MUCOSAL IMMUNOLOGY

Causal effects of the microbiota on immune-mediated diseases

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The mammalian immune system has evolved in the presence of a complex community of indigenous microorganisms that constitutively colonize all barrier surfaces. This intimate relationship has resulted in the development of a vast array of reciprocal interactions between the microbiota and the host immune system, particularly in the intestine, where the density and diversity of indigenous microbes are greatest. Alterations in the gut microbiota have been correlated with almost every known immunological disease, but in most cases, it remains unclear whether these changes are a cause or effect of the disease or merely a reflection of epidemiological differences between groups. Here, we review recent efforts to demonstrate a causal role for the microbiota in health and disease, outline experimental advances that have made these studies possible, and highlight how changes in microbial composition may influence immune system function.

INTRODUCTION

We are constitutively colonized by trillions of viral, fungal, bacterial, and eukaryotic microbes at all barrier surfaces, which are collectively referred to as the microbiota, and these microorganisms can have marked effects on the immune system in health and disease (*1*, *2*). In humans, the gastrointestinal tract contains the largest number and greatest diversity of bacteria; every individual harbors a distinct microbial community composed of hundreds of bacterial species and strains, and the collective human microbiota contains thousands of species spanning more than 10 phyla (*3*). This community is shaped by a wide variety of both extrinsic and intrinsic factors, including microbial exposure, diet, medical drugs, host genetics, and the immune response itself.

Although the presence of indigenous microbes in the gut has been known since before Metchnikoff, there has been a marked resurgence in interest in host-microbiota interactions over the past two decades. The initial phase of this revival was nucleated by developments in next-generation sequencing, which enabled a variety of culture-independent methods to determine microbiota composition and function (*3*–*5*). In particular, reductions in the cost of 16*S* ribosomal RNA (rRNA) gene sequencing have resulted in an overwhelming number of observational reports of changes in microbial community composition in diseases as diverse as inflammatory bowel disease (IBD), obesity, metabolic syndrome, and autism (*6*). However, these findings also made apparent the major limitation of observational studies of the microbiota: Correlation does not equal causation. Because the microbiome can be influenced by innumerable distinct exogenous and endogenous factors, imputing cause and effect by observation alone is nearly impossible. Drawing causal connections between specific microorganisms and their impacts on the host has important implications for understanding the role of the microbiota in human disease and for informing the development of microbiota-targeted therapeutics. Thus, we are now entering what could be considered the second wave of microbiome studies, in which many groups are striving to transition from correlation to causation and subsequently to elucidate the specific molecular mechanisms by which the microbiota influences host physiology and pathology. In this Review, we outline recent advances toward these goals and the theoretical and technical challenges that remain and speculate on future opportunities and challenges.

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EMERGING TOOLS FOR DISSECTING CAUSAL RELATIONSHIPS

It is now well documented that differences in microbiota composition are observed in individuals with immunological disease (*2*, *7*). However, much less is known about whether these changes have biological relevance. Because causality cannot be readily assessed in humans, many groups have begun to use germ-free mice colonized with human gut microbes to start to address causation and to assign specific functions to individual microbes or groups of microbes based on their impacts on the host. These "humanized" gnotobiotic mouse–based approaches range from monoassociations with individual human gut microbes to transplantations of complete gut microbial communities from healthy or diseased individuals (Fig. 1). Each of these methods has its own advantages and limitations.

Monocolonizations

Monoassociations of germ-free mice with a single microbe (monocolonizations) are often used to test how specific human gut microbes affect immune system development. For example, monocolonization studies have demonstrated that the commensal *Bifidobacterium adolescentis* can potently stimulate T helper 17 (T_H17) differentiation (*8*), that specific *Clostridia* and *Bacteroides* species can stimulate regulatory T (T_{reg}) cell differentiation (*9*–*11*), and that *Bacteroides fragilis* polysaccharide A (PSA) can promote gut colonization (*12*). Furthermore, a recent study used monocolonizations to determine the impacts of 53 human microbes on various aspects of the immune response and observed diverse effects of specific microbes on the immune system (*10*).

Although monoassociation approaches are useful for pinpointing the effects of individual organisms, they also have many limitations. First, monocolonizations are inherently artificial. For example, they often allow bacteria that typically occupy a limited niche to spread throughout the intestinal tract, which may lead to abnormal host responses. In addition, isolating individual strains away from their natural microbial communities eliminates potentially critical interactions with other bacterial, fungal, or viral species, which may alter the natural effect of that single organism on the host. Second, it is often difficult to identify

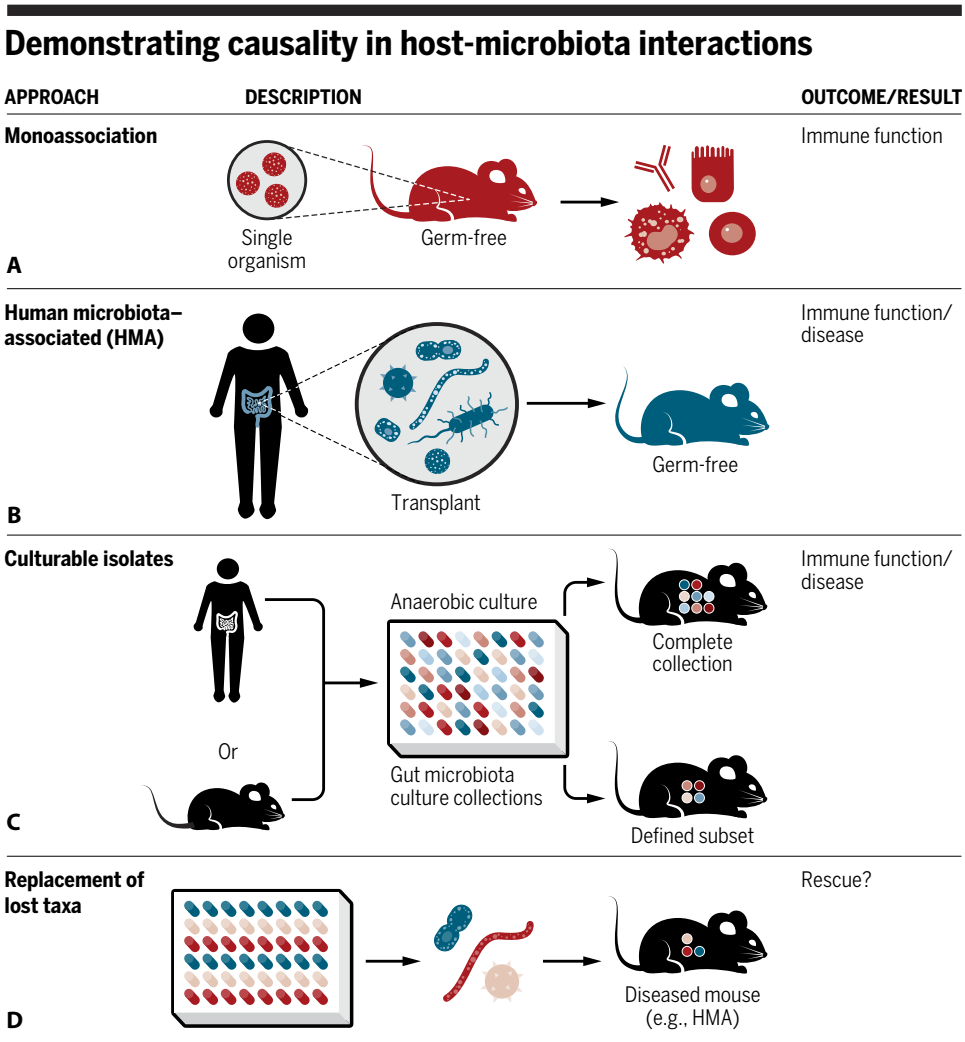

Fig. 1. Demonstrating causality in host-microbe interactions. Germ-free mice colonized with specific microorganisms or microbial communities have emerged as the gold standard for demonstrating causal roles for the microbiota in shaping host physiology and disease. These approaches range from highly reductionist models, such as monocolonization, all the way to colonization with complete microbial communities from human patients. Each of these methods has particular advantages and limitations. (**A**) Monoassociation of germ-free animals with a single microorganism. Germ-free mice can be monocolonized with bacteria from many sources including, most commonly, isolates from human or mouse gut microbial communities. Advantages: It allows for precise interrogation of the activity of a single organism, it may reveal the functions of low-abundance organisms that are masked in the presence of a complex community, and bacterial mutants can be used to understand gene-function relationships. Limitations: It ignores the complexity of the human microbiota and the importance of microbe-microbe interactions, it is highly nonphysiological, and it is often difficult to choose proper controls for comparison. (**B**) HMA mice. Complete gut microbial communities obtained from individuals with a particular disease (such as IBD and Parkinson's disease) and healthy controls can be used to colonize germ-free mice. Advantage: Conferral of a given phenotype proves microbial causation. Limitation: Xenotransplantations of microbial communities face multiple hurdles, including loss of species because of oxygen sensitivity, an inability of certain or particular human gut microbes to colonize rodent hosts, and mechanisms of host-microbe interaction that are specific to the human host. (**C**) Introduction of culturable isolates into germ-free mice. Recent studies have taken the HMA model one step further by colonizing germ-free mice with culturable isolates from the human gut microbiota. Advantages: It allows for determination of the effects of specific groups of microbes on the host, defined subsets of the microbiota can be assembled rationally according to phylogenetic or functional profiling and predictions, and cultured microbes can be studied in vitro and in vivo to determine mechanisms by which a given organism affects the host. Limitations: Certain gut microbes are difficult or impossible to culture in vitro, and isolate-based experiments are very low throughput due to the effort and time needed to construct culture collections. (**D**) Replacement of "beneficial" bacterial taxa. Conventional mice or HMA mice with dysbiosis or microbiota-driven disease are ideal tools to test the ability of beneficial species to correct dysbiosis and prevent disease. The advantages and limitations of these models are similar to those described for HMA mice and culturable isolates.

appropriate experimental controls for a given monocolonization: Is the proper comparison to germ-free mice, to mice colonized with a complete microbiota, or to monoassociations with other bacterial species? Last, and maybe most importantly, monocolonizations do not allow for determination of the collective effect of complex microbial communities on the host.

Human microbiota–associated mice

Many investigators have recently begun to use human microbiota–associated (HMA) mice (germ-free mice colonized with a complete gut microbial community from an individual human) to model the effects of complex human microbial communities on disease. HMA mice were first used to examine the role of the microbiota in obesity by transplanting gut microbial communities from a twin pair that was discordant for obesity into two groups of germ-free mice (*13*). In this experiment, the recipients of the "lean" microbiota remained lean, whereas recipients of the "obese" microbiota became obese. Since these initial studies, HMA models have been used to determine the role of the microbiota in a variety of disease states, including asthma (*14*), pregnancy-induced increases in adiposity (*15*), behavioral differences associated with irritable bowel syndrome (*16*), IBD (*17*), multiple sclerosis (*18*, *19*), and Parkinson's disease (*20*). Together, these studies highlight the ability of HMA mice to illuminate causal roles of the microbiota in a diverse array of disease states.

Although HMA models have led to remarkable successes, they also suffer from multiple unavoidable caveats that can limit their ability to accurately reflect the role of the gut microbiota in human disease (*21*). Many of these limitations result from inherent differences between mice and humans in intestinal anatomy and physiology, and therefore in gut ecology. In addition, environmental, behavioral, and genetic factors will naturally differ between HMA mice and their human donors; all of these factors affect microbial communities in diseased humans and are often difficult or impossible to replicate in mouse models. Thus, it is perhaps unsurprising that microbial communities in HMA mice do not perfectly reflect their human donors. For example, a considerable number of human gut microbiota species (~15%) fail to colonize the mouse gut after human microbiota transplantation

into germ-free mice (*22*). In addition, microbial community structures in HMA mice (the relative abundance of the various species and strains) are substantially altered as compared with their human donors. Thus, states of "dysbiosis" resulting from simple changes in community structure, rather than from gain or loss of specific species, are difficult to replicate in HMA models. Last, certain commensals have evolved mechanisms of host interaction that are exquisitely host-specific; for example, rat-adapted segmented filamentous bacteria (SFB) fail to adhere tightly to the epithelium and drive T_H17 responses in mice (*23*). Thus, even human-adapted species that successfully colonize mice may not exert their natural functions or activities in the context of a mismatched host.

The substantial limitations of current HMA models suggest that novel animal models will be necessary to fully illuminate the causal role of the microbiota in human disease. For example, immunodeficient germ-free mice containing transplanted human hematopoietic cells (humanized mice) may allow for dissection of host-specific interactions between the microbiota and the immune system (*24*), and gnotobiotic piglets more closely approximate the anatomical and physiological characteristics of the human gastrointestinal tract. However, in addition to their complexity and cost, these approaches also have major caveats. For example, even the most sophisticated humanized mouse models remain highly immunologically abnormal, and epithelial cells in humanized mice are mouse rather than human; thus, epithelial-microbiota interactions, which are a dominant mechanism of host-microbiota interaction in the gut, will remain mismatched. Given these challenges, and the advantages of traditional mouse models more generally, HMA mice will likely continue to be the dominant in vivo system for determining the role of the microbiota in human disease.

Using the immune system to identify causal microbes

The reciprocal relationship between the immune system and the microbiota is central to the impact of the microbiota on disease (*1*, *2*); thus, immunologically important gut microbial species and strains are likely to have outsized effects on human disease. An approach referred to as immunoglobulin A sequencing (IgA-seq) takes advantage of mucosal antibody responses to the microbiota to identify immunologically relevant microbes that may play causal roles in disease (*17*, *25*–*27*). IgA-seq simply involves purification of IgA-coated bacteria and noncoated bacteria by cell sorting, followed by 16*S* rRNA gene sequencing to determine which specific organisms are targeted by IgA. One of the first papers to use this method used IgA-seq to identify putative disease-driving bacteria in IBD and went on to isolate these organisms and demonstrate that these microbes, but not organisms displaying low IgA coating, severely exacerbated mouse models of colitis (*17*). Similar approaches have shown that IgA-targeted microbes can influence enteropathy in undernourished individuals (*28*) and can drive the development of Crohn's disease–associated spondyloarthritis (*29*). Collectively, these studies demonstrate that, despite the complexity of the microbiota, causal members can be identified, isolated, and tested in animal models.

Emerging and future approaches to dissecting host-microbiota interactions in human disease

Future developments in "omics"-based profiling techniques—such as shotgun metagenomics, proteomics, and metabolomics, as well as the development of new functional profiling approaches such as IgA-seq—promise to enable the identification of causal organisms in a variety of diseases in the future (*6*). For example, functional profiling–based approaches can theoretically be used to identify specific organisms that affect a variety of physiological features that are regulated by the microbiota, such as regulation of epithelial permeability or production of specific bioactive metabolites. Studies such as these will naturally lead to a plethora of new hypotheses regarding potentially causal roles for the microbiota in disease. However, the number of hypotheses generated will inevitably exceed our capacity to test these emerging hypotheses one by one using experimental models. One emerging solution to this problem is to integrate omics and experimental data by using advanced computational approaches (such as machine learning) to reduce the number of experimental tests necessary to establish robust and predictive models. Integration of various data sets to create more sophisticated models may eventually allow for many emerging hypotheses to be tested in silico rather than in vivo or in vitro (*6*). Given the sheer magnitude of the problem, creating synergistic and self-reinforcing interactions between experimental data and omics data will be critical to building a complete picture of the causal role of the microbiota in human health and disease.

Culture-based studies of the gut microbiota

Elucidating the roles of individual commensal microbes in host physiology inevitably requires the ability to capture a given microbe of interest in monoculture. For many years, it was assumed that the vast majority of gut microbes are unculturable; this conclusion likely stemmed from the simple observation that very few gut microbiota species, which are primarily obligate anaerobes, grow on standard media under aerobic conditions (*30*, *31*). However, recent high-throughput anaerobic microbial culture approaches that use next-generation sequencing to classify large numbers of bacterial isolates have revealed that a much greater proportion of the human gut microbiota can be captured in monoculture than was previously thought. These efforts have revealed that the vast majority of gut microbiota species are culturable, often under relatively standard anaerobic culture conditions. One of the first studies in this area successfully cultured ~50% of all detectable bacterial species from an individual's gut microbiota by using a single rich medium (*32*). More recently, a broader effort to capture increased gut microbial diversity in culture, combined with a meta-analysis of previously isolated species, concluded that >75% of all known species inhabiting the human gut have been captured in monoculture at some point in time (*30*). Last, large-scale culture studies using diverse media have suggested that up to 95% of species present at >0.1% relative abundance in fecal samples are theoretically culturable (*31*). One interesting finding of recent culture-based studies is that certain species observed in these studies are missed when the same samples are analyzed using culture-independent sequencing-based methods. These findings highlight limitations of culture-independent microbiota analyses, which include biases in DNA recovery and 16*S* amplification, as well as relatively shallow limits of detection based on sequencing depth.

An additional factor that highlights the importance of culture-based studies is the phenomenon of strain variation. Many studies have demonstrated that different strains of the same species (with 97% nucleotide identity at the 16*S* rRNA gene often used as a proxy for species-level classification) can have distinct, and sometimes even opposite, effects on the host (*17*, *33*). Thus, species-level classifications of the microbiota are often insufficient to capture critical functional differences between bacterial strains. Therefore, use of a previously cultured strain (such as a type strain from the American Type Culture Collection) to test the role of a particular species in disease is a poor alternative to direct isolation of that species from the gut microbial community of interest. Thus, although culture-independent methods have revolutionized

microbiota research, the resurgence of culture-based studies of the microbiota will be critical to build a complete picture of microbial contributions to human disease.

Together, these studies suggest that cultivation of the majority of gut microbes is now possible. Perhaps even more importantly, multiple groups have now shown that cultivatable fractions of the human gut microbiota can confer human phenotypes in HMA mice (*17*, *28*, *34*, *35*). These studies thus suggest that many of the species and strains responsible for shaping human physiology can be cultured, opening the door to more detailed explorations of the mechanisms by which specific human gut microbes influence disease.

WHAT WE HAVE LEARNED ABOUT CAUSAL ROLES OF THE MICROBIOTA IN HUMAN DISEASE: DIVERSITY MATTERS

Biodiversity contributes to the stability and resiliency of diverse ecosystems. Resiliency is the amount of disturbance that the community can absorb while still maintaining its functional state. If a community is highly durable, then the community can reorganize and renew when a particular species is lost, whereas communities with low biodiversity are more sensitive to losses of individual species. Highly diverse ecosystems likely contain organisms with redundant functions, making the loss of a single species tolerable because a functionally redundant species can rapidly take over this niche and replenish that function. Low-diversity communities contain fewer members, making functional redundancy less probable, with specific functions being fulfilled by only one member (*36*). Therefore, loss of that one member can have a catastrophic effect on the rest of the ecosystem. On the basis of this, it is often accepted that, within any given ecosystem, a more diverse community is preferred.

One theme that has emerged from many large surveys of gut microbial communities in a variety of settings is that reduced microbial diversity (species richness) is almost invariably associated with disease. Reductions in the total number of bacterial species present have been observed in humans with eczema (*37*), asthma (*38*), multiple sclerosis (*39*), arthritis (*40*), type II diabetes (*41*), obesity (*42*), autism (*43*), and IBD (*44*), to name just a few. Similar to this, loss of microbial diversity is present in many animal models of disease (*45*). These observations suggest that harboring a more diverse gut microbial community may prevent disease. The potential mechanisms responsible for the beneficial effects of diversity fall into at least three nonmutually exclusive categories: loss of community functions, immunological imbalance, and mismatched niche occupancy (Fig. 2).

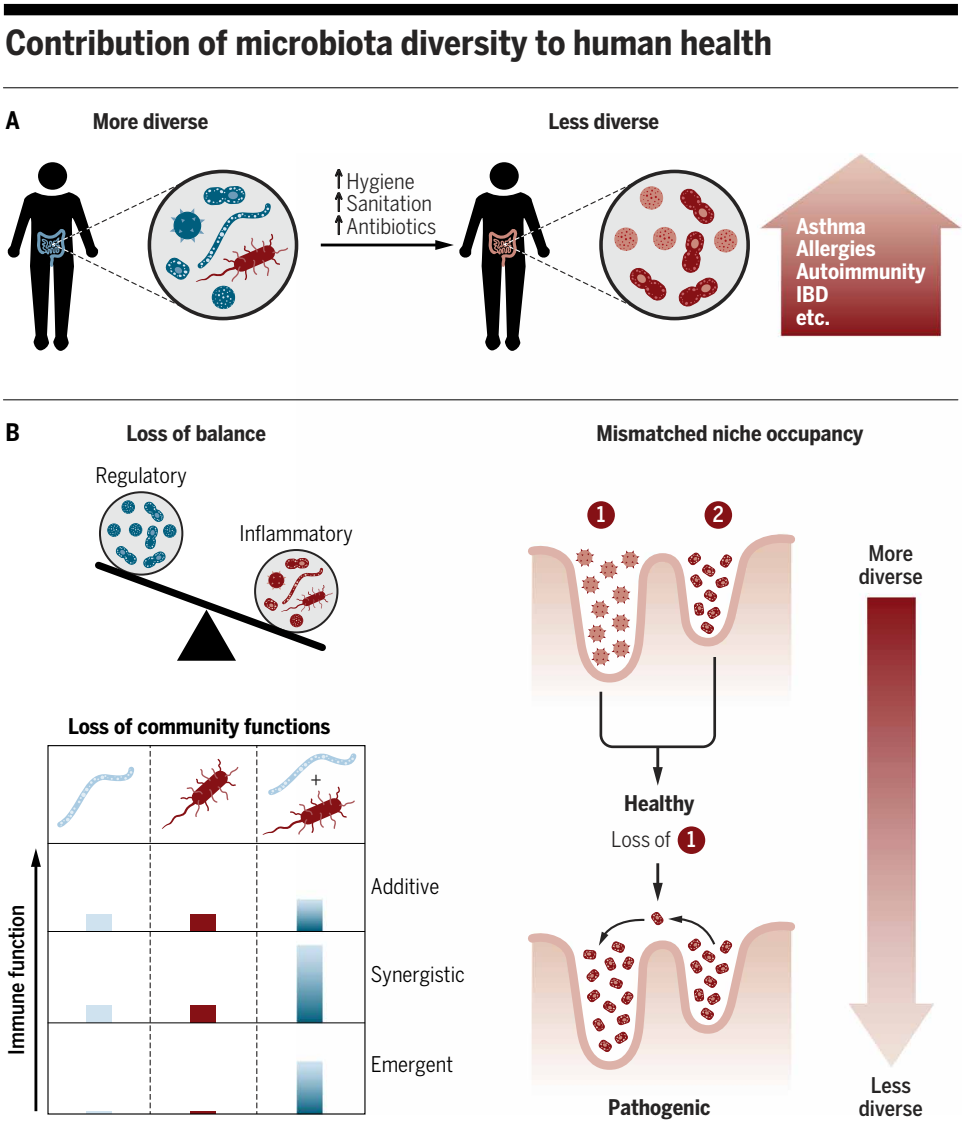

Fig. 2. Contribution of microbiota diversity to immune health. (**A**) Higher levels of α diversity (number of different taxa within a given microbiota) are almost invariably associated with reduced incidences of disease both within modern societies and between modern society and the developing world. (**B**) Potential mechanisms by which reduced microbial diversity may contribute to the development of dysbiosis and increased susceptibility to disease. Loss of balance: This model posits that the impact of the microbiota on disease depends mainly on the relative balance of inflammatory versus immunoregulatory taxa present in a given microbial community. Thus, selected loss of regulatory taxa without concomitant loss of inflammatory taxa might lead to a loss of homeostasis and increased disease development. Loss of community functions: The effects of a complex microbial community can scale owing to additive and synergistic effects, as well as emergent properties. Thus, decreases in microbial diversity may lead to erosion, or even complete loss, of specific beneficial functions of the microbiota. Mismatched niche occupancy: This model posits that particular bacterial taxa have coevolved with the host to inhabit exquisitely defined niches, where they can coexist symbiotically with the host and provide colonization resistance against neighboring species. The loss of a "niche-matched" symbiont would lead to invasion of the newly empty niche by neighboring microbes that have not evolved to occupy that niche. This mismatched niche occupancy might result in the initiation of an inappropriate and potentially pathogenic host response.

Loss of community functions

Complete microbial communities may provide activities that individual organisms cannot confer on their own. Loss of community functions can thus occur because of additive effects of multiple taxa, synergistic effects between taxa, or emergent properties of a microbial community. One experimental example of this is that monocolonizations with a variety of organisms are insufficient to reverse the hyper-IgE phenotype in germ-free mice; in contrast, colonization with a complex microbial community restores normal IgE levels (*46*). Another example is the observation that microbiota-dependent microglia maturation cannot be restored by a consortium of three common gut commensals (*47*). Last, induction of T_{reg} cells in the gut by a consortium of spore-forming Clostridia species also requires diversity: Whereas 15 Clostridia strains induced strong T_{reg} cell responses, collections of one to five members could not replicate this effect (*9*). Although these studies support the idea that complexity of the microbiota may be necessary for proper immune development, there are also examples in which monocolonization can restore specific immune parameters to the levels of a complex community. For instance, monoassociation of germ-free mice with 23 individual species of bacteria identified multiple bacterial species that could restore normal development of RAR-related orphan receptor–γt^+ ($Ror\gamma t^+$) T_{reg} cells (*11*).

Immunological imbalance

Immune health requires the maintenance of a homeostatic balance between pro- and anti-inflammatory responses (*7*). This equilibrium is particularly crucial at mucosal surfaces, where tolerance of commensal microbes needs to be carefully balanced with resistance to infection (*2*). The immunological balance in the intestine is primarily determined by the composition of the microbiota (*1*, *7*). Although an overabundance of inflammatory microbes can lead to chronic intestinal diseases, such as IBD, harboring excessive tolerogenic organisms can make the host more vulnerable to infection. Although most studies have focused on how a single bacterial species drives either inflammation or tolerance, most microbiota species actually elicit mixed responses. Thus, although SFB are best known as inducers of T_H17 cell responses in the mouse gut (*48*, *49*), they can also induce T_{reg} cell and T_H1 responses (*49*). Furthermore, a recent study that broadly characterized the immune response to 53 different bacterial strains found that many microbes simultaneously induce inflammatory and immunoregulatory responses; in some cases, one of these responses is dominant, whereas in other cases, the response is balanced (*10*). This study reinforced the idea that distinct microbes can elicit both specialized and redundant immune functions. Overall, these studies suggest that colonization with a diverse array of organisms ensures a robust and balanced development of immune functions and promotes optimal health.

Immunological imbalance due to altered microbiota composition can also arise when the immune system fails to interact appropriately with the microbiota. In one model of immune-microbiota interaction, the immune system might be thought of as a predator. Predators have an outsized effect on ecological communities by controlling the abundance and diversity of species in lower trophic levels. Thus, in the absence of predatory species, a small number of dominant community members can expand unchecked and displace competing species. Supporting this, multiple lines of experimental evidence suggest that immunological defects lead to reductions in microbiota diversity and function (*50*–*52*).

The immune system can potentially affect the microbiota via multiple mechanisms (Fig. 3), including secretion of antimicrobial peptides, lectins, complement, and secretory IgA. All of these immune effectors can directly bind to microbes and potentially influence microbial abundance and function. Knockout and overexpression studies have demonstrated a role for adenosine 5′-monophosphates (AMPs), lectins, and complement in regulating microbiota composition (*50*, *51*). Whereas lectins and antimicrobial peptides target broad classes of bacteria, secretory IgA can target individual microbial taxa

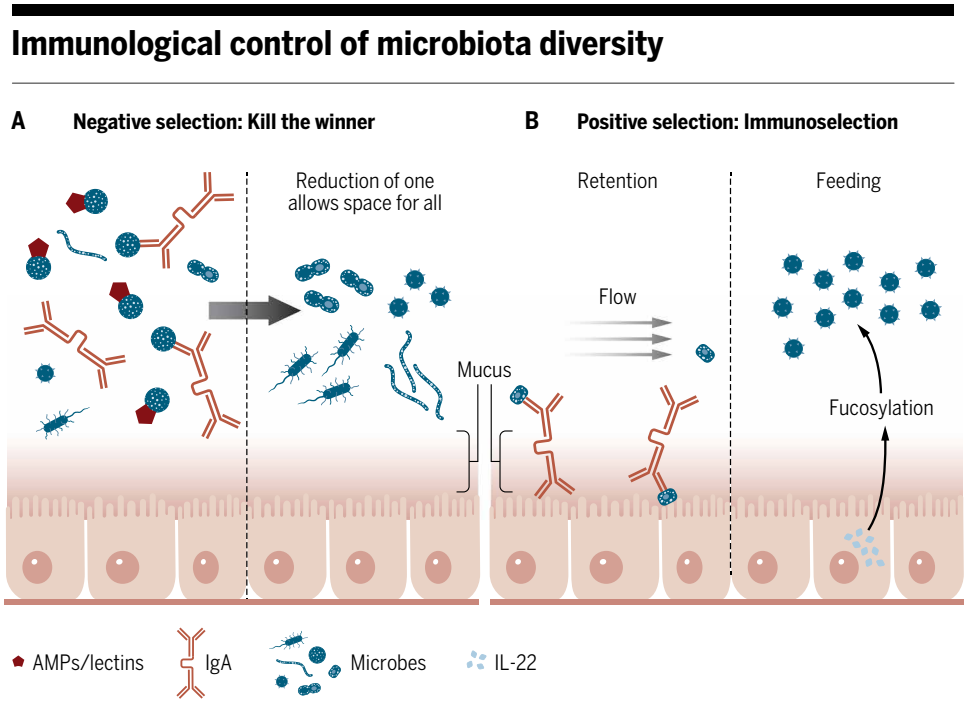

Fig. 3. Immunological control of microbial diversity. (**A**) Negative selection: kill the winner. Studies in macroecosystems demonstrate that predation increases biodiversity by serving to control particularly abundant and well-adapted species. Controlling population growth of these species can liberate niches and resources that allow other organisms to thrive. The immune system can control gut microbial communities via negative selection through multiple mechanisms. AMPs can mediate direct killing, and IgA can cause aggregation and elimination of specific organisms, as well as down-regulation of proteins involved in bacterial motility, invasion, or toxicity, such as flagellin. (**B**) Positive selection: immunoselection. The immune system might also serve to select particular organisms for residence within the gut. In addition to mediating negative selection, IgA may also help retain specific bacterial taxa by promoting retention of slow-growing species in the mucus or by enabling residence in protected niches, such as the colonic crypt. The immune system can also support survival and growth of specific taxa by inducing lumenal deposition of specific nutrients; for example, interleukin-22 (IL-22) induces epithelial fucosylation, which nourishes particular beneficial bacterial taxa.

in a highly specific manner. Correspondingly, elimination or alteration of the IgA response leads to reductions in overall microbiota diversity and outgrowth of particular members of the microbiota that are usually targeted by IgA (*52*).

IgA can influence microbial populations through down-regulation of invasive proteins such as flagellin (*53*), aggregation of dividing bacteria for elimination (*54*), and toxin neutralization. However, precisely how IgA controls the diversity of the gut microbiota remains an active area of investigation. We propose that the immune system might promote microbiota diversity by restricting the most abundant members of a given niche, thus providing space for other microbes to thrive. Because many of the functions of IgA result in the elimination of microbes, IgA binding to the most abundant members might result in "kill-the-winner dynamics," described mathematically by the Lotka-Volterra model, which predicts that predators will rapidly reduce the population of the most abundant species (*55*). In support of this model, there is a strong correlation between microbiota abundance at the mucosa and IgA binding (*26*). However, recent reports have determined that IgA does not always target the most abundant members but perhaps can selectively bind to organisms that are immunologically relevant (*17*). Immunologically relevant members could include those that instigate trouble at the mucosal barrier (such as inflammatory species) or those that are beneficial (such as immunoregulatory species). This idea raises the possibility that IgA might also function to selectively contain "beneficial" bacteria within their niche—for example, by preventing slow-growing bacteria from being washed away by peristalsis (*56*). Consistent with this hypothesis, IgG can selectively "hold" certain species within mucus of the vagina (*57*).

Mismatched niche occupancy

Immunologically potent taxa with varied, and sometimes opposing, activities have been identified in both mice and humans, but what distinguishes immunologically important members of the microbiota from the remainder of the microbial community remains mostly unknown (*2*). However, one emerging theme in the study of immunologically important bacterial taxa is that they often occupy specialized niches in the intestine that other commensal species are unable to colonize—for example, the inner mucus layer in the large intestine, the base of the colonic crypt, or the epithelial surface in the terminal ileum (*58*). All of these niches either are sterile or exhibit very low bacterial density under homeostatic conditions, owing to active immunological defense mechanisms, such as antimicrobial peptide and mucus production (*51*, *59*, *60*). These niches are also proximal to the host epithelium and the underlying immune cells in the lamina propria; thus, one might expect that they are under high immunological surveillance and that occupancy of these niches might naturally lead to the induction of an immune response. For example, bacteria that exacerbate the development of colitis have been shown to occupy the inner mucus layer in the colon, which is largely devoid of bacteria in healthy humans and mice (*61*). Furthermore, specific bacteria thought to be responsible for the development of inflammasome-mediated dysbiosis colonize the base of the colonic crypt (*62*). Occupancy of immunologically important host-proximal niches is not restricted to proinflammatory species, and many noninflammatory or immunoregulatory species have also been found at these sites. For example, the model symbiont *B. fragilis* has also been shown to occupy colonic crypts and induces mainly immunoregulatory responses rather than inflammatory responses because of the production of specific symbiosis-inducing factors (*12*, *63*).

These studies suggest that niche occupancy alone is insufficient to predict the type of immune response that will be induced. Instead, additional signals based on innate sensing of certain features or behaviors of particular classes of bacteria, and production of specific immunomodulatory factors by the microbe, are likely involved in determining the specific outcome of immune activation. Defining these features and behaviors, their corresponding innate sensors and signals, and the hierarchy of these signals is a substantial future challenge for the field.

The gastrointestinal tract is a complex ecosystem comprising a large number of microhabitats. Changes along the length of the small and large intestine include differences in oxygen concentration, pH, mucus, and nutrient availability, to name just a few (*58*). Many resident microbes have evolved to occupy highly specialized niches within this complex ecosystem. For example, multiple commensal organisms have evolved to adhere to and degrade mucus, which allows colonization of the mucus layer (*64*). Furthermore, *B. fragilis* has evolved specific commensal colonization factors that enable it to live deep within the colonic crypts and exclude other bacterial strains, including different strains of *B. fragilis* (*63*). On the basis of these examples, it is likely that other members of the microbiota have evolved to occupy highly defined niches throughout the human body. Currently, most studies examine immune parameters along the entire length of the colon or small intestine because of technological limitations. However, many gut microbes likely exert their effects locally or hyperlocally, and these effects may be missed when examining whole tissues in mice colonized with a complex community; in these cases, monoassociation may sometimes allow for amplification of normally confined effects.

The idea that specific bacterial taxa in the gut occupy highly defined niches raises another possible model for how gut microbiota richness may support host health. In this model, which we will refer to as the mismatched niche occupancy model, we posit that each taxon in the intestine has evolved to occupy a specific niche where it coexists symbiotically with the host. Given the diversity of distinct niches present in the intestine, it is likely that some of these niches may be occupied by only a few species (or, in some cases, an individual species). By occupying a specific niche, these species prevent colonization of that niche by other species or strains. In organisms with an optimally diverse microbial community, each niche is thus occupied by an "ideal" symbiont that is coevolutionarily matched to that given niche. By contrast, individuals with low microbial diversity may lack specific bacteria that normally occupy particular niches, which would then leave these locations open to colonization by neighboring bacterial species that did not coevolve to colonize that niche. This may lead to induction of an aberrant immune response because of mismatched niche occupancy or outgrowth of species that are usually restricted to a minor niche. An extreme example of this second phenomenon is illustrated by *Clostridium difficile*, which exists in small numbers (in a restricted niche) in many healthy individuals but can cause severe colitis in individuals whose microbial communities have been disrupted by antibiotics.

Microbial diversity in the gut is a strong predictor of overall health, but we are only just beginning to understand the mechanisms underlying this fascinating epidemiological phenomenon. This area promises to be a fruitful field of investigation in the coming years and will enrich our understanding of how we have coevolved with our resident gut microbes to optimize our own physiological functions.

EMERGING AREAS OF HOST-MICROBIOTA RESEARCH

We have only touched on a small proportion of studies of immune-microbe interactions here. There are also many emerging areas of research that highlight the pervasive effects of immune-microbiota interactions on the host. Two particularly vibrant emerging areas of research are microbiota–nervous system interactions and elucidation of the intricate spatial organization of gut microbial communities.

Recent studies have revealed an intimate bidirectional interaction between the microbiota and the nervous system, with the immune system acting as an essential intermediary (*65*). This interaction can be observed locally in the gut but also appears to affect more distal sites of the nervous system, including the central nervous system (CNS). For example, the microbiota influences the maturation of microglia in the CNS (*47*), blood-brain barrier permeability (*66*), myelination of neurons (*67*), serotonin production (*68*), and behavior (*69*). Future investigations in this area will undoubtedly reveal previously undiscovered mechanisms by which the gut microbiota can interact with and instruct the nervous system, as well as the specific microbes responsible for these effects.

A given microbe's effect on host immunity is very likely influenced, if not predetermined, by the specific niche that it occupies. Development of new technologies to better track and image the locations and activities of distinct microbial populations will be essential to illuminate critical organizational principles that affect host-microbe interactions. Recent strides in this area have used genetic engineering and chemical biology approaches to image specific microbes in vivo and ex vivo (*70*, *71*) and to precisely control microbial gene expression (*72*–*74*). Future developments that use alternative imaging techniques, such as ultrasound and magnetic resonance imaging, will further advance imaging of host-microbiota interactions (*75*) and allow for a more in-depth mechanistic understanding of microbial dynamics and function.

Looking forward, we appear to be entering an exciting new era in our understanding of host-microbiota interactions. Collectively, the field has recognized and risen to the challenge of transitioning from mostly correlative studies to the development and implementation of a variety of tools and technologies that now allow us to demonstrate causal roles for the microbiota in mammalian health and disease. Such studies are an essential first step toward the development of microbiota-based therapeutics for a wide array of immune-mediated diseases.

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Abstract

One-sentence summary: This Review summarizes current strategies for modeling host-microbiome interactions in the mammalian gastrointestinal tract.

Causal effects of the microbiota on immune-mediated diseases

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