

Exploring New Horizons in Microbiome Research

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Leading scientists in microbiome research met at Lake Titisee, Germany, in April 2014 to discuss the current state of the field, the most urgent and unresolved questions, state-of-the-art technological advances, and new avenues of future research. We summarize some of the concepts and themes discussed at this meeting.

In the beautiful setting of Lake Titisee in the southern Black Forest, Germany, around 50 scientists gathered on April 9–13, 2014, on the occasion of the 109th International Titisee Conference, entitled “Microbiome-Host Mutualism in the Shaping of Host Immunity.” Reaching back to the early 1960s, the Titisee Conferences organized by the Boehringer Ingelheim Fonds, a foundation for basic research in medicine, have a longstanding tradition in bringing together leading scientists to discuss current progress and open questions in multiple fields of biomedical research. The recent exciting advances made in our understanding of the microbiota and its tremendous effect on host physiology and pathophysiology led the organizers Dan Littman (Skirball Institute), Samuel Miller (University of Washington), and Philippe Sansonetti (Institut Pasteur) to assemble a group of researchers from various disciplines and methodological approaches (Figure 1). They discussed the current state of the field and the problems that need to be overcome by the scientific community to enable meaningful insight into the functional state of microbial communities and their impact on host physiology and pathophysiology, standardization and reproducibility between cohorts and laboratories, and translation of results from basic research into therapeutic approaches.

Toward the Disease Metagenome

A decade after the completion of the Human Genome Project, the scientific community has realized that deciphering the molecular underpinnings of common human disease requires far more than the decoding of human genomes and polymorphisms. Instead, many multifactorial human diseases have been associated with a causative “environmental” component. In addition to their own cells, comprising around 20,000 genes, human beings harbor trillions of microorganisms whose collective metagenome is estimated to be around 100- to 1,000-fold larger. As such, a considerable part of the variability in human physiology and pathophysiology that has so far been ascribed to “environmental” influences is potentially due to interindividual variability in their microbial metagenome. Recently, two large-scale consortia, the Human Microbiome Project (HMP) and the Metagenomics of the Human Intestinal Tract (MetaHIT) project, have attempted to capture and classify this variability. Analogous to the post-Human Genome Project years dominated by genome-wide association studies, the challenge ahead now is to functionally link this variability to disease outcome using metagenome-wide association studies. This concept is currently applied to a multitude of pathologic conditions, including inflammatory bowel disease (IBD), metabolic diseases, stem cell transplantation, and skin disease.

Peer Bork (European Molecular Biology Laboratory) presented recent data from the MetaHIT project. Network analysis of species abundance in various cohorts further supports the concept that intestinal community structures can be classified into recurrent clusters, designated “enterotypes.” On a deeper level, each individual might have a unique metagenome, due to polymorphisms in bacterial genomes and individual-specific strains (Schloissnig et al., 2013). Curtis Huttenhower (Harvard School of Public Health) presented follow-up studies to the HMP that focus on characterizing the microbiome involved in IBD and its various clinical manifestations and complications. The main effort in these studies is to determine characteristic compositional and functional microbiota profiles associated with disease states and to develop predictive measures for microbiome-based diagnosis. Recently, a large pediatric cohort of Crohn’s disease patients revealed a signature of microbial communities associated with this disease (Gevers et al., 2014). Richard Blumberg (Harvard University) focused on the host side of the complex etiology of IBD. Genome-wide association studies of IBD patients have highlighted three recurrent pathways to be involved in the genetic susceptibility to IBD: innate immune pathways of the NOD-like family of receptors, genes involved in autophagy, and the ER stress and unfolded protein response. A unifying concept is emerging by which these pathways do not act in isolation, but rather integrate into a network of cooperation and compensation (Adolph et al., 2013). Bringing together the microbial profiles associated with disease and the genetic susceptibility loci of the host will remain a central challenge of the next decade in IBD research.

Eric Pamer (Memorial Sloan-Kettering Cancer Center) described human fecal microbiota analyses in patients following allogeneic hematopoietic stem cell transplantation that correlated a marked survival benefit with increased commensal flora diversity. Marcel Van den Brink (Memorial Sloan-Kettering Cancer Center) presented work establishing a connection between the gut microbiota dysbiosis and intestinal inflammation secondary to graft-versus-host disease (GVHD) (Jenq et al., 2012). Certain members of the microbiota can function as biomarkers for GVHD severity, and modulation of the microbiota composition prior to transplantation affects the outcome of GVHD. Pamer and Van den Brink also discussed the challenges facing the field in translating laboratory-derived findings into therapeutic, microbiota-altering modalities. At the clinical level, ongoing efforts must integrate data from multiple study centers and establish common readouts and clinical endpoints. In the laboratory, the mechanisms involved in



Figure 1. Participants of the 109th International Titisee Conference Organized by the Charitable Boehringer Ingelheim Fonds on “Microbiome-Host Mutualism in the Shaping of Host Immunity” at Lake Titisee, Germany

microbiota-mediated modulation of the host must be defined. Some microbiota-generated metabolites are providing glimmers of hope that they may function as therapeutic agents or clinical targets.

Eugene B. Chang (University of Chicago) demonstrated an example of how Western diets can trigger experimental colitis in genetically susceptible IL-10-deficient mice. A diet high in saturated fats promoted taurine conjugation of hepatic bile acids, and in turn, taurine, which is a rich source of organic sulfate, caused a bloom of sulphite-reducing bacteria, resulting in a proinflammatory immune response and exacerbation of colitis in the IL-10-deficient host (Devkota et al., 2012). These data suggest that environmental factors (e.g., diet), together with host factors that affect gut microbial assemblage, can determine immune response, risk of disease, and clinical phenotype. Since metabolite abundances in the intestinal lumen and lamina propria are reflective of the functional state of the microbiota, sensing of metabolites, rather than mere bacterial presence, may be the most informative variable according to which the host adapts homeostatic set points in pathways communicating with the microbiota.

In addition to intestinal inflammation, the microbiota also strongly influences metabolic diseases. Jayne Danska (Hospital for Sick Children, University of Toronto) provided an interesting perspective on the role of early-life microbial exposure in type 1 diabetes. After puberty, male and female nonobese diabetic mice feature distinct microbial configurations, which modulate sex hormone levels and control susceptibility to autoimmunity (Markle et al., 2013). Similar recent findings by the group of Alexander Chervonsky (University of Chicago) (Yurkovetskiy et al., 2013) fuelled new efforts to determine interventions against type 1 diabetes that are independent of the microbiota. Such interventions should feature higher robustness across age and gender. Based on recent findings, Chervonsky discussed various novel dietary approaches with microbiota-independent

effects as a new avenue for clinically related microbiome research.

Samuel Miller (University of Washington) applied the concept of disease-associated dysbiosis to cystic fibrosis, which includes gastrointestinal inflammation and malabsorption of nutrients. Children with cystic fibrosis feature significantly higher levels of fecal *Escherichia coli*, a signature that is commonly observed in inflammatory settings (Hoffman et al., 2014; Stecher et al., 2013). This bloom of Enterobacteriaceae during intestinal inflammation was elaborated on by Andreas Bäuml (University of California at Davis). The inflammatory response

induces alterations in gene expression in intestinal tissue. One prominent change involves the production of reactive nitrogen species that are converted to nitrate in the inflamed intestine. The majority of commensal Enterobacteriaceae are equipped with nitrate-reducing enzymes that confer competitive advantage under such situations of nitrate enrichment (Winter et al., 2013). This new knowledge is now exploited to assess selective interventions targeting anaerobic respiration of Enterobacteriaceae. This example shows how mechanistic knowledge about host-microbiota crosstalk in the disease setting can lead to powerful interventional strategies.

Julie Segre (National Institutes of Health) is characterizing the disease-associated microbiota at a different site, the skin. The fungal microbiome of the skin is dominated by the genus *Malassezia* at most body sites; only foot colonization features higher diversity (Findley et al., 2013). Analogous to the intestinal microbiome, current efforts are focusing on characterizing dysbiosis involved in skin disease. First insights into the microbiota associated with atopic dermatitis suggest that dysbiosis of the skin may be useful as a diagnostic indicator and potentially as therapeutic target.

Toward a Systematic Understanding of Colonization Principles

In addition to profiling of microbiota configurations associated with specific diseases, the convergence of bioinformatics, data processing, genomic sequencing, and high-throughput technologies now enables systematic approaches to understanding host-microbiota interactions. Andrew Goodman (Yale School of Medicine) has developed a transposon-based approach that enables screening for genomic components in members of the commensal microbiota that facilitate membership and interaction within the bacterial community. This approach is able to uncover components of the metagenome that promote microbial fitness within the intestinal ecosystem. For instance, the

prominent human gut commensal *Bacteroides thetaiotaomicron* encodes for three vitamin B₁₂ transporters that appear to be nonredundant, but rather confer competitive advantage during intestinal colonization (Degnan et al., 2014). Systematic exploration of the metagenome for factors involved in colonization of the gut ecosystem will be instrumental for generating a more comprehensive map of genetic factors involved in host-microbial and intermicrobial crosstalk. Another promising approach is the generation of metagenomic libraries that can be screened for their effect on the host. Joël Doré (French National Institute for Agricultural Research) presented a strategy employing functional metagenomics that complement proteomics screening efforts to generate metabolite profiles characteristic of distinct bacterial compositions. Such a bacterial protein profile was recently generated for Crohn's disease patients (Juste et al., 2014).

Nassos Typas (European Molecular Biology Laboratory) further emphasized the high dimensionality of microbe-microbe interactions, within and across animal kingdoms, which take place in the human intestine. Screening of bacterial mutants in the presence or absence of other members of the commensal microbiota, an approach complementary to the abovementioned transposon-based approach *in vivo*, yields interesting insight into interdependencies of microbial species and the network architectures of intestinal colonization. Julie Pfeiffer (University of Texas Southwestern Medical Center) characterized the interplay between intestinal bacteria and enteric viruses. Following the initial observation that the microbiota promotes virus replication and pathogenesis, her group has recently uncovered direct binding of viruses to bacterial LPS, thus increasing virion stability and transmission (Robinson et al., 2014). Functional consequences of bacterial-viral interactions and parasite-viral interactions on the immune system were discussed by David Artis. His laboratory is using coinfection models to decipher the modulatory capacity of distinct classes of microbes on the host immune system and the consequences for subsequent exposure to different microorganisms.

Another instance of interbacterial crosstalk was discussed by Jon Clardy (Harvard Medical School) in the context of his talk on the use of host-microbe systems to trace evolution. Using two bacterial strains isolated from the red seaweed as a model, the siderophore avaroferrin was discovered as an inhibitor of bacterial swarming, the rapid movement over a surface (Böttcher and Clardy, 2014). Nicole King (University of California, Berkeley) employs the choanoflagellate *Salpingoeca rosetta* to study host-bacterial interactions in a simple model organism. Her work revealed that molecules generated by prey bacteria modulate colony formation of the host. This model system is well suited to discover fundamental principles of interkingdom coevolution that can be applied to mammalian colonization by bacteria.

Complementary to the systematic exploration of the metagenome by genetic means and bacterial interactions by using microbial ecology, the application of mathematical modeling to symbiotic communities has recently yielded new ideas about the concepts underlying microbial colonization of the intestine. One of these concepts is the idea that the host can influence microbial colonization to yield a precise biogeography of the intestine. Kevin Foster (University of Oxford) reported on inter-

esting computer simulations that suggest that nutrients (Schluter and Foster, 2012) and antibodies secreted from the epithelium can strongly affect the positioning of commensals in the intestine.

Ultimately, a mechanistic understanding of host-microbiota interaction might require architectural understanding of molecules involved in this crosstalk. The central challenge is to capture and visualize protein transport across membranes, which is pivotal for intercellular communication and information flow. Thomas Marlovits (Institute for Molecular Biotechnology, Vienna) demonstrated examples for the successful structural characterization of such information flow. His group has recently characterized protein transport across bacterial type III secretion systems, a major mechanism involved in host-microbial and intermicrobial communication (Radics et al., 2014).

Taken together, understanding host-microbiota interactions requires the application of principles derived from ecology and genomics to a new context: a high-dimensional multikingdom ecosystem of prokaryotic-eukaryotic interplay. This challenge can be met from various perspectives, and a combination of mathematical modeling, genomic screening, and classical microbiology will prove fruitful in future research.

Toward Mechanisms of Host Immune Shaping

One of the most striking effects of intestinal microbial colonization on the host is the modulation of the immune system. First studies of germ-free mice had noted altered architecture of intestinal lymphoid structures and distorted compartments of lymphoid cells. This was followed by seminal studies identifying direct induction of immune cell populations by distinct microbes. Dan Littman (Skirball Institute) and Kenya Honda (RIKEN Yokohama) presented exciting new findings following the initial discovery of segmented filamentous bacteria (SFB) as inducers of T helper 17 (T_H17) cells and *Clostridia* as inducers of regulatory T (T_{reg}) cells. Littman demonstrated that T_H17 cells induced by SFB recognize SFB-specific antigen, even in the presence of additional, T_H1-inducing microbes in the intestine (Yang et al., 2014). These findings suggest an interesting concept, namely a direct match between antigen specificity of a T cell and the type of bacteria that influence the T cell's effector function. Honda elaborated on the specific properties of SFB as inducers of T_H17 cells and presented interesting insights into differential induction of ROR γ t-expressing T_H17 cells and ROR γ t-expressing innate lymphoid cells (ILCs). He also focused on recent work identifying a cocktail of *Clostridia spp.* as inducers of another subset of T_{reg} cells, a finding with great potential for the therapeutic modulation of intestinal inflammation (Atarashi et al., 2013). The fact that interactions between the host immune system and the microbiota are not a one-way communication of bacterial induction of T cells was discussed by Sidonia Fagarasan (RIKEN Yokohama). She took the example of T_{reg} cells to describe the importance of immune homeostasis for a stable microbial community. Interestingly, aberrations in T_{reg} function and germinal center reactions lead to alterations in microbial diversity in the intestine.

The challenge ahead in deciphering microbiome-host mutualism in the shaping of host immunity is to integrate the many variables and pathways that are involved in this crosstalk. Such a regulatory network has emerged from the work of Fiona Powrie (University of Oxford) over the last decade. Her group has

defined roles for members of the IL-1 and IL-12 family of cytokines for the interplay between T cell subsets in the intestine and the intestinal microbiota. Intestinal T_{reg} cells play a critical role in maintaining tolerance against commensal antigens. New data now suggest a role for the alarmin IL-33 in this complex network.

Another layer of signal integration happens at the epigenetic level. Koji Hase (University of Tokyo) presented a new mechanism for the control of intestinal T_{reg} homeostasis. Gut microbial colonization induces the DNA-methylation adaptor Uhrf1 in T_{reg} cells. This leads to methylation and silencing of the gene encoding for cyclin-dependent kinase inhibitor p21, thereby driving the proliferation and functional maturation of colonic T_{reg} cells (Obata et al., 2014). The importance of epigenetic regulation in intestinal epithelial cells was emphasized by David Artis (University of Pennsylvania). Deletion of histone deacetylase 3 (HDAC3) in epithelial cells results in a loss of Paneth cells, development of dysbiosis, and enhanced susceptibility to intestinal inflammation. Interestingly, these phenotypes are dependent on the presence of the microbiota, suggesting that epigenetic remodeling is necessary for appropriate integration of microbial signals to the host (Alenghat et al., 2013). John Rawls (Duke University Medical Center) reported on a genome-wide approach to decipher the impact of microbial colonization on chromatin accessibility and transcript levels in the intestinal epithelium. Comparison of both small and large intestine between germ-free and colonized mice indicated that transcriptional responses to microbiota are not accompanied by changes in chromatin accessibility, but are instead linked to altered expression of specific transcriptional regulatory factors.

A further aspect of transcriptional control of intestinal lymphocyte function was discussed by Lora Hooper (University of Texas Southwestern Medical Center). Her group recently found that T_H17 cells in the gut are controlled by elements of the circadian clock. The transcription factor Rev-Erb α , a component of the molecular clock, is negatively regulating another transcription factor, Nfil3, which in turn negatively controls ROR γ t, the lineage-defining transcription factor of T_H17 cells (Yu et al., 2013). This finding suggests that intestinal immunity may be controlled by regulatory principles of whole-organism physiology that have so far been overlooked. It will be interesting to determine in the future whether more such regulatory systems are involved in the shaping of intestinal immunity.

Yasmine Belkaid (National Institutes of Health) focused on the interaction between the microbiota and the immune system at the skin. Studies from her laboratory have defined a role for the skin microbiota in controlling the local T cell response to commensals and pathogens (Naik et al., 2012). The concept that emerged from these studies suggests that each mucosal site is defined by autonomous mutual feedback loops of host-microbiota mutualism.

These recent findings about antigen specificity of microbiota-induced T cells and the strict compartmentalization of tissue-specific antibacterial immunity suggest the existence a regulatory relay that links microbial recognition to T cell activation. Such a relay is likely provided by antigen-presenting cells. Belkaid elaborated on distinct functional assignments to subsets of mononuclear phagocytes (MNPs) in the skin. Maria Rescigno (European Institute of Oncology) studies such MNP populations

in the intestine and recently discovered antigen transfer between the two major classes of lamina propria MNPs: CX₃CR1⁺ and CD103⁺ cells. This antigen transfer was essential for the induction of oral tolerance and might be an example of how MNP subsets with distinct functions can cooperate to maintain tolerance at mucosal surfaces, while at the same time retaining the ability to rapidly react against pathogenic insults (Mazzini et al., 2014).

Antigen-presenting cells link microbial signals to lymphocyte activation. However, the direct recognition of microbial products happens further upstream and is performed by receptors of the innate immune system. Dana Philpott (University of Toronto) focused on a family of innate immune receptors called Nod-like receptors (NLRs). NLRs have recently emerged as central regulators of the intestinal immune response to bacteria (Philpott et al., 2014), and mutations in NLRs are frequently found in IBD patients. Philpott provided insight into the role of a yet poorly understood member of the large NLR family, NLRC3. Her work pointed to a role of this protein in T cell regulation. Innate immune receptors are a highly conserved class of proteins, and many homologs are found in nonvertebrates. Bruno Lemaitre (Ecole Polytechnique Fédérale de Lausanne) has made pivotal contributions to understanding the innate immune system of *Drosophila melanogaster* and its interactions with the microbiota. He highlighted the key role of intestinal stem cells and epithelial repair in the defense against oral bacterial infection. He also showed how the *Drosophila* immune response is compartmentalized along the digestive tract (Buchon et al., 2013) and presented interesting new results on the impact of the *Drosophila* microbiota on gut compartmentalization and tissue renewal (Broderick et al., 2014). Filipe Cabreiro (University College London) studies the impact of microbial drug metabolism on longevity in *Caenorhabditis elegans*. He recently discovered that the biguanide drug metformin alters microbial metabolism (Cabreiro et al., 2013). In addition to *Drosophila* and *Caenorhabditis*, the zebrafish *Danio rerio* is a versatile model to study host-microbiota interactions. Karen Guillemin (University of Oregon) has set up a germ-free zebrafish system, which allows for systematic assessment of immune cell responses to microbial colonization in this transparent model organism.

In addition to lymphocytes, nonhematopoietic cells have recently been recognized as important immune cells in the orchestration of host-microbiota interactions. Mucus-secreting goblet cells promote the spatial segregation between the epithelial layer and the majority of microbes in the intestinal lumen. Over the last decades, the work of Gunnar Hansson (University of Gothenburg) has revealed the composition and function of the mucus layer secreted by goblet cells. In the colon, the inner mucus layer is normally devoid of bacteria. Interestingly, recent findings indicate that this spatial separation is lost in IBD patients (Johansson et al., 2014). Thaddeus Stappenbeck (Washington University) has recently defined epithelial autophagy as a control mechanism regulating paneth cell and goblet cell function (Patel et al., 2013). He presented interesting new findings on the function of mucus-associated commensal *B. thetaiotaomicron* and a new means of communication between the bacteria and the intestinal mucosa. Andrew Macpherson (University of Bern) elaborated on the role of mucus in the stratification of bacterial colonization in the intestine. His laboratory has developed a tool to reversibly colonize germ-free mice (Hapfelmeier

et al., 2010), allowing differentiation between colonization and persistence. In addition, reversible colonization can be used to determine the impact of distinct phases of microbial exposure during life. The arguably most critical phase of microbial colonization is the neonatal period. Early colonization profoundly affects immune system maturation, with lifelong consequences. Matthias Hornef (Hannover Medical School) characterized the interdependency of immune system development and gut microbial ecology after birth (Renz et al., 2012) and presented interesting new findings on the consequences of this process for disease susceptibility.

Together, the next decade in research on shaping of immunity by the microbiota will aim at deepening our mechanistic understanding of bacterial functions necessary to induce appropriate host responses, myeloid cells as communicators between immune compartments, and the role of epithelial cells as mediators of host immune responses.

Toward New Perspectives

Ruslan Medzhitov (Yale School of Medicine) presented, in an inspiring keynote lecture, an evolutionary perspective on host-microbiome interactions and a conceptual overview of the challenges that the field is facing in the years to come. While many examples have been found for functional complementation leading to functional dependency between host and microbiota (such as the one found in vitamin biosynthesis pathways), a similarly important, albeit much less studied, aspect of host-microbiota interactions is the niche construction ability of microorganisms, i.e., their ability to alter the capacity of an ecological niche in favor of the microorganism. He hypothesized that the types of interactions between the host and members of the microbiota might be classifiable into a limited number of categories based on commonalities in the functional consequences for both sides. This means that coevolution of host and members of the microbiota might have followed common repetitive patterns with respect to hallmark variables of microbial colonization (including microbial density, attachment to host cells, and metabolite production) and the host appropriate host response (including development of a specific immune response and metabolic adaptation). Understanding these features will be a challenging yet promising avenue of future research.

Furthermore, the evolution of host genes may appear in a new perspective when considered under the circumstance of host-microbiota coevolution. For instance, the adaptation of a host organism to a new environment during evolution might have occurred not only by direct adaptation but rather by facilitating adaptation through the microbiome. In other words, the function of some host genes might be to modulate the adaptation of the microbiome to selective pressure. Eran Elinav (Weizmann Institute of Science) presented evidence that immune genes involved in host-microbiota interactions may serve as “buffers” that enable the response of the microbiota to changes in environmental conditions. He presented his work on the role of the inflammasomes, host sensors of the innate immune system, in intestinal epithelial cells, where they are involved in shaping of microbial composition and in creating a physical barrier between host and microbial cells by promoting mucus secretion (Wlodarska et al., 2014). Importantly, the functional conse-

quence of dysbiosis for host fitness in the absence of inflammasomes is context dependent, and thus inflammasomes might provide a bone fide example for host pathways facilitating the adaptive capability of the microbiota to environmental conditions.

The resilience of the microbiota to a change in external conditions was also discussed by Eric Alm (Massachusetts Institute of Technology), who followed changes in the human microbiome over the course of a year. He elaborated on factors controlling the stability of the microbiota and presented interesting insight into the differential roles of α and β diversity in this regard.

The meeting also aimed at formulating the conceptual difficulties that this young field is facing and at discussing conditions that need to be met for a productive future. Samuel Miller raised the question of whether the current taxonomical species concept still captures the relevant variables when analyzing a large microbial community like the intestinal microbiota, as functions could be contained across species, and thus functional classifications might be far superior to the species concept. Furthermore, he noted that standardized model systems will be of great benefit for the field moving ahead. Tools envisioned include well-defined microbial communities for mouse models, animal models of microbiota-mediated disease and their human correlates, human epithelial model systems, and standards for the implementation and evaluation of fecal transplantation.

Taken together, this meeting reflected on the tremendous progress made over the recent years in our understanding of host-microbiota interactions and the multiperspective approach that needs to be taken when studying this fascinating part of human physiology and pathophysiology. It also sought to define new standards that must be applied when analyzing the many variables characterizing this community, as the importance of the majority of these variables remains unknown. A systematic understanding of the microbiome has the potential to transform modern medicine, and this is an important time for establishing the right conditions for a productive decade ahead in microbiome research.

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