Immunity

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Voices

# Microbes and metabolites in immunity

The immune system has a vital, albeit complex, relationship with the microbes residing within us, one that we are only beginning to understand. We asked investigators what they felt were the fundamental challenges we currently face in unraveling the impacts of microbes and their metabolites on host immunity and to discuss key opportunities toward achieving future insights and innovation.

**Eran Elinav**
Weizmann Institute of Science; German Cancer Research Center

## Innovation through chemistry

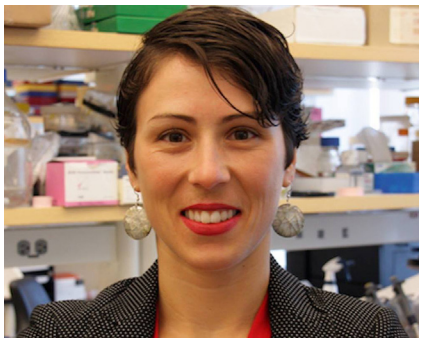
I never enjoyed chemistry classes—the field felt complex, intimidating, and out of context to me. I now realize how mistaken I was. Chemistry has become a critical tool in decoding the intricacies of host-commensal interactions and their physiological impacts. Microbiome research began as a global quest to describe microbial configurations correlating with human health and disease states. Although the generated microbiome atlases are invaluable, we gradually recognized that the most noted associations were driven by "passenger" microbiome community changes secondary to disease-related epiphenomena rather than impacting its course. This realization led to a first "maturation phase" utilizing technologies such as bacterial gnotobiotic transfers and commensal-organoid co-cultures to establish a cause-and-effect relationship between a minority of microbial strains and their hosts. The revelation that microbiomes may produce, degrade, and modulate thousands of metabolites capable of reaching any cell and organ through the bloodstream fostered a paradigmatic shift toward identifying and characterizing these microbial-modulated molecules. While the breathtaking chemical diversity and complexity of such bioactive molecules present new challenges, integration of such chemical exploration advances the microbiome field toward improved appreciation of the molecular nature of microbial impacts on the human host. Of no less importance, harnessing these chemical insights may enable the development of long-awaited, reproducible, and scalable microbiome-based treatments while bypassing the marked human interindividual microbiome variability. Returning to my old chemistry notebooks remains as intimidating as ever but is now accompanied by bright prospects. The future of microbiome research, viewed through the chemical lens, is not just promising—it's indispensable.

**Suzanne Devkota**
Cedars-Sinai Medical Center

## Uncovering therapeutic metabolites

The functional unit of the microbiome is the collection of metabolites they produce. There are many beautiful examples in nature in which metabolite exchange among microbes, and between their environments, forms the bedrock of entire ecosystems. The human body is no different, where the primary inputs for this exchange are largely derived from our diet, and the bi-directional outputs shape everything from how we sense satiety to the expansion of regulatory T cells. But we are entering a new era of microbial chemical biology as we consider the over 3,000 biosynthetic gene clusters encoding small molecules of secondary microbial metabolism, such as terpenes and polyketides, many never before thought to derive from our microbiome and many of unknown function. Plant and fungal analogs of these metabolites have been successfully developed as anti-cancer, cholesterol-lowering, and antimicrobial agents. The nascent bacterial pharmacopeia we are amassing naturally fills us with hope and wonder, like a rainforest of opportunity for manipulating health. While large sequencing efforts identified these cryptic gene clusters, deeper experimentation to mine their activities and byproducts is essential for therapeutic development. The challenge lies in their silence under standard lab-grown conditions. This necessitates creativity on the experimental approaches and conditions used to coax our microbiome to tell us its secrets. Tractable tools for genetic manipulation combined with differing media formulations, temperatures, pH, oxygen tension, and nutrient availability are all

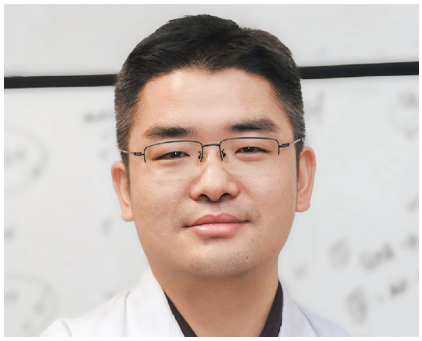
opportunities for tapping into microbial metabolism. Focused innovation addressing this issue will usher in a new day for microbiome-based therapeutics.



**Marlies Meisel**
University of Pittsburgh School of Medicine

## Leveraging diet for immunity

Over the past two decades, we have discovered that one key mechanism by which the gut microbiota modulates local and systemic immunity—both during homeostasis and in diseases such as autoimmunity and cancer—is through the metabolites they produce. With this discovery, however, comes the exciting new question of how to direct the gut microbiota metabolome in order to drive certain immune responses. Diet is a crucial environmental factor that shapes the composition and function of the gut microbiota. Essentially, we are "eating for two," providing both nutrients for ourselves and substrates for bacteria, thus influencing the diversity of the microbiome and its metabolic profile. The diet is a complex entity, composed of a vast array of macro- and micronutrients. Therefore, it is important to identify how we can use diet as a tool to precisely and selectively modulate microbial metabolic pathways to tailor immunity to our needs. To enable comprehensive conceptual advances, we must investigate the impact of single nutrients (e.g., amino acids, carbohydrates, vitamins) on the microbiota metabolome and its subsequent impact on immunity one at a time. Technologically, we need to apply a combination of interdisciplinary multi-omics approaches (metabolomics, culturomics, metagenomics, and genomics) along with traditional mechanistic studies to test the impact of identified metabolites on immune cell functionality. Advances in this line of research will be crucial for breakthroughs in precision dietary approaches that can fine-tune immunity by directing the metabolic machinery of our gut microbiota to maintain health and combat disease.



**Shu Zhu**
University of Science and Technology of China

## Food, microorganisms, and the immune system

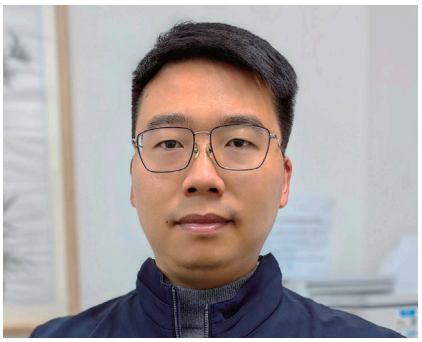
The intestine serves as a crucial interface where food, microorganisms, and the immune system interact and influence human health. Immunologists are beginning to understand how our immune system recognizes food components and commensals as "non-self" while still maintaining homeostasis and tolerance. Additionally, we are uncovering how specific nutrients (e.g., fiber, amino acids, fatty acids, vitamins) and their microbial derivatives (e.g., secondary bile acids, short-chain fatty acids, tyramine) shape and affect the intestinal immune system. However, due to limitations in knowledge and technical constraints, a comprehensive understanding of this dynamic triad remains elusive.

A multidisciplinary approach integrating nutrition, microbiology, immunology, gastrointestinal physiology, and computer science is required to understand three key aspects of the field. First, advancements in techniques—including spatial metabolomics and metabolic flux, microbial engineering, *in vivo* functional screening, and machine learning—could begin to unravel the intricacies of innate sensing and microbial recognition. Ultimately, this would help construct a chemically based network linking nutrients, microbes, and the immune system. Second, high-throughput screening systems are needed to decipher antigen specificity of adaptive immune cells. Applying large language models to comprehensive databases of T or B cell receptors and their cognate antigens could enable epitope prediction or identify the type of corresponding receptor based on antigen sequence characteristics. Finally, insights from the above aspects could lead to more tailored approaches in immunotherapy and immune modulation, including dietary adjustments and administration of commensal microorganisms or metabolites. By leveraging individual immune profiles and understanding diet-microbe-immune interactions, precision immunotherapy would be better suited to improve health outcomes and optimize disease treatment efficacy.

**Hiutung Chu**
University of California, San Diego

## Inflammatory impact on microbiota

Advances in metabolomics and computational methodologies have unveiled an unprecedented number of diverse microbial metabolites produced or modified by the gut microbiota. Research over the last two decades has revealed that specific bacterial metabolites (e.g., bile acids, short-chain fatty acids, lactate, succinate) can, under certain conditions, impact immune cell function. However, it is important to emphasize that the interaction between the host and microbiota is bi-directional, whereby the immune system can also shape the microbial composition and reprogram bacterial metabolism. Inflammatory conditions, in particular, often result in a transition from an anaerobic to a more aerobic environment in the gut, leading to selection and expansion of microbes that can adapt and survive in the presence of oxygen. Moreover, metabolic reprogramming of immune cells leads to a shift from oxidative phosphorylation to glycolysis. This depletes local glucose and drives gut bacteria to utilize alternative carbon sources, resulting in distinct changes in microbial physiology and metabolite production. These metabolic adaptations in gut bacteria dramatically alter the microbial metabolite pool, further modulating their impacts on the host. Understanding these complex and coordinated effects is essential for developing strategies to alter bacterial metabolism and regulate immune responses. The dynamic nature of bacterial metabolism, influenced by the host environment and, specifically, the immune system, is central to the multifaceted interplay between gut microbiota and host health. Decoding this intricate dialogue will provide new insights into the mechanisms underlying the maintenance of immune homeostasis and modulation of disease.



**Haiwei Chen**
Shanghai Medical College, Fudan University

## Technical innovation for gut microbes

During the past two decades, multi-omics-dependent cohort studies have revealed the correlations between gut microbiota dysbiosis and diseases. However, the mechanistic investigations of how commensal species modulate host physiologies and pathologies need to be strengthened. *In vitro* bioactivity screenings facilitate the identification of bioactive microbial metabolites, whose potential targets include membrane receptors (e.g., G-protein-coupled receptors, pattern-recognition receptors, and cytokine receptors), cytosolic proteins (e.g., kinases and enzymes), and nuclear transcription factors. Because the activation, differentiation, and function of immune cells are dictated by the integration of various cell signals, these commensal-derived bioactive chemicals can act as powerful tools directing host-microbiota interactions in health and diseases.

In addition to mechanistic studies, another challenge in this field is how to illuminate the biological functions of millions of human microbial genes. Functional profiling of microbial genes not only advances mechanistic insights into complex diet-microbiota-host interactions,but also expedites therapeutic harnessing of microbial genes for metabolic syndromes or immune disorders affected by microbiota dysbiosis.

Novel technologies are required to overcome these challenges. For example, multiplexed bioactivity screening platforms that build off signal transduction will enable systemic identification of commensal-derived molecules that can modulate host cell signaling pathways. Also, a universal genetic tool that is suitable for the majority of bacterial species and executes genome-wide loss-of-function screenings in the host will facilitate functional profiling of microbial genes at scale. Both *in vitro* bioactivity screening and *in vivo* genetic screening will accelerate functional and mechanistic studies of host-microbiota interactions in health and diseases.

**Jens Puschhof**
German Cancer Research Center

## Microbes and tumor immunotherapy

The success of cancer immunotherapy in patients is linked with fecal microbiome patterns. However, the tumor-resident microbiome has only recently come into focus. A detailed understanding of the distinct mechanisms and relative importance of immune modulation by the gut and tumor microbiome is essential for harnessing bacterial constituents of the tumor microenvironment and improving immunotherapy efficacy.

The vast majority of the body's microbiome resides in the gut. In contrast, the biomass of any tumor's microbiome is expected to be dramatically lower and—while found consistently in colorectal cancer and other tumors of organs with an endogenous microbiome—remains controversial for different cancer entities. Even so, the proximity of tissue-resident microbes to the tumor and infiltrating immune cells would likely shape signaling gradients and even contact- or invasion-dependent phenotypes.

The impact of the gut microbiome on immune cell training, translocation, and metabolite availability is becoming increasingly clear in diverse cancer contexts. Similarly, the first intriguing reports on tissue-resident microbial effects are emerging, ranging from recruitment of tumor-targeting immune cells to immunosuppressive competition for nutrients. Technological advances combining low-biomass microbiome sequencing with spatial transcriptomics approaches are beginning to map the tumor ecosystem at unprecedented depth. Transforming these omics-based atlases into a detailed understanding of microbe-immune interplay through systems biology approaches will be essential for modulating microenvironment components toward improved immunotherapy outcomes.

Ultimately, understanding the homing and interaction mechanisms of tumor-resident microbes with local immune cells and the tumor mass promises to yield new approaches for microbiome-informed immunotherapy of diverse tumor entities.


**Florencia McAllister**
MD Anderson Cancer Center

## Tackling the tumor ecosystem

Although methodologies to quantify and identify tumor microbes are still in development, most tumors likely contain microbes. The inherent complexity of the tumor microenvironment presents challenges in unraveling microbe interactions with host cellular and molecular components. Tumor microbes likely drive biology, but they also impact responses to therapy, due to their influences on drugs metabolism and interactions with cells in the tumor environment. Moreover, tumors' intrinsic heterogeneity suggests that variability may occur within the same tumor. Thus, resolving microbial contributions within a tumor warrants spatial examination at the single-cell level to factor in differences in oxygen, pH, and blood flow—all key components dictating microbial behavior.

Metabolites are the language of microbes. Nevertheless, given their capacity to cross cellular membranes, metabolites likely not only play a role in the microbial communities but also alter functionality of host cells. Because of their easy diffusion within tissues, metabolites derived from tumor microbes may affect not only tumors but also adjacent tissue, consequently influencing tumor expansion and/or affecting metastasis formation in distant tissues. Beyond this, host-derived metabolites may also affect tumor microbial function and, ultimately, tumor behavior. Metabolic spatial heterogeneity likely also exists within tumors, again calling for single-cell evaluation to study these complex functional interactions. As tumor microbial metabolites are identified, new therapeutic strategies should be developed through synthetic biology to ultimately establish functional relevance. Future tumor assessments at diagnosis may require obtaining not only genetic information but also microbial and metabolic information so that therapies can be tailored to tackle the tumor ecosystem.

**Randall Jeffrey Platt**
ETH Zurich

## Engineered bacteria for human health

Commensal bacteria have profound impacts on human health. Uncovering the mechanisms underlying these impacts holds immense potential for guiding strategies that promote health and/or diagnose and treat disease. Engineered bacteria are being developed with fantastic designer functionalities such as priming the immune system, sensing diagnostic biomarkers, destroying toxic molecules, delivering therapeutic payloads, and non-invasively recording intestinal composition and function. Increasing our capacities and advancing these discoveries to the clinic will lead to medical breakthroughs, but numerous challenges need to be overcome.

Microbial engineering in the context of human health is confronted with biological, technical, and practical challenges. Biologically, most of our understanding is based on association rather than causation, making subsequent engineering efforts futile from the outset. Technically, it is extremely challenging to control bacteria and their gene circuits in complex environments. Current technologies are largely restricted to *E. coli*, which limits potential. Practically, academia, biotech, and healthcare systems are not set up to support the full range of development, regulatory, and commercialization activities required to translate engineered bacterial products.

To overcome these challenges, we must demand causal mechanistic understanding, go beyond preliminary proof-of-concept demonstrations toward achieving efficacy and safety in translational models, embrace the challenge of establishing new model systems beyond *E. coli*, and overcome the convenience of conventional technologies to fearlessly pursue new, creative technological frontiers. We must also build bridges to broader stakeholders—funding bodies, investors, regulators, healthcare agencies, and the public—to pave the way for realizing the full potential of bacteria in promoting human health.

**Kenya Honda**
Keio University School of Medicine

## Exploring uncharted microbiota metabolites

The human gut microbiota, a prolific source of over 100,000 bioactive metabolites, plays a crucial role in maintaining local gut health and broader systemic processes. Key metabolites such as short-chain fatty acids and aromatic amino acid derivatives are vital in immune regulation and neuromodulation, helping maintain homeostasis across multiple organ systems. Secondary bile acids not only prevent colonization by gram-positive pathogens but also modulate immune responses and reduce inflammation in the gut and liver, potentially promoting longevity. The clinical application of microbial metabolites, or the knowledge derived from them, opens promising avenues for treating and preventing age-related diseases, including cancer and infections, as well as metabolic, immune, and neurological disorders. Despite advancements in liquid chromatography-mass spectrometry technology, over 90% of these metabolites remain structurally and functionally unannotated, constituting a vast "dark matter" in microbiota research. The microbiota's cooperative nature, involving multiple bacterial species, complicates the task of attributing specific effects to individual microbes. To effectively decipher the structures, biosynthetic pathways, and functions of these metabolites (including their interactions with receptors), holistic approaches are essential. These include integrating generative AI to predict metabolite structures from mass spectrometry data, employing precision synthetic organic chemistry, and conducting co-abundance analysis with known compounds. Furthermore, linking specific metabolites to phenotypic outcomes through gene manipulation of microbes and testing them *in vivo* using gnotobiotic techniques is crucial for developing targeted therapeutic interventions and substantiating the use of live biotherapeutic products with defined mechanisms of action. Engaging in this dynamic and transformative field is both challenging and a privilege.

### DECLARATION OF INTERESTS

E.E. is a scientific cofounder of BiomX and GutBiome and an advisor to Purposebio, Aposense, and Zoe in topics unrelated to this work. S.Z. is a cofounder of Ibiome, which studies microbial regulation of immune responses in topics unrelated to the subject of this work. F.M. is a scientific advisory board member at Neologics Bio. R.J.P. has patents related to this topic: "Non-invasive assessment of gut function with transcriptional recording sentinel cells," EP22166075 (2022) and "Transcriptional recording by CRISPR spacer acquisition from RNA," PCT/EP2019/074267 (2018).