resistant cells. Accordingly, we revealed that double knockout of DNMT1 and DNMT3B could confer permissiveness against the ICP0 mutant HSV-1. The study demonstrates that deregulated DNA methylation can inhibit cellular intrinsic anti-viral immunity, and proves ICP0 HSV-1 as a promising therapeutic targeting cancers with hypomethylated genome.

C. difficile-associated Antibiotics Prime the Host for Infection by a Commensal-independent Mechanism

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Clostridioides difficile infection (CDI) is a major public health threat; the CDC estimates CDI accounts for 29,000 deaths and over 4 billion dollars in related healthcare costs annually. The most clinically relevant risk factor for CDI is recent antibiotic treatment. Though many broad-spectrum antibiotics significantly disrupt the structure of the gut microbiota, only certain ones increase CDI risk. This discrepancy led us to hypothesize that host-dependent factors may also contribute to CDI following antibiotic treatment. To test this, we used our established *in vitro* human gut epithelial system to treat mature mucosal barriers with antibiotics having CDI odds ratios ranging from one to 17. To gain an unbiased understanding of the system's response, we quantified transcriptional changes using RNA-seq. A Self-Organizing Map was employed to discover shared patterns of gene expression, which suggested CDI-associated antibiotics might have decreased innate immune and mucosal barrier functions. To validate these predictions, we used Luminex, phagocytosis and bacterial killing, qPCR, and Alcian blue assays, which confirmed a marked loss of barrier and immune function with CDI-associated antibiotic pretreatment. This was distinct from pretreatment with an antibiotic unassociated with CDI, which did not alter innate immune or mucosal barrier function. Finally, we determined that these changes have consequences for CDI: pretreatment with CDI-associated antibiotics sensitized mucosal barriers to *C. difficile* toxin activity compared to an antibiotic unassociated with CDI and controls. These data implicate commensal-independent host changes in the increased risk of CDI with specific antibiotics. Future efforts will address the molecular mechanism of antibiotic-induced host sensitization to *C. difficile* toxin and explore host-directed therapies for CDI.

Alcohol Exacerbates Intestinal Dysbiosis in a Mouse Model of Colitis

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Alcohol has been shown to trigger ulcerative colitis (UC) disease flares and onset, though the underlying mechanism has not been established. In this study, we assessed the effect of alcohol on the intestinal microbiome using a mouse model of dextran sulfate sodium (DSS)-induced colitis. UC patients develop an intestinal dysbiosis, characterized by increased relative abundance of proinflammatory bacteria. Our hypothesis is that alcohol exposure would further alter the structure of the microbiome during intestinal inflammation. To test this, male C57BL/6J mice were administered either normal drinking water or a 2% (w/v) solution of DSS ad libitum for 5 days. On day 5, DSS was stopped and mice received a gavage of either 3g/kg ethanol or water per day until day 7. Mice were euthanized three hours after the last gavage. In total, four treatment groups were used - Control+Vehicle (Ctrl+V), Ctrl+Ethanol (Ctrl+E), DSS+Vehicle (DSS+V), DSS+Ethanol (DSS+E). Microbial communities in cecal contents were analyzed using DNA extraction, high-throughput sequencing of 16S ribosomal RNA gene amplicons, and targeted quantitative PCR. Global community changes revealed significant differences in alpha diversity between treatments. Cecal content from mice in the DSS+E treatment had lower microbial diversity relative to those in the DSS+V treatment. Beta diversity, assessed by Unifrac, was globally significant ($p<0.01$) between treatments, and there was a trend for significance in pairwise comparison between DSS+V and DSS+E treatments ($q=0.084$). Bacteria from the family Enterobacteriaceae were significantly higher in DSS+E, as assessed by the linear discriminant analysis effect size (LEfSe) method. These global community and specific taxonomic changes suggest a synergetic effect between colitis and ethanol, leading to putative dysbiosis. Such dysbiosis may explain the ability of alcohol to worsen intestinal inflammation. (Support: R21AA022324, T32AA013527, F30AA027442, F31AA025536).

Investigating Microbiota Species that Influence Postnatal Growth

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The intestinal microbiome influences host gastrointestinal development, nutritional status, and mucosal immunity, and thus plays important functions in health and disease. However, under some conditions, constituents of the microbiota can become pathogenic to the host and cause disease. The factors that maintain the mutualistic relationship between the host and microbiota and those that drive microbial virulence remain incompletely understood. To determine such factors that promote microbial virulence, we performed a candidate screen using different strains of *Escherichia coli* to observe the effect of the colonization of individual *E. coli* strain on host development. We identified an *E. coli* strain that behaves as a commensal when colonizing the adult murine intestine, but when colonizing the neonate intestine, it displays pathogenic behavior by impairing the host's neonatal development. The *E. coli*-induced growth retardation results in decreased circulating insulin-like growth factor 1 (IGF-1) levels and increased pro-inflammatory cytokines. Supplementation of recombinant IGF-1 is sufficient to ameliorate growth impairment, but not through genetic ablation of an inflammatory mediator. Furthermore, the *E. coli*-colonized neonates display reduced feeding during lactation period and cross-fostering by germ free dam rescues *E. coli*-induced growth inhibition. These results show that the *E. coli* colonization affects maternal factors to inhibit neonatal milk intake, which causes a state of undernutrition that downregulates early developmental processes.

Unraveling the Role of Histo-Blood Group Antigens in Shaping the Gut Microbiota and Susceptibility to Gastrointestinal Pathogens

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Diarrheal diseases are the second leading cause of death in children under five. Blood group antigens are a polymorphic trait that have been associated with altered susceptibility to infection. In the PROVIDE cohort of 700 Bangladeshi urban slum-dwelling children, we sought to determine if blood group antigens were associated with altered susceptibility to gastrointestinal pathogens. We found that children negative for Lewis A antigen (encoded by FUT3) were significantly more likely to have *Shigella spp*. diarrhea in the first year of life (p=0.02, log-rank test). Protection was not associated with secretor status (FUT2). Interestingly, the presence of Lewis A antigen in mother's breast milk was not associated with protection (p=0.5, log-rank test). In an *in vitro* model of *Shigella flexneri* invasion, blocking H antigen, with a monoclonal antibody significantly decreased the number (p=0.046) and size of infection foci (p=0.007) in HT29 cells. This suggests that H antigen may serve as a receptor for *Shigella flexneri* invasion of epithelial cells, and explain why Lewis A (-) children are more susceptible. We are currently conducting fecal metagenomics and metabolomics from the PROVIDE children to test for additional contributions of histo-blood group antigens to altered structure and/or functional capacity of the gut microbiota.

Novel Foxp3 Regulators Discovered by a Genome-wide CRISPR Screen

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Regulatory T cells (Tregs) play a pivotal role in suppressing auto-reactive T cells and maintaining host immune homeostasis. Tregs development and their suppressive function are dependent on a transcription factor called Forkhead box P3 (Foxp3). Understanding the mechanism that regulating Foxp3 expression can potentially lead to the development of therapeutics to manipulate Tregs for treating autoimmune diseases or enhancing cancer immunotherapy. Although a large body of works has been devoted to unraveling the molecular mechanisms controlling Foxp3 expression, a systemic, genome-wide approach is still needed to understand Treg development and function. In this study, we developed tools to perform a genome-wide CRISPR-Cas9 based screen to identify the regulators of Foxp3 expression in mouse primary T cells. The outcomes of this screen not only confirmed a number of known Foxp3 regulators but also revealed many novel factors that control Foxp3 expression. Gene ontology analysis showed that positive regulators of Foxp3 are highly enriched with genes involved in chromatin remodeling including subunits in the SWI/SNF complex and SAGA complex, indicating an underappreciated role for chromatin organization in Treg development. Among the negative regulators of Foxp3, a set of genes involved in cell cycle progression are highly enriched, suggesting active regulation of Foxp3 protein degradation during cell division. Taken together, our study generated a comprehensive picture of genes and pathways controlling Foxp3 expression, and uncovered multiple novel regulators that could be exploited to manipulate Tregs. The tools and strategy used in our study can also be applied to screen for factors determining other traits in mouse primary T cells.

The Role of the Microbiome in Immune Responses against *Vibrio cholerae*

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Despite improvements in treatment, the diarrheal disease cholera caused by *Vibrio cholerae* remains a significant global health burden. Several studies show that oral cholera vaccines elicit a reduced antibody response in populations from developing versus industrialized regions. We hypothesize that one source of variation in vaccine responses is differences in gut microbiome structure. Our studies examine the role of commensal microbes on vaccination, with a view to develop effective microbial interventions that can boost immune responses to vaccines.To examine the role of different human microbiomes, we assembled a "susceptible" community comprised of Streptococcus species, and a "resistant" consortium of Bacteroidetes and Firmicutes. These groups represent respectively an "unhealthy" community, similar to the post-diarrhea gut microbiome, and a normal adult microbiome. We used adult CD-1 mice treated with an antibiotic cocktail to deplete the murine microbiome, and then introduced our defined communities along with *V. cholerae*. We characterized changes in antibody response after Vibrio infection by ELISA and quantified the antibody efficacy by using an *in vitro* vibriocidal assay. Another measure of protection is the ability of antibodies from each of our microbiome contexts to limit infection of naïve animals. Suckling mice were gavaged with *V. cholerae* that had been incubated with purified antibodies from feces of adult mice infected with either microbiomes. While the susceptible group had higher vibriospecific IgA, the resistant group shows a ~ 6 log better difference in killing efficiency as compared to the susceptible group. We show that a standardized quantity of fecal antibody from animals with a resistant microbiome were better able to prevent infection in naïve animals, compared to the susceptible microbiome group. These data provide insights into how gut microbiome configurations contribute to variable mucosal vaccine responses.

AI-based NLP re-analysis of Microbial Metabolites Role in Host Disease Pathways Reveals Newly-associated Functions and Mechanistic Predictions

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Systems biology or phenome analysis relies on the integration of data across scales of biological data. In a metabolomics context, we group metabolites onto their biological metabolic pathway with the associated genes and proteins. Then, manual deciphering or interpretation of what role a pathway plays in the disease presentation is required. Incorporating AI (Artificial Intelligent)-NLP (Natural Language Processing)-based pathway and disease searches can be used to find the role of dysregulated biological pathways in the disease state efficiently. We have recently been re-analyzing metabolomics data sets associated with the gut microbiome and different diseases with the IBM Watson for Drug Discovery platform. Specifically, we take the dysregulated metabolic pathways from a comparison of healthy to diseased sample and search those against the disease of interest. We have found direct literature evidence for the function of these pathways in the disease that were not reported in the metabolomics study. Additionally, in some cases, we can make testable predictions about the mechanism of causation between the metabolite, dysregulated pathway and disease state.

Elucidating the Metabolic Response to *Legionella pneumophila* Infection in Young and Aged Mice

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Legionella pneumophila (Lp) is a bacterial pathogen that colonizes the lung by replicating in alveolar macrophages. Recent *in vitro* work has determined that Lp infection shifts macrophage metabolism to a glycolytic state which impairs clearance of the pathogen. The influence of Lp infection on *in vivo* systemic host metabolism, however, is unknown. The elderly are more susceptible to Lp infection than younger individuals, possibly due to defects in glucose metabolism, since 80% of the elderly (>65 years) display hyperglycemia and glucose intolerance. This project aimed to characterize young and aged hosts metabolic responses to Lp infection and impacts on disease outcomes. We found that intranasal infection of young (7-week-old) mice with Lp led to muscle and fat-specific weight loss, decreased core body temperature, and anorexia. At 48 hrs post-infection, young mice developed hypoglycemia, increased glucose tolerance, and underwent active lipolysis of white adipose tissue. Despite elevated obesity and hyperglycemia, aged mice (73-week-old) showed similar levels of hypoglycemia and glucose tolerance to young mice at 48 hrs post-infection, though, aged mice lost percent body mass at a slower rate and underwent less lipolysis of white adipose tissue than young mice. Analysis of serum prior to, during, and post-infection with Lp revealed age and infection-specific changes in circulating metabolites, with alterations in abundances of tricarboxylic acid cycle intermediates, amino acids, and numerous free-fatty acids. These results demonstrate that Lp infection alters host glucose metabolism, independent of age. However, changes in other metabolic parameters in response to Lp, such as lipid metabolism, are age-dependent. This suggests that deviations in host metabolism that occur with aging alter metabolism in response to pathogen challenge and may ultimately change the course and severity of an infection.

The Microsetta Initiative: Creating a Map of the Global Gut Microbiome

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Populations differ in host genetics, diet, lifestyle and environment. Within that variation, unattributed factors drive variation in the gut microbiome, a result that has been replicated by multiple independent research groups. However, the data from these studies are often not directly comparable for technical reasons. Here, we bring together data from The Microsetta Initiative, which spans multiple populations, and integrate these data in a meta-analysis with other population datasets that also used the Earth Microbiome Project protocols. The combined analysis spans over 30,000 samples. We tested for technical vs. biological determinants of microbiome structure across populations, and constructed a hierarchy of population similarity. Specifically, 16S rRNA V4 amplicon data from The Microsetta Initiative and other studies using common molecular protocols were integrated using Qiita. With previously recorded sample collection and sample storage information, we tested whether population effects are readily driven by sample handling practices or true biological signal. Finally, using the subset of countries represented by The Microsetta Initiative, we determined which health and lifestyle effects tracked by a participant questionnaire translate between populations. These data recapitulate and extend Yatsunenko et al. (2012, Nature), showing that European, Australian, and North American populations are more similar to one other than each is to South American or sub-Saharan African populations. Unexpectedly, individuals from Bangladesh, the Philippines and Iran exhibit an intermediate relationship between Western and non-Western groupings. Defining how populations relate is a critical step in translating microbiome results. This backdrop carries medical implications, as it is presently unknown whether such massive differences in microbial community composition among populations have the potential to modulate microbiome therapies.

Early Life Effects of Juvenile Western Diet and Exercise on Adult Microbiome Composition in Mice

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Increases in availability of energy-dense foods and simultaneous reductions in physical activity of Western industrialized societies has created an environment that promotes obesity, but alterations in the gut microbiome may also play a role. As with other organismal characteristics, the microbiome may be influenced by factors experienced early in life. We previously demonstrated that early-life wheel access for 3 weeks, starting at weaning, increases adult wheel running in both selectively bred High Runner (HR) and non-selected Control (C) lines of mice. We extended these studies by examining effects of early-life treatments on the gut microbiome, as well as potential correlations between microbial composition and individual variation in activity levels, behavior, body weight regulation, and physiology. Mice were subjected to early-life wheel access and/or Western diet from weaning until sexual maturation, then housed individually without wheels until 14 weeks of age, when fecal samples were taken. Total DNA was extracted from fecal samples using MoBio Laboratories PowerSoil DNA Isolation kit, ITS was PCR amplified, libraries were sequenced using an Illumina MiSeq, and OTUs were chosen using QIIME. We expect to find significant baseline differences in gut microbiome composition between HR and C mice. We also expect significant effects of early-life exercise and diet manipulation. Some of these effects may be interactive, e.g., because mice from HR lines run ~3-fold more per day than those from C lines and are partially protected from the obesogenic effects of Western diet. Supported in part by NIH grant R21HD084856.

Establishing a Model of Lung Cancer-driven Cachexia to Elucidate the Role of IGF-1 Signaling in Disease Progression and Severity

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Cachexia is a metabolic syndrome that occurs in nearly half of all cancer patients, driven by chemo/radiation therapies, the tumor, or both. Cachexia in cancer is characterized by skeletal muscle loss with or without fat loss. Research has shown that insulin growth factor 1 (IGF1) plays a critical role in the prevention of muscle loss during disease. For instance, in mouse models of infection, circulating IGF1 decreases, leading to loss of muscle mass. We have shown this is preventable by gut colonization with a commensal *E. coli* (021), which increased circulating IGF1 by stimulating production from white adipose tissue, resulting in retained muscle mass and increased survival. Although recognized during infection, the role of tissue-specific IGF1 production in protection against muscle loss and survival in cancer is unknown. Our goal was to establish a lung cancer model in mice and determine the role of tissue-specific IGF1 production in mouse survival. Tail vein injections containing 105 Lewis lung carcinoma (LLC) cells were administered to female 6-week-old C57BL/6 mice. Mice developed lung tumors, increased lung weight, and increased free water mass by 3 weeks post-administration. To evaluate the role of IGF1 in lung cancer, several female tissue-specific cre/loxP IGF1 knockouts were injected with LLC cells and monitored for survival (Fabp4- adipose, LyzM- myeloid, and Albumin- hepatocytes). By 4 weeks post-LLC administration, 50% mortality occurred in myeloid-specific and hepatocyte-specific IGF1 knockout mice. In comparison, cre- littermates had a mortality rate of 15%, and 0% mortality was observed in adipose-specific knockouts. Here, we have established a mouse model of lung cancer and our data suggests that IGF1 production by various tissues alters the severity of cancer. Future research will characterize cancer-induced cachexia in IGF1 knockouts and evaluate the potential of *E. coli* 021 in prevention of wasting and mortality in lung cancer.

Anti-fungal Therapeutics Show Contrasting Results Against *Bachtrochium dendrobatidis*

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Emerging infectious diseases are among the leading causes of global wildlife species extirpations. *Batrachochytrium dendrobatidis* (Bd) is a highly virulent fungal pathogen, which has led to amphibian mass mortality worldwide. Bd causes the epidermal skin disease chytridiomycosis, which disrupts normal skin function and causes hyperkeratosis—leading to problems with regulation of electrolytes in the epidermal skin layer. Bd infection elicits one of two possible responses in amphibians: a susceptible response resulting in death via asystolic cardiac arrest, and a reservoir response, where an infected individual continues to shed zoospores but remains otherwise healthy. Typically, the anti-fungal itraconazole is used to treat Bd infection, however, application requires five-minute baths for five to ten consecutive days, and thus clearing infection with itraconazole is a time-consuming process. Direct side effects of itraconazole can range in severity from skin depigmentation to death. Because of itraconazole's toxicity and time-consuming application process, we tested a novel anti-fungal treatment—BMP-NTf2, an ionic liquid chosen for its success in clearing Bd *in vitro*. BMP-NTf2 requires a single application, and was non-toxic to uninfected frogs in doses ranging from 4µL to 120µL, as well as non-toxic to a reservoir frog species infected with Bd. Contrary to these findings, 69% of Bd-susceptible infected frogs treated with BMP-NTf2 died immediately after application, showing the ionic liquid is lethal when paired with disease susceptibility and infection. Further, surviving infected susceptible frogs treated with BMP-NTf2 showed a 33% increase in infection intensity by the end of the experiment, where 100% of itraconazole treated frogs cleared their infection by the same time point. Novel anti-fungals are needed in ecology to counter the current amphibian mass extinction; ionic liquid treatments are promising in their clearance of the fungal pathogen *in vitro*, yet more research is needed into the differences between susceptible and reservoir species reactions to both the ionic liquid and Bd infection itself.

Gut Microbiome Control of *Vibrio cholerae* Colonization Via Processing of Bile Acids

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Vibrio cholerae (*V. cholerae*) is the causative bacteria of the severe diarrheal disease cholera, affecting 1.3-4 million people yearly in regions with low sanitation practices and high rates of contaminated drinking water, underlining the need for ways to prevent infection. *V. cholerae* requires signals to initiate pathogenicity, one being the bile salt taurocholate (TCA) in the intestine. Prior work shows intestinal bacteria can change bile salt levels *in vivo* via enzymes, such as Bile Salt Hydrolase (BSH), that process TCA into the less efficient stimulus cholic acid. Our work focuses on identifying bacteria that process TCA, affecting *V. cholerae* pathogenesis.We screened human gut bacterial strains chosen based on prevalence in 'healthy' microbiomes in normal adults or 'unhealthy' communities typical of individuals affected by diarrhea or malnutrition. These bacteria were tested first *in vitro* with PBS+TCA then inducing activation of a transcriptional reporter with the Ptcp promoter that regulates production of the key virulence genes. We tested for the organisms ability to process TCA in infant mouse intestinal homogenate to confirm our *in vitro* results. We then identified a BSH homolog from Blautia obeum and expressed it in an E.coli strain unable to process TCA, to see if the BSH enzyme alone was able to control *V. cholerae* colonization.Blautia obeum was shown to process TCA into a non-TCP activating form while Streptococcus salivarius showed no ability to reduce TCP induction. In comparison to *E. coli* with the corresponding empty vector, *E. coli* expressing Blautia obeum BSH enzyme reduced *V. cholerae* colonization in infant mice, suggesting TCA modification *in vivo* can result in differential *V. cholerae* infectivity.The next step is an *in vivo* germ-free infant mice experiment, testing the ability to reduce infectivity with LCMS testing to quantify levels of TCA. The same will be done with fecal pellets of mice colonized with resistant or susceptible microbiomes.

Vitamins in Host Defense Against an Enteric Pathogen

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Upon infection, the host mounts multifactorial responses to defend against disease. Early host defense can be categorized as resistance, disease tolerance, or anti-virulence defenses. While resistance mechanisms encoded by the immune system promote health of the host by killing the pathogen, disease tolerance and anti-virulence defenses promote host health while having a neutral to positive effect on pathogen fitness and are executed by the cooperative defense system. Disease tolerance involves mechanisms that induce health. Anti- virulence mechanisms are physiological responses of the host that prevent the engagement of pathways that lead to disease by preventing virulent behavior of microbes. One known regulator of immunity is the calcitriol receptor, commonly known as the Vitamin D receptor (VDR). In response to enteric bacterial infections, VDR has been implicated in mediating host resistance against infection. Utilizing multiple murine models to investigate the role of VDR during infection with an attaching and effacing (A/E) pathogen, our data implicate the VDR in disease tolerance or anti-virulence defenses in response to infection. Intact Calcitriol receptor promotes host health during infection but does not alter pathogen burden. We are currently investigating how this pathway mediates cooperative defenses against infection focusing on VDRs role in mineral metabolism and homeostasis.

CRTAM Shapes the Microbiota and Enhances the Severity of Gut Infection

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Gut lymphocytes and the microbiota establish a reciprocal relationship that tunes the host immune response. For example, the gut microbiota is required for T helper (Th)-17 cell differentiation; Th17 cytokines, in turn, stimulate the intestinal epithelium to produce antimicrobial proteins that control the gut microbiota. Residency and maintenance of T cells in the gut is controlled by Class I-restricted T cell-associated molecule (CRTAM). We thus evaluated whether CRTAM influences the gut microbiota composition. We found that CRTAM expression caused a shift in several members of the gut microbiota under homeostatic conditions. As the gut microbiota and T cells contribute to host defense against intestinal pathogens, we tested whether CRTAM plays a role during infection with the enteric pathogen *Salmonella*. We found lower levels of *Salmonella* colonization in the gut of Crtam-/- mice, along with lower expression of the Th17 cytokines *Il17* and *Il22* in the cecum. *Salmonella*-infected *Crtam*$^{-/-}$ mice exhibited fewer gut T cells and IL-17-producing cells. To investigate whether the altered gut microbiota composition of *Crtam*$^{-/-}$ mice influenced the host response to infection, we transplanted the fecal microbiota from WT or *Crtam*$^{-/-}$ littermate mice into germ-free mice, and then infected the mice with *Salmonella*. Relative to mice transplanted with "WT" fecal microbiota, mice transplanted with "*Crtam*$^{-/-}$" microbiota exhibited lower numbers of T cells and IL-17-producing cells in the gut upon infection. Our results thus suggest an interplay between CRTAM expression and the gut microbiota, which ultimately impacts the mucosal immune system and the host response to enteric pathogens.

Interaction of *Actinobacillus pleuropneumoniae* With Pig Aortic Endothelial Cells

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Actinobacillus pleuropneumoniae (App) is a respiratory system pathogen and etiological agent of porcine pleuropneumonia, which is characterized as an exudative, fibrinous, hemorrhagic and necrotizing pleuropneumonia and is considered one of the main causes of economic losses of the swine industry due to the high mortality rates, reduction of average daily weight gain, medical expenses, sacrificial losses and intervention expenses. *A. pleuropneumoniae* is highly disseminated worldwide, with serotypes 1, 5 and 7 being the most prevalent in North America. In Mexico, particularly, serotypes 1, 3, 5 and 7 present the highest prevalence rate and the disease that it produces affects from 60 to 80% of the pig farms in the state of Aguascalientes.The virulence factors are those that allow a pathogen colonization, invasion, evasion and survival to the host organism's immune response. One of them is the capacity to adhere to the surface of the host cell and the internalization in it. The virulence factors of *A. pleuropneumoniae* are not currently fully identified, however, it is known that App can adhere to host's cell membrane. This work demonstrates that App serotypes 1, 3, 5b and 7 have the capacity to internalize in the host and to lodge in a perinuclear way, it was also found inside vesicles implying a possible vacuolizing capacity.These results serve as a basis to know the mechanisms of infection of the bacteria and to better understand their virulence factors, which once properly described, will serve to develop a specific treatment for the disease and reach its future eradication.

Adipose Tissue Physiology in Host-Parasite Interactions

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Traditionally, white adipose tissue was believed to function solely as an energy storage tissue, however, evidence from recent studies have shown WAT is a critical regulator of immune responses. This is best appreciated in the context of metabolic diseases where the morbidities such as hyperinsulinemia, hyperglycemia, and chronic low-grade inflammation, can be associated with improper immune activation within the WAT. A role for adipose tissue during infections is less well understood. Multiple pathogens have been found to localize in adipose tissue during chronic infection including parasites, bacteria, and viruses. We are interested in why pathogens localize to the adipose tissue for a chronic infection. To address our goal, we are utilizing the eukaryotic, single cellular parasite, Trypanosoma brucei. *T. brucei* is the causative agent of African sleeping sickness in humans and trypanosomiasis in animals. The parasite has previously been thought to mainly reside in the blood before entering the central nervous system before causing death. However, it has recently been found to localize to adipose tissue and has more parasites in the adipose tissue than in any other tissue, including blood, once the disease enters the chronic phase. Here I describe our initial studies characterizing the host metabolic responses to *T. brucei* over the course of the infection.

Cholera Toxin Promotes Pathogen Acquisition of Host-derived Nutrients

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Vibrio cholerae is the causative agent of cholera, a potentially lethal enteric bacterial infection. Cholera toxin (CT) is required for *V. cholerae* to cause severe disease and is also thought to promote transmission of the organism in that victims can shed many liters of diarrheal fluid that typically contains in excess of 10^{11} organisms. However, how the pathogen is able to reach such high concentrations in the intestine during infection remains poorly understood. Here we show that CT-mediated disease enhances pathogen growth and induces a distinct *V. cholerae* transcriptome signature that is indicative an iron-depleted gut niche. We show that *V. cholerae* heme and vibriobactin utilization genes confer a growth advantage to the pathogen only when CT is produced. Furthermore, CT-induced capillary congestion pathology in the terminal ileum correlated with an increased bioavailability of luminal heme. CT-induced disease in the ileum also led to increased luminal concentrations of long-chain fatty acids (LCFAs) and L-lactate metabolites, as well as upregulation of *V. cholerae* iron-sulfur cluster-containing TCA cycle enzyme genes. Genetic analysis of *V. cholerae* suggested that heme and LCFA uptake-dependent growth of *V. cholerae* occurs during infection but only in a strain capable of producing CT *in vivo*. We conclude that CT-induced disease creates an iron-depleted metabolic niche in the gut that selectively promotes the explosive growth of this pathogen through acquisition of host-derived heme and fatty acids as nutrients.

Identification of a Novel Mechanism for Genetic Switch of Lambdoid Prophages in *Bacteroides cellulosilyticus* WH2, a Commensal of the Human Gut

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The human gut microbiome is composed of a variety of bacteria many of which perform essential metabolic functions. One such bacterium is *Bacteroides cellulosilyticus* WH2, a commensal symbiont necessary for the breakdown of otherwise indigestible polysaccharides of the human diet. Its presence in the gut contributes to a mature composition of the gut microbiome in healthy individuals. The genome of WH2 harbors two prophages, one of which is inducible both *in vitro* and *in vivo*. This study seeks to unveil a new mechanism of lambdoid prophage induction in WH2. To identify candidate genes involved in this mechanism, a *Bacteroides cellulosilyticus* WH2 INSeq library was treated with a known prophage inducer and mutagenic antibiotic, Carbadox (CX). This method allows for the simultaneous genetic screening of thousands of isogenic mutants, each of which harbors a transposon insertion in one gene of the genome. Our results showed one isogenic mutant highly enriched after CX treatments, revealing an alternative pathway for prophage lysogenic-to-lytic switch regulation. A gene located within the WH2 genome codes for Guanosine-3'2C5'-bis(diphosphate) 3'-pyrophosphohydrolase (*spoT*), an enzyme responsible for the synthesis of (p)ppGpp and ppGpp, and for their hydrolysis into GTP and GDP, respectively. This study demonstrates that when *spoT* is disrupted by a Tn, the prophage cannot be induced, suggesting that the levels of the alarmones (p)ppGpp and ppGpp serve as a regulator for phage lambda induction. These results propose a novel induction mechanism for lambdoid prophages, that contributes to the understanding of human gut microbiome composition dynamics as well as latent viral infections beyond the human gut.

Muscle wasting is a maladaptive host metabolic response to infection

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During infection, hosts endure significant metabolic changes that can dramatically influence host defense responses and pathogen behavior. Muscle wasting is one of the most highly conserved metabolic changes observed across a wide range of infections and across the animal kingdom. The function of muscle wasting during infection, if any, is not understood. It has been proposed that muscle wasting is a host metabolic response that modulates energy allocation during infection. However, current experimental and clinical evidence show that muscle wasting decreases host survival and impairs recovery from severe infections. Using a transgenic murine model lacking a critical mediator of muscle atrophy (*FoxO1*) in skeletal muscle, we will determine why and how muscle wasting leads to a more severe outcome of infection in the context of *Salmonella* Typhimurium infection. Mice deficient for muscle Foxo1 had increased survival when challenged with a lethal dose of *S.* Typhimurium. Consistent with our previous report, we found that the protection from muscle wasting was not accompanied by changes in pathogen burden. This suggests that muscle wasting increases disease severity by promoting pathogen virulence or impairing a disease tolerance mechanism rather than host resistance.

Microbiomes of Autism Spectrum Disorder Patients Induce Behavioral Deficits in Mice

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Autism spectrum disorders (ASD) manifest with deficits in social communication and repetitive, stereotyped behaviors. The gut microbiome differs between typically developing (TD) and ASD individuals, however it is unclear whether or not it contributes to etiology. To directly test the role of the gut microbiome in ASD, we colonized wild-type germ-free mice with fecal samples from TD and ASD children and tested their offspring for ASD-relevant phenotypes. We show that exposing mice to some ASD microbiomes is sufficient to induce behavioral deficits. We observed extensive effects on alternative splicing in genes known to contribute to ASD. Moreover, we observed changes in the availability of various metabolites in the colon. We further show that the administration of putatively protective metabolites, elevated in TD-colonized mice, to an idiopathic mouse model of autism from gestation can attenuate behavioral deficits and decrease excitation in L5 pyramidal neurons in the prefrontal cortex of these mice. Conversely, the administration of some “pathogenic” metabolites, that were elevated in ASD mice, induced deficits in locomotion. These results suggest that the altered metabolism by the gut microbiome contributes to behavioral deficits in ASD.

The IBD Candidate Gene, *PTPN2*, Regulates Segmented Filamentous Bacteria Mediated Th17 Response and Intestinal Barrier Protection against Adherent-Invasive *E. coli*

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The inflammatory bowel disease (IBD) candidate gene, protein tyrosine phosphatase non-receptor type 2 (*PTPN2*), maintains intestinal homeostasis by restricting cytokine-induced epithelial barrier dysfunction. The aim of this study was to identify if *PTPN2* loss alters intestinal barrier function by changing the balance between barrier-modifying pathobionts (e.g., adherent-invasive *Escherichia coli* [AIEC]), and beneficial commensal bacteria (e.g., Segmented Filamentous Bacteria [SFB]).

Mucosal-associated microbes (small and large intestine) from *Ptpn2* wild-type (WT), heterozygous (Het), and knockout (KO) mice were analyzed by internal transcribed spacer (ITS) region sequencing. IL-17A and SAA1/2 levels in mouse intestinal tissue lysates were examined by ELISA and qPCR, respectively. Prevention of *m*AIEC induced barrier defects by SFB (viable [*v*SFB], heat-killed [*hk*SFB]) pre-treatment was measured by transepithelial electrical resistance (TER) and fluorescein isothiocyanate (FITC) 4kDa dextran (FD4) permeability studies on Caco-2 epithelial monolayers.

Bacterial communities in all three genotypes were distinct while increased mucosal *m*AIEC in *Ptpn2*-KO mice inversely correlated with decreased SFB (r=–0.47, P=0.018). *v*SFB prevented *m*AIEC adherence to epithelial monolayers *vs*. *m*AIEC alone (P<0.001, n=8-10). *m*AIEC decreased TER (P<0.001, n=3), increased FD4 permeability (P<0.001, n=5), and altered tight junction protein expression. SFB prevented these effects. *Ptpn2*-KO mice had a significantly reduced Th17 response with decreased intestinal serum amyloid A (SAA1/2) and IL-17A *vs*. WT mice (P<0.001, n=3-6).

Ptpn2 loss reduced SFB abundance and the Th17 response while promoting colonization of *m*AIEC. Our data suggest that *Ptpn2* serves as a “microbial modulator” to regulate the balance of pathobionts *vs*. commensals thereby protecting intestinal barrier function.

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Gut Microbiota Dysbiosis Leads to the Development and Progression of Multiple Sclerosis

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Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system, and is the leading cause of neurologic disabilities in young adults. Most patients initially present with the relapsing-remitting form, but some advance to the secondary progressive phase with cumulative disability. The number of patients suffering from this disease is increasing in developed countries. Considering the physical and financial burden of MS, the cause of this tendency should be investigated. This trend is particularly obvious in Japan. Westernization of Japanese life style and dysbiosis of the gut microbiota comprise a leading explanation for this phenomenon. Notably, the incidence of classical Western-type MS (CMS) is rapidly increasing. Here, we compared the gut microbial 16S rRNA and metagenomic gene sequence data of 62 relapsing-remitting CMS patients, 15 secondary progressive CMS (SP-CMS) patients, 21 atypical MS patients, 20 neuromyelitis optica spectrum disorder (NMOSD) patients, and 55 age-matched healthy controls, and tried to detect the specific bacterial species and gene function that are related to the pathogenesis of CMS. All results obtained from 16S rRNA analyses were verified by metagenomic analyses. After a newly incorporated rational selection process using clinical severity score, we identified two representative butyrate-producing bacterial species, both of which are abundant in healthy Japanese gut microbiota. These two species were found to be specifically reduced in both the development and progression phase of CMS, which was confirmed by metagenomic functional butyrate production results. Additionally, several bacterial species were specifically abundant in the SP-CMS gut microbiota and positively correlated with clinical severity score. Our research suggests rational candidates of specific bacterial species and gene function that are involved in the development and progression of CMS, leading to treatment that normalize gut microbiota.

Novel Live Biotherapeutic for the Treatment of Chronic *Pseudomonas aeruginosa* Lung Infections

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Chronic lung infections are a primary cause of morbidity and mortality for individuals with cystic fibrosis (CF). The formation of anaerobic biofilms support tolerance to antibiotics like tobramycin which require oxygen and metabolically active *P. aeruginosa* to be effective. A novel live biotherapeutic with activity against chronic multi-drug resistant infections in CF lungs represents an innovative treatment strategy against the expanding antibiotic resistance public health crisis. Patented Microbial Enhanced Recombinants Generated by Evolution (MERGE) technology combined metabolically beneficial and therapeutic traits from probiotic strains, such as bacteriocin production and anaerobic growth, into a modified biotherapeutic strain. Strain characterization was completed using Etest and biological assays for mucolytic activity. A modified cross-streak assay and the drop test were used to characterize bactericidal activity against 43 multi-drug resistant *P. aeruginosa* clinical isolates, 23 *S. aureus*/MRSA clinical isolates. An acute murine (BALB/c) lung infection model evaluated preliminary safety following intranasal administration. The MERGE technology combined multiple therapeutic traits into a single strain that is mucolytic and produces a bacteriocin that kills multi-drug resistant *P. aeruginosa*. Safety tests in healthy BALB/c mice have shown that intranasal administration of the live biotherapeutic at a concentration of 1 x 10^8 CFU is nontoxic (100% survival) with no adverse effects after 5 days compared to mice treated with 1 x10^7 CFU *P. aeruginosa* (0% survival). These preliminary studies suggest MERGE derived live biotherapeutics facilitate a targeted antibacterial and mucolytic activity that may be administered locally to deliver pluripotent therapeutics directly to the lungs to treat chronic multi-drug resistant.

Nephrocytes Mediate Immune Tolerance to Microbiota by Removing Peptidoglycan from Systemic Circulation

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Maintenance of immune tolerance to microbiota is essential to organismal homeostasis. Here, we report that the *Drosophila* nephrocyte, a cell type with molecular, anatomical, and functional similarity to the glomerular podocyte of the vertebrate kidney, promotes immune tolerance to Gram-positive microbiota. Flies deficient for *Klf15*, a transcription factor required for nephrocyte development and function, are viable but lack nephrocytes. *Klf15* mutants display constitutively elevated Toll pathway activity, and as a consequence, are both shorter-lived and more resistant to microbial infection. Our analysis revealed that nephrocytes uptake Lys-type peptidoglycan from systemic circulation and degrade it inside lysosomes. Without nephrocyte function, microbiota-derived peptidoglycan accumulates in circulation, triggering Toll pathway activation even in the absence of infection. These results unveil a role for hemolymph (insect blood) filtration in the maintenance of immune tolerance to microbiota and also identify a potential root cause for the chronic immune activation observed in animals suffering from impaired blood filtration.

Whole Genome Evolutionary Fingerprinting Suggests Novel Primate Gene Functions in Evolutionary Arms Races

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Previous positive selection analyses have helped to elucidate differences in primate adaptation. However, many of these studies have been focused on small gene families and genes known or suspected to play a role in the host innate immune system. With the genome sequencing of numerous primate species, we are now capable of comparing the evolutionary profiles of primate genes across the whole genome. Using these evolutionary profiles as guides, we identify new gene functions that are rapidly evolving and insight on the biological pressures that affect them.

Pathogen Evasion of Intracellular Complement in Macrophages Enables Systemic Colonization and Bacteremia

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A primary function of the mucosal immune system is to contain infections and prevent pathogen dissemination through the bloodstream to systemic sites. Interleukin (IL)-22 is one arm of the host response that promotes mucosal barrier function and controls dissemination of many pathogens from the mucosa. However, IL-22 does not protect against infection with the gut pathogen Salmonella enterica serovar Typhimurium (STm). Here we identified a new means by which STm evades IL-22-mediated immunity to cause systemic infection. We found that, during infection, upregulation of complement component C3 by IL-22 occurs indirectly via S100A9 upregulation, and that this was dependent on the presence of the gut microbiota. To counteract this host defense pathway, STm expresses the serine protease PgtE, which enables the pathogen to evade intracellular C3-dependent autophagy in mouse and human macrophages, and to achieve high levels of colonization at extraintestinal sites, including the blood. By contrast, PgtE did not confer an advantage to STm in colonizing the gut, or during infection of mice deficient in upregulating C3 (Il22-/- mice, S100A9-/- mice), or lacking C3 (C3-/- mice, cobra venom factor-treated mice). Altogether, our study has identified an IL-22 / S100A9 / C3 axis that leads to the elimination of bacteria through activation of autophagy in macrophages. Moreover, we identified the mechanism by which STm evades this important mucosal barrier defense to cause bacteremia.

Investigating the Molecular Details of Bacteroides and Human Colon Epithelial Cell Interactions

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The human gut microbiota is composed of 100 trillion microbes that tightly interact with each other and with the host, playing a fundamental role in human health. Compositional changes in the gut microbiota are linked to the development of the immune system, susceptibility to cancer, inflammatory diseases, and mental health. An ever-growing number of studies reveal that gut microbiota and epithelial cells directly or indirectly interact. Microbial ligands and other factors that are produced by gut microbiota are bound by receptors on epithelial cells, which in turn results in alteration of the immune response by gut epithelium. Our long-term research goal is to discover and characterize mechanisms mediating bacteria-host interactions for the purpose of informing strategies for better treatments or development of novel therapeutics. Our preliminary studies show that bacteriophage tail-like structures called Contractile Injection Systems (CIS) from a marine bacterium *Pseudoalteromonas luteoviolacea* can lyse murine-derived macrophages, suggesting that bacterial CIS elements can directly interact with mammalian cells. We hypothesize that the members of the human gut microbiota produce CIS that can modulate human physiology and health. In this study, we investigate the interactions between Bacteroides species and the human colon epithelial cells (HT-29) using both qualitative (microscopy) and quantitative approaches (cell proliferation/death assays and RNAseq). Our ongoing and future studies will reveal whether Bacteroides-mammalian cell interactions are mediated through the CIS elements. In addition, we developed a flow-cell system with human gut epithelial cell lines that will allow us to investigate Bacteroides-host cell interactions in three dimensional epithelial cell culture model. Understanding the mechanisms of host-microbiota interactions will inform strategies for manipulating the gut bacterial community to promote our well-being.

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