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REVIEW

Cross-Domain and Viral Interactions in the Microbiome

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SUMMARY The importance of the microbiome to human health is increasingly recognized and has become a major focus of recent research. However, much of the work has focused on a few aspects, particularly the bacterial component of the microbiome, most frequently in the gastrointestinal tract. Yet humans and other animals can be colonized by a wide array of organisms spanning all domains of life, including bacteria and archaea, unicellular eukaryotes such as fungi, multicellular eukaryotes such as helminths, and viruses. As they share the same host niches, they can compete with, synergize with, and antagonize each other, with potential impacts on their host. Here, we discuss these major groups making up the human microbiome, with a focus on how they interact with each other and their multicellular host.

KEYWORDS archaea, bacteria, bacteriophage, cross-domain, fungi, helminths, microbiome, protozoa, virus

INTRODUCTION

Over the past several years, the importance of the microbiome to human health and disease has become increasingly recognized. The trillions of microbes, outnumbering even our own cells, that live in and on us can protect us from colonization by

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pathogens, promote immunoregulation and tolerance by our own immune systems, and digest many of the foods that we ourselves cannot. However, they can also contribute to disease, if their balance is disrupted by antibiotics, immune dysregulation, or other disturbances. The focus of this field has largely been on the bacterial members of the microbiome, as they make up the largest proportion of the living organisms which constitute the microbiota. However, the bacteria exist alongside a diversity of organisms from other domains of life, including archaea, fungi, other unicellular eukaryotes, and in some cases helminths, as well as various families of viruses. All of these components can interact with each other and the host to impact health and disease. In this review, we discuss the various elements of the microbiome, with particular focus on the cross-domain interactions within the microbiota and with the host.

BACTERIA AND ARCHAEA

Perhaps the clearest cross-domain interaction related to the microbiome occurs between the commensal bacterial and archaeal microbiota and the eukaryotic host. Colonizing microbes play a number of significant roles in the health of their host, and studies of germfree animals have revealed that a lack of microbiota results in metabolic and immunological differences in comparison to conventional animals with a normal microbiota. Here, we provide an overview of the composition of the human bacterial and archaeal microbiota and briefly review two major impacts of these commensals on the human host: liberation of energy and nutrients from food components and stimulation of the immune system to promote a tolerogenic environment.

Of all of the research on the microbiome, it is the bacterial component, sometimes called the "bacteriome" to differentiate it from other members of the microbiota, that has received the lion's share of the attention. Of that work, the majority has examined the gut bacteriome, with publications on that topic dwarfing the combined works on the oral, skin, and urogenital microbiota (1). A number of robust tools and pipelines have been developed and made available for researchers to assess both the taxonomic classification and function of bacteria at multiple body sites and associated with various disease states (2–4). Importantly, these methods allow analysis of the bacterial microbiota without the need to culture the species present; researchers can instead extract DNA or RNA from samples of interest and use next-generation sequencing technologies to assess the composition and/or function of the microbes.

The most common method to analyze the composition of the gut bacteria is marker gene sequencing, generally using the 16S rRNA gene. Universal primers to amplify various regions of the 16S rRNA gene have been developed, and several databases exist to use such amplicons to taxonomically classify the bacterial sequences present within a biological sample (3–11). This method has the benefit of being relatively simple and inexpensive and has thus been used extensively for bacteriome research. More recently, methods have been developed to predict bacterial metagenomes from 16S rRNA gene sequencing data (12, 13). However, there are several limitations: technological limits on amplicon length have led to the use of various subsections of the 16S rRNA gene rather than its full length, the primers used for each of these subsections may introduce biases for or against certain taxa during amplification, and different bacterial taxa have different numbers of copies of the 16S rRNA gene (2–4). Additionally, while this method can also be used to study archaea, the primers are typically optimized to detect bacterial communities and frequently fail to amplify archaeal 16S rRNA gene sequences in useful numbers. Furthermore, the databases for archaeal sequences are less complete, potentially leading to an underrepresentation of archaea (14).

Accordingly, there is increasing interest in using shotgun metagenomics to profile the microbiome, as this removes some of the biases of marker gene amplicon sequencing and has the added benefit of assessing the functional potential of all of the genes present in a microbial community (2–4, 15, 16). Furthermore, metagenomic approaches can assess the entire breadth of the community of interest, including eukaryotes, archaea, and viruses, rather than simply the bacterial members (2–4, 15, 16). Even

metagenomics, however, can provide information only about the composition of the community, and tools like multiorganism transcript arrays, metatranscriptomics, metaproteomics, and metabolomics are required to analyze the actual functions being performed by the communities at a given time (17–21). However, these -omics methods are relatively expensive and hard to implement, and they suffer from a lack of complete and fully annotated reference databases; as such, the ability to define the contributions of so-called "microbial dark matter" not represented in databases (including many archaea) is limited (14, 22, 23). Thus, at this time, -omics methods are less common than 16S rRNA gene sequencing, but they are becoming more widespread and are revealing important information about the microbiota (17–19).

Composition of the Bacterial and Archaeal Microbiota

Bacteria and archaea are present along the gastrointestinal tract, with the greatest density present in the colon, and have received much research attention due to their roles in digestion and immune function (24). Unsurprisingly, given the largely anaerobic environment of the gastrointestinal tract, the gut microbiota are primarily facultatively or strictly anaerobic (25, 26). The specific taxonomic composition can vary significantly between individuals, impacted by different lifestyles, diets, and ages, although generally, they are fairly stable over time within the same individual (27, 28). Insights from metagenomics have led to the conclusion that rather than a set of specific taxa comprising a "core microbiota," there may instead be core functions that can be provided by different bacterial taxa in different individuals (27, 29, 30). However, metatranscriptomics suggests that there is still interindividual variation in transcription levels, which is intermediate between the highly idiosyncratic taxonomic composition and the more conserved functional capacity (31, 32). In the human gut, there appears to be a core metatranscriptome composed largely of housekeeping genes, with a much larger variable metatranscriptome of specialized pathways, suggesting that gut community transcription is context-specific and adaptive to the individual environment (32).

Despite this variation, sequencing, particularly large-scale efforts including the Human Microbiome Project and Meta-HIT, has revealed some common patterns of bacterial composition (27, 33). The human gut is generally colonized by hundreds of species-level bacterial taxa, which typically are dominated by members of only a few phyla; *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* are most abundant, with *Actinobacteria* and *Verrucomicrobia* making up smaller proportions (Fig. 1) (25, 27, 30, 34). It should also be noted that the gut microbiota is not a single, homogenous community but instead displays significant three-dimensional organization. First, the gut is comprised of several unique environments, in particular the stomach, the small intestine (divided into the duodenum, jejunum, and ileum), and the large intestine (colon), each of which has different properties and harbors its own community (35). To date, the vast majority of research has focused on the colon due to the comparative ease of obtaining fecal samples and the fact that it contains by far the highest density and numbers of bacteria (24). Second, even within a given compartment, bacteria may differ along the transverse axis, with different populations found in the lumen versus the mucosa (35).

Despite the difficulties in studying the stomach and small intestine, techniques including endoscopy and biopsy have allowed profiling of these microbial communities. In general, the microbial community becomes increasingly anaerobic along the gastrointestinal tract, with the stomach and small intestine containing a greater proportion of facultatively aerobic taxa than the largely anaerobic colon (36). Work in the stomach has frequently focused on the species *Helicobacter pylori*, given its close association with the gastric mucosa and public health relevance as an organism linked to gastric ulcers and cancers (37–40). However, the presence and levels of *H. pylori* vary between individuals, and a combination of culturing and amplicon sequencing techniques has revealed that other genera can be found in the gut despite the harshly acidic conditions. While exact findings have differed, *Streptococcus* has been consistently observed in relatively high proportions, along with genera including *Prevotella*,

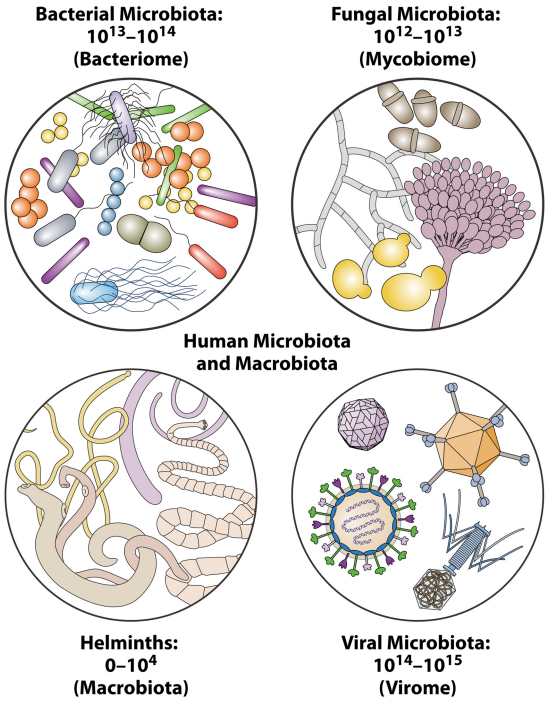


FIG 1 Outline of the major components of the human microbiota, summarized across body sites, including the gastrointestinal tract, oral cavity, vaginal mucosa, and skin. (Top left) Bacteria are the most abundant and include members of the phyla *Firmicutes* (*Clostridium*, *Lactobacillus*, and *Enterococcus*), *Bacteroidetes* (*Bacteroides* and *Prevotella*), *Proteobacteria* (*Escherichia* and *Acinetobacter*), *Actinobacteria* (*Bifidobacterium*), and *Verrucomicrobia* (*Akkermansia*). (Top right) Based on metagenomics, human-associated fungi are significantly outnumbered by the bacteria; they are mainly members of the phylum *Ascomycota* (*Candida*, *Saccharomyces*, *Aspergillus*, and *Malassezia*), but some *Basidiomycota* are detectable. Humans may also be infected with nonfungal eukaryotic pathogens, which are not shown here. (Bottom right) Viruses in the human microbiota are primarily bacteriophage and likely outnumber the bacterial population by at least 10-fold. The virome is largely composed of *Caudovirales* (*Siphoviridae*, *Myoviridae*, and *Podoviridae*) and *Microviridae*, along with some eukaryotic host viruses. (Bottom left) Helminths are now typically absent from humans in high-income nations but still parasitize billions worldwide to various degrees of severity. They include trematodes (flatworms), nematodes (roundworms), and cestodes (tapeworms).

Lactobacillus, *Rothia*, *Veillonella*, and *Propionibacterium* (41–45). Additionally, while the stomach lumen certainly contains transient microbes from the mouth and nose, the gastric community was shown to be distinct from either of these groups (43).

The small intestine also contains a distinct community of bacteria, typically containing the genera *Streptococcus* and *Veillonella*; other frequently encountered taxa include *Escherichia*, *Clostridium*, *Turicibacter*, and *Lactobacillus* (46). Like the stomach, it is less hospitable to bacterial life than the colon, with a faster transit time, higher acidity, more antimicrobial molecules, and an influx of bile acids, and therefore, it is less densely populated along most of its length (46). Studies of effluent from subjects with ileostomies suggest that the small intestinal bacterial microbiota tends to be more temporally variable than that of the colon, likely due to an increased short-term sensitivity to dietary intake given the small intestine's primary role in host nutrient absorption (47). Indeed, metatranscriptomics indicates that the maintenance of the small intestinal bacteria is driven by the rapid uptake and utilization of simple carbohydrates, which could make this population particularly sensitive to the composition of ingested food (48). In particular, the genus *Streptococcus* expressed genes for these functions at high levels, matching their high relative abundance in the population (48). However, the community is not necessarily consistent along the entire small intestine, and there is evidence that the bacterial composition becomes more similar to that of the colon in the terminal ileum (46).

The colon is more diverse, densely colonized, and anaerobic. *Firmicutes* and *Bacteroidetes* make up the majority of bacteria, although *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia* are typically present in lower proportions. Within these phyla, a number of commonly prevalent bacterial families may be identified, including *Bacteroidaceae*, *Clostridiaceae*, *Prevotellaceae*, *Eubacteriaceae*, *Ruminococcaceae*, *Bifidobacteriaceae*, *Lactobacillaceae*, *Rikenellaceae*, *Verrucomicrobiaceae*, and *Enterobacteriaceae* (1, 30, 34, 46). Interestingly, within the phylum *Bacteroidetes*, individuals tend to be dominated by either *Bacteroides* or *Prevotella* based on their diet and lifestyle. Studies show that urban subjects eating a "Western" diet high in protein and fat tend to be dominated by *Bacteroides*, while members of rural communities eating more plant-based, fiber-rich diets are dominated by *Prevotella* (49). Additionally, the archaeal genus *Methanobrevibacter*, which feeds on metabolites from other gut microbes and produces methane, is typically found in the human colon and is highly active; along with other less dominant methanogenic archaea, these organisms drive bacterial metabolism by removing hydrogen from the local environment and thereby making polysaccharide fermentation more thermodynamically favorable (1, 30, 32, 34, 50–53).

Furthermore, it has become increasingly recognized that the gut microbial community displays a transverse organization, with a distinct composition in the lumen relative to the mucosa (25, 46, 51, 54). One reason for this is that luminal and fecal samples contain long-term residents alongside transient bacteria and DNA from the digesta, which is less true for mucosal communities. Another reason is that the intestinal mucus, composed of highly glycosylated mucin proteins, provides a distinct niche for certain microbiome members. In the colon, a continuous mucus barrier covers the epithelium, organized into a dense inner layer that blocks most bacteria and a loose outer layer adjacent to the lumen; in the small intestine, there is only a single layer, and it is patchier than in the colon (46, 55, 56). The outer layer is home to a number of bacteria, including primarily mucolytic species such as *Akkermansia muciniphila*, mucolysis-capable species such as *Bacteroides thetaiotaomicron* and some *Bifidobacterium* species, and nonmucolytic (and even asaccharolytic) species that can feed on downstream metabolites from this process (46, 54, 57). There is also an oxygen gradient in the intestines, with high oxygen concentrations at the epithelium relative to the largely anaerobic lumen (58). This gradient tends to favor an enrichment of species that are more aerotolerant closer to the epithelium, including facultative anaerobes and those possessing mechanisms, such as catalase and superoxide dismutase, to deal with oxidative stress (58, 59). Finally, some bacteria have adaptations for penetrating the mucus layers and coming into close contact with the epithelium, such as the segmented filamentous bacteria (SFB) (sometimes known as "*Candidatus* Arthromitus" or "*Candidatus* Savagella"), while others can shelter in the crypts of the small intestine or the folds of the proximal colon (59–61). As a result of these factors, a number of studies in humans and animal models have found that the communities and transcripts of the lumen or feces are distinct from those associated with the mucus and/or epithelium in the same individual (46, 51, 57–59, 62–65). Interestingly, even species found in both the lumen and mucus may behave differently based on their location, with work demonstrating differential transcriptional profiles observed between luminal and mucus-associated members of the same species (57).

In contrast to the bacteria in the colon, the oral community displays relatively low interindividual variation (known as beta diversity) but has comparably high levels of diversity within any given individual (or alpha diversity) (30). The oral community is frequently dominated by members of the genus *Streptococcus* but also contains *Prevotella*, *Veillonella*, *Haemophilus*, *Neisseria*, *Corynebacterium*, *Actinomyces*, and *Rothia*, among others; it may also contain archaea, including *Methanobrevibacter* (1, 27, 66). However, like the gastrointestinal tract, there are several distinct regions within the mouth, including the gingiva, tongue, and teeth, that harbor somewhat distinct communities (27, 66). Similarly, the skin does not harbor a single unified bacterial community, and the composition depends on the characteristics of the site sampled, for example, dry skin, oily (sebaceous) skin, or moist skin (1, 27, 67–69). In contrast to

the gut, the skin is dominated by *Actinobacteria*, followed by *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*; common genera include *Staphylococcus*, *Propionibacterium*, and *Corynebacterium* (1, 27, 68–70). In particular, the lipophilic genus *Propionibacterium* is associated with sebaceous sites (68–70). Additionally, the skin is colonized by the *Thaumarchaeota* phylum of archaea, possibly involved in ammonia oxidation (14, 71–73). Finally, the vaginal bacterial community is an interesting demonstration of the fact that grouping bacteria at higher taxonomic levels can hide the diversity at lower levels. The vaginal community in most individuals is dominated by the genus *Lactobacillus*, giving it an apparent low diversity at this level, but the species and strains present are diverse and variable (27). However, recent work has also revealed that a significant subset of individuals possess a more diverse vaginal bacterial microbiota, including *Gardnerella*, *Atopobium*, *Megasphaera*, *Streptococcus*, and *Prevotella* (74, 75).

Mutualistic Metabolism: Gut Microbes in the Digestive Tract

As might be expected given their residence in the gastrointestinal tract, the gut bacterial and archaeal microbiota play an important role in digestion and metabolism. Collectively, gut bacteria possess the ability to extract energy from a wide variety of molecules that are indigestible by the host alone. Generally, these molecules are plant-derived polysaccharides, including fibers and starches, which are broken down into metabolites that can be used by the host or other microbes (76–78). Indeed, metatranscriptomics studies indicate that carbohydrate transport and metabolism are highly expressed functions across individual microbiomes, despite taxonomic variation (79). The importance of including such molecules in the diet is highlighted by studies that suggest that in their absence, gut microbes may instead overdigest the mucus layer, potentially allowing epithelial access to pathobionts (80–82). Additionally, nonfermentative members of the microbiota may form cooperative metabolic networks with the fermenters; for example, methanogenic archaea in the gut remove excess hydrogen from the local environment, driving fermentation by increasing the thermodynamic efficiency of the process (52, 53). In fact, metatranscriptomics studies have indicated that methanogens are particularly active relative to some other members of the gut microbiota (31).

The importance of the gut microbiota in harvesting energy from food can be demonstrated by studies in germfree animals, which lack any microbiota and display metabolic differences from their conventionally raised counterparts. Germfree mice are leaner than conventional mice despite consuming more food on a standard diet, but they lose this phenotype when they are colonized with the gut microbes of their conventional counterparts (83, 84). This effect arises from the reduced capacity of germfree mice to extract energy from food, thereby decreasing the caloric intake from the same amount of food, as well as the ability of the gut bacteria to promote fat deposition by the host (84). Another study found that when on a high-fat diet, germfree mice actually consumed less food than conventional mice, while also displaying increased lipid excretion and less-efficient food utilization. Together, these effects resulted in lower weight gain than in conventional mice, suggesting a degree of resistance to the ill effects of the high-fat diet (85). Recent work further confirms this observation, as germfree mice on a high-fat diet were shown to gain less weight, deposit less epididymal and mesenteric fat, excrete more triglycerides in the stool, and absorb significantly less lipid into the bloodstream than conventional mice; together, these data suggest that gut microbes play an important role in lipid digestion and absorption (86, 87).

Further studies have demonstrated that not all microbiomes are equal. For example, the bacterial microbiota of genetically obese mice have been shown to be more efficient at extracting dietary energy than those of their lean littermates. Obese mice showed an enrichment in bacterial genes for indigestible polysaccharide breakdown, produced more short-chain fatty acids (SCFA), and had lower fecal energy content, suggesting a greater ability to extract energy from their food. Furthermore, transferring microbiota from an obese mouse to a germfree mouse resulted in a significantly larger

body fat percentage increase than transferring microbes from a lean mouse (88). In fact, transplanting fecal microbiota from humans has a similar effect; mice given microbiota from an obese human gained more weight and fat than mice given microbiota from the donor's lean twin (89). Interestingly, cohousing both types of mice together led the obese-transplant mice to resemble their lean-transplant counterparts in both bacterial microbiota and body composition, suggesting that the low-fat, high-fiber diet that the mice were provided with selected for the lean-associated microbes (89).

In fact, it is widely recognized that diet is a major factor that influences the makeup and function of the gut microbiota. For example, researchers comparing the gut bacterial microbiota of children in urban Italy and rural Burkina Faso found dramatic differences, including a high prevalence of fiber-digesting taxa and a significantly reduced *Firmicutes*-to-*Bacteroidetes* ratio in the African children compared with their European counterparts. Those authors attribute these differences to the high-fiber, low-animal-protein diet of the African cohort, which promotes the growth of bacterial taxa capable of digesting dietary fibers and starches (90). More experimental studies have further demonstrated the importance of diet in the makeup of the gut microbiota. One study found that a high-fat diet led to a reduction in the abundance of *Bacteroidetes* and increases in the abundances of *Firmicutes* and *Proteobacteria*, even in an obesity-resistant mouse model (91), while another found that weight loss in obese humans was associated with increases in the abundances of *Bacteroidetes* (92). Diet may also interact with microbes in the small intestine to regulate lipid absorption; recent work has found that colonizing germfree mice with jejunal microbiota from mice fed a high-fat diet increases their capacity for lipid absorption even on a low-fat diet, while transferring microbes from mice fed a low-fat diet did not have the same effect (87). Strikingly, Turnbaugh et al. found that switching mice from a low-fat, plant-rich diet to a high-fat, high-sugar diet could change microbial composition and metabolism in as little as a day (93), and the same group demonstrated alterations to the human gut microbiota after only 4 days on a plant-based or animal-based diet (94). As such, there is significant research interest in the microbial contributions to obesity and metabolic disorders, as well as in whether the gut microbiota present a therapeutic target to treat or prevent these conditions. However, these efforts have been generally complicated by conflicting results and difficulty in finding a consistent signature of metabolic disruption across experiments (95, 96).

In addition to simply liberating more energy from the diet, gut bacteria produce important metabolites that may promote host health. Many gut bacteria produce vitamins, particularly vitamin K and several B vitamins, although the amount absorbed by the host relative to the microbiota is unclear. More importantly, many of the gut-resident bacteria produce SCFA, primarily butyrate, acetate, and propionate, as end products of fermentation of undigested fiber, starches, and plant polysaccharides in the colon; in contrast, branched-chain fatty acids, including isobutyrate, methylbutyrate, and isovalerate, can also be produced as amino acid metabolism by-products (77). Acetate is produced by many enteric microbes, including the mucolytic *A. muciniphila*, *Bacteroides* species, and *Bifidobacterium* species. It enters peripheral circulation and is the primary SCFA detectable in blood, and some functions include serving as a fuel source for the liver and muscles and being used in the synthesis of molecules such as cholesterol (77, 97). Acetate can also be used by other gut microbes to produce butyrate (77). Propionate, produced by microbes including members of *Bacteroidetes* and the *Negativicutes* class of *Firmicutes*, is almost wholly metabolized in the liver and has impacts on gluconeogenesis (77, 97). Butyrate, which has received a significant amount of research interest, is primarily produced by members of *Firmicutes*, such as *Faecalibacterium prausnitzii*. It is the primary fuel source for the colonic epithelium and has been implicated as an anti-inflammatory influence that helps to maintain intestinal homeostasis (97, 98). The concentrations of SCFA decline along the length of the colon, reaching 70 to 140 mM in the proximal colon and 20 to 70 mM in the distal colon, and SCFA also form a concentration gradient from the lumen outwards; furthermore, they are present at different molar ratios, with acetate being the most abundant, followed

by butyrate and propionate at approximately similar fecal levels, although this likely does not accurately represent the ratios in the colon itself due to differences in absorption (77, 96, 97, 99, 100). In addition to their role in host metabolism, SCFA are implicated as important signaling molecules mediating interactions between the gut bacteria and the host immune system, as described in more detail below.

Immunomodulation and Bacterial "Old Friends"

In addition to their role in metabolism, the human microbiota play an important role in the immunity of the host, which must be able to differentiate between commensal and/or symbiotic microbes and potentially pathogenic bacteria. Therefore, there is an important balance that develops, involving a limitation of contact between the microbiota and the local mucosa in addition to immunoregulatory mechanisms, allowing beneficial microbes to persist while preventing autoimmunity or self-damage by the host. The contributions of commensal microbes to immunoregulation form an important part of the "old friends" (formerly "hygiene") hypothesis (101–105). In short, this hypothesis posits that changes that have occurred in developed nations, including water sanitation, increased usage of antibiotics, higher rates of caesarean sections, more time indoors, and shifts to a low-fiber Western diet, have reduced early-life exposure to and colonization with helminths and beneficial microbes (old friends that humans coevolved with) that help to regulate the immune system, thereby leading to increases in autoimmune and allergic disorders in their absence (101–105). Here, we focus on the bacterial component of this hypothesis, but we discuss the contributions of helminths in a later section.

Humans are colonized with commensal microbes during and shortly after birth and must develop an immune system that can tolerate bacteria at many body sites without losing the ability to defend against pathogens. According to the old friends hypothesis, if there is insufficient exposure to diverse commensal or environmental microbes, it can lead to a failure to properly train immunological tolerance to harmless stimuli and subsequent overreactions to allergens or innocuous microbes. At the same time, many beneficial commensal microbes actively regulate the immune response, helping to prevent inappropriate immune activation to both the microbes themselves and other "bystander" antigens (101, 106, 107). Without this influence, particularly in early childhood, the risk for diseases of immune hyperreactivity, such as asthma, type 1 diabetes, multiple sclerosis, and inflammatory bowel disease (IBD), increases. In fact, adults who immigrate from low- or middle-income nations to high-income nations tend to retain protection against such disorders, but their children or those who immigrate when very young develop these diseases at higher rates more similar to those of indigenes of the new country (104, 108–114).

There are several mechanisms by which key members of the commensal microbiota modulate the immune response. First, the mere presence of gut microbiota is required for proper immune development; studies in germfree mice have revealed a number of immunological irregularities. For example, the microbiota are important for the development of the gut-associated lymphoid tissue (GALT), which allows the uptake and presentation of gut antigens to local immune cells. Accordingly, germfree mice have underdeveloped GALT compared to conventional animals. Specifically, they have small Peyer's patches with fewer germinal centers, reduced numbers of $CD4^+$ T cells in the lamina propria, and low levels of secretory IgA-producing plasma cells (115–118). They also show signs of a T_H2-biased immune system, even in peripheral locations such as the spleen, and a decreased ability to develop oral tolerance to ingested antigens (119–123). They also have increased accumulation of invariant natural killer cells in the colonic lamina propria, although they may be hyporesponsive (124, 125). Generally, such defects can be corrected by colonization with microbes at an early age, but not always in adulthood, supporting the importance of an early-life "critical window" for the microbiota to stimulate normal immune development (125–127). Even certain single species of bacteria can serve to normalize some aspects of immune function in germfree mice; for example, *Bacteroides fragilis* monocolonization can correct T_H2 bias

and promote immunological balance (123), *Bifidobacterium infantis* can correct oral tolerance defects when administered to neonatal mice (128), and SFB (a lineage within the family *Clostridiaceae*) can direct balanced T-cell maturation comparable to that of a complete mouse microbiota (129).

The presence of the microbes is also important to the "education" of the adaptive immune system, training it to discriminate between innocuous commensals and harmful pathogens and thereby promoting tolerance of microbiota-derived antigens. A key component of this process is the development of forkhead box P3$^+$ (FoxP3$^+$) regulatory T cells (T_{reg} cells), which can suppress effector CD4$^+$ T-cell subsets and thereby promote immune tolerance. Traditional T_{reg} cells arise from the thymus, with the objective of suppressing self-reactivity by the immune system (130–132); while these thymic cells (tT_{reg} cells) play a role in intestinal homeostasis, there is also an important role for naive T cells recognizing commensal antigens that are induced to differentiate into T_{reg} cells in the colon (iT_{reg} cells) (126, 133–136). This occurs in part through the action of tolerogenic CD103$^+$ dendritic cells in the epithelium, which preferentially sample the luminal bacteria and favor the differentiation of naive CD4$^+$ cells into iT_{reg} cells (137–141). As might be expected, germfree mice can display defects in their T_{reg} populations, although they do not lack them entirely (142, 143). The presence of colonic iT_{reg} cells with a diverse repertoire of receptors recognizing commensal antigens helps to prevent inappropriate responses to the microbiota and other bystander antigens, which have been implicated in the pathogenesis of IBD (144–146). This is thought to be particularly important during early life; the microbiota of humans is temporally unstable for the first several years and is theorized to provide a sampling window for the training and development of immunoregulatory responses (122, 144–146).

In addition to simply serving to educate the adaptive immune system, several types of commensal microbes have been found to actually direct certain immune responses, often promoting tolerance (147, 148). For example, the common gut microbe *B. fragilis* (of the phylum *Bacteroidetes*) has been found to activate development of T_{reg} cells and increase immunoregulatory cytokine production via the molecule polysaccharide A (PSA) (149–151). Accordingly, this molecule has been found to be protective against certain inflammatory diseases in mouse models (149–151). The related species *B. thetaiotaomicron* may be able to downregulate intestinal inflammation, even in the face of inflammatory challenge, by repressing host NF-κB signaling (152, 153); some other microbes, including *Lactobacillus* species and nonvirulent *Salmonella* strains, have demonstrated similar capabilities (153, 154). In addition, members of the class *Clostridia* (of the phylum *Firmicutes*) can induce the expansion of thymic T_{reg} cells and the development of colonic T_{reg} cells; this effect is at least partially mediated by the production of SCFA, particularly butyrate (140, 155–160). Specifically, the species *F. prausnitzii* has been found to be anti-inflammatory at least in part via its production of butyrate, inducing T_{reg} cells and anti-inflammatory cytokine production. Accordingly, it has been suggested to be protective against the development of IBD (161–163). Furthermore, colonization with altered Schaedler flora, a defined mix of eight commensal bacterial species that robustly colonize mice, including *Lactobacillus*, *Clostridium*, and *Bacteroides* species, has been shown to increase the levels of T_{reg} cells in the colonic lamina propria and promote intestinal immune homeostasis (164, 165). Finally, some *Lactobacillus* species have demonstrated an ability to drive T_{reg} development and subsequent interleukin-10 (IL-10) production (166). Contrarily, SFB have been found to associate closely with the mucosa and induce a T_H17 response; the T_H17 response is a generally proinflammatory pathway that can help to protect against bacterial pathogens but potentially contribute to autoimmune pathology (60, 148, 167).

The commensal bacterial microbiota also promote the function of the gut epithelial barrier, the integrity of which is important for preventing inappropriate immune activation and invasion by pathogens. A barrier of mucus, antimicrobial peptides, and secretory IgA serves to keep most microbes at a safe distance (168–173), although some are able to come into fairly close contact with the epithelium (61, 174). The commensal

microbiota appear to serve as a stimulus for increased mucus production, as germfree animals have been observed to have impaired mucus production, which can be rescued via colonization with a normal microbiota or even administration of bacterial products, including lipopolysaccharide (LPS) (56, 175–178). The production of butyrate may contribute to this effect, as it has been demonstrated to promote epithelial production of the major mucus component mucin-2 (33, 179–181). Butyrate can also promote epithelial barrier function and integrity (182–184). Additionally, gut bacteria may stimulate the production of IgA and antimicrobial peptides (120). While some of these impacts may seem counterproductive to the gut bacteria, they ultimately help both host and microbiota by maintaining a tolerant, anti-inflammatory environment. Furthermore, some commensal bacteria may be able to use host immune factors such as IgA to aid them in stable gut colonization (185).

Finally, commensal bacteria and archaea provide resistance to host infection with pathogens, a phenomenon termed colonization resistance. Commensal microbes occupy the readily available niches of the sites that they colonize and stimulate the local immune system, preventing potential pathogens from effectively establishing infections. They can compete for nutrients, produce antibacterial or inhibitory molecules, or even kill other bacteria through type VI secretion systems; in contrast, commensal bacteria can also indirectly encourage resistance to pathogenic infection by promoting antimicrobial peptide production, epithelial barrier integrity, and T_H17 responses, as described above. In one example of such immune-mediated competition, the Gram-negative organism *B. thetaiotaomicron* can stimulate the production of the antimicrobial peptide RegIIIγ, which primarily acts against Gram-positive bacteria (174, 186, 187). Additionally, some interactions require a combination of interbacterial competition and host immune involvement; the probiotic *Escherichia coli* strain Nissle 1917 can antagonize *Salmonella enterica* colonization by competing for iron, but only when the host produces the innate immune molecule lipocalin-2 to limit bacterial iron availability (188). As might be expected, germfree mice or antibiotic-treated mice or humans are more susceptible to colonization with certain pathogens, including *S. enterica*, *Clostridium difficile*, *Klebsiella pneumoniae*, and pathogenic *E. coli* (174, 189).

Given the importance of the commensal bacterial microbiota to immune regulation and colonization resistance, there is interest in using probiotics (specific strains or cocktails of bacterial species) and/or prebiotics (food or nutrients, typically fibers, meant to foster the growth of beneficial bacteria) as therapeutic agents. Of greatest interest are lactic acid bacteria, which are generally well tolerated by humans and are often present in fermented foods. *Lactobacillus* and *Bifidobacterium* species are most commonly studied, although some other microbes, including *Streptococcus*, *Lactococcus*, *Enterococcus*, and *E. coli* Nissle 1917, have been studied as well (190). Both mouse and human studies have examined the potential for probiotics, sometimes in combination with prebiotics, to prevent or alleviate a wide variety of disorders, including antibiotic-related *C. difficile* infection, IBD, *H. pylori* infection, atopic disorders, and necrotizing enterocolitis in preterm infants, among others (191–194). In this work are potentially promising results, although many studies are of a small size or have methodological limitations, so it is difficult to draw robust conclusions in some cases. However, large and well-designed studies can demonstrate the potential of pre- and probiotics; for example, Panigrahi et al. included over 4,000 subjects in a randomized, double-blind, placebo-controlled study of a combined pre- and probiotic ("synbiotic") that showed a reduced risk of sepsis in full-term infants in rural India (195).

Summary

The bacterial and archaeal microbiota, particularly within the gastrointestinal tract, perform a number of important functions beneficial to the eukaryotic host. Most directly, they play a major role in digestion, allowing the host to extract energy from dietary components that the host does not possess the capacity to break down. In doing so, the gut microbiota produces SCFA, including butyrate, which serves as a primary fuel source for the colonic epithelium. Furthermore, the microbiota and their

metabolites have significant impacts on the development and function of the host immune system. They stimulate innate mechanisms to shield the gut epithelium, protect the host against pathogenic colonization, and direct adaptive immune cell populations, particularly T_H17 and T_{reg} cells; in fact, the lack of a diverse community in early life may contribute to the development of immunological disorders in the genetically susceptible. In return, the eukaryotic host provides its microbial passengers with a sheltered niche and an array of nutrients, maintaining a tolerant environment despite the huge numbers of nonself organisms found in and on its body.

FUNGI AND OTHER UNICELLULAR EUKARYOTES

While the vast majority of human microbiome sequencing to date has been performed on bacteria, interest in the fungal component, the "mycobiome," has been growing. Fungi are a normal part of human microbial communities, found alongside bacteria in the skin, oral, gut, and vaginal microbiomes, and can interact with the resident bacteria in both mutualistic and antagonistic ways. In terms of cell numbers, fungi are a much smaller component of the microbiota than bacteria by orders of magnitude, although fungal cells are generally much larger than their bacterial neighbors (33, 196). In addition, humans may become infected with a number of nonfungal unicellular eukaryotes, including amoebozoans, heterokonts, metamonads, trypanosomatids, and apicomplexans.

Like bacteria, the fungi of the microbiome can be studied via either metagenomics or amplicon sequencing; the internal transcribed spacers (ITSs) between either the 18S and 5.8S rRNA genes (ITS1) or the 5.8S and 28S rRNA genes (ITS2) are typically the markers of choice, although sometimes the 18S gene itself is used (196–199). However, these types of analysis are more difficult for fungi than for bacteria, in large part due to the comparatively underdeveloped databases for fungal genomes. These databases are less complete, less accurate, and less well annotated than those for bacteria; in particular, the sexual (teleomorph) and asexual (anamorph) phases of some fungi may be classified as separate species despite identical genomes (200). Regardless, significant progress is being made to characterize the normal or "core" mycobiome, to associate fungal taxa with various disorders, and to understand how fungi interact with their bacterial neighbors across host colonization sites (196, 201).

Members of the Mycobiome

Primarily using ITS sequencing, there have been a number of studies attempting to catalogue the resident gastrointestinal fungi and determine the composition of a core healthy mycobiome (Fig. 1). ITS sequencing of human feces has had somewhat variable results, although they generally support the conclusion that the gut mycobiome has relatively low diversity and varies significantly between individuals. In contrast to observations of the bacterial gut residents, the number of fungal operational taxonomic units (OTUs) detected pales in comparison to those seen for bacteria, and longitudinal sampling from the same individual over time indicates that the detectable fungi fluctuate significantly. This observation is supported by data from mice, which also have longitudinally variable gut mycobiota despite living under controlled conditions (202, 203).

Despite this, ITS sequencing studies have identified various fungal genera associated with the human gut. Most studies indicate that the healthy human gut contains *Candida* and *Saccharomyces* species, with other frequently detected taxa including *Malassezia*, *Debaryomyces*, *Cladosporium*, *Aspergillus*, *Pichia*, and *Alternaria*, among others (202, 204–206). There is also some research into gut-resident fungi in the context of human disease, and there is evidence that *Candida* itself and fungus-associated antibodies may be related to IBD. Specifically, anti-*Saccharomyces cerevisiae* antibodies (ASCA), which, despite the specificity of their name, can recognize antigens from other yeasts, including *Candida*, have been associated with IBD (207–210).

One issue is that there is significant variability between studies, in both the specific taxa detected and their relative abundances. Differential DNA extraction protocols,

marker genes, primers chosen, analysis techniques, and sample populations may be to blame, in addition to inherent mycobiome variability. An issue acknowledged by many authors is that it is difficult to differentiate true gut colonizers from food-associated and environmental fungi that may simply pass through; this could potentially help to explain the high variability in fungal composition over time, as it may be influenced by recent diet and environmental exposures (202, 204). For example, while *Saccharomyces* is frequently found in the fecal mycobiota, *S. cerevisiae*, known more commonly as baker's or brewer's yeast, is used in the production of bread and beer and may present a confounding influence on analysis. The variable and uncontrolled diet of human subjects makes this a difficult question to answer. However, the studies that have been performed suggest that while dietary fungi can be detected at appreciable levels in the fecal mycobiome, they may be metabolically active while in the gut and therefore contributing to the local community, even if transiently (94).

There are also a number of fungi found in the human oral cavity, both on the mucosal surfaces and in subgingival plaque (199). A few groups have used ITS sequencing in humans to identify potential members of a core oral mycobiome, including *Candida*, *Cladosporium*, *Alternaria*, *Aspergillus*, *Fusarium*, *Cryptococcus*, and *Aureobasidium* (211–213). Unsurprisingly, those studies found that *Candida* was particularly common, reflecting previous results from culture-based studies (211). Ghannoum et al. also identified *Saccharomycetales*, *Dothioraceae*, *Teratosphaeria*, and *Glomus* as commonly occurring mycobiome members, while Dupuy et al. identified *Malassezia*, *Irpex*, *Cytospora*, *Lenzites*, and *Sporobolomyces* (212, 213). As in the gut mycobiome, differences in core taxa identified may be attributable to study populations, sampling type, extraction of fungal DNA, and methods of filtering and analysis, which were somewhat different between the two studies. Furthermore, food-associated and environmental contaminations are difficult to rule out. Finally, Ghannoum et al. were able to use community-level analysis to determine that subjects' gender and ethnicity were associated with their mycobiota, with analysis indicating significant differences between samples from females, white males, and Asian males (213).

Fungi have also been identified on the skin, particularly the lipophilic yeast genus *Malassezia* (200, 214, 215). A comprehensive survey of the skin mycobiota sampled and analyzed the fungi at 14 skin sites of healthy adults (216). Most were dominated by *Malassezia*, with species differing between sites, although three foot-based sites showed a greater diversity of fungal genera including *Aspergillus*, *Cryptococcus*, *Rhodotorula*, and *Epicoccum* (216). Similarly, another group found that *Malassezia* species dominate much of the skin mycobiota, while other genera, including *Candida*, *Meyerozyma*, *Rhodotorula*, *Trichosporon*, *Cladosporium*, *Aureobasidium*, and *Alternaria*, could also be identified (217). Furthermore, another study demonstrated a similar composition of skin mycobiota in a non-Western population; samples from Chinese participants in Hong Kong were dominated by *Malassezia* at multiple sites, with other commonly occurring genera including *Aspergillus*, *Penicillium*, *Candida*, and *Cryptococcus* (218). Finally, recent work found that the skin mycobiome of children is more diverse and variable between individuals and that the domination by *Malassezia* becomes established by adulthood, potentially due to the activation of sebaceous glands that occurs during puberty (219, 220). There may also be some differences in facial carriage of *Malassezia* by gender, with women harboring fewer fungi, although this has been suggested to be attributable to the use of cosmetics rather than inherent differences between men and women (220–222).

When discussing the presence of fungi in the female reproductive system, much of the focus has been on vulvovaginal candidiasis, or "yeast infections." However, the vagina may be colonized by a number of fungi, and opportunistic pathogens such as *Candida* colonize many women asymptomatically in the absence of an immune or vaginal bacteriome disruption (223, 224). In fact, *Candida* has been found in multiple studies to be the most frequent colonizer of the vaginal mycobiota, even in healthy individuals. In a study using an 18S rRNA gene clone library to identify fungal taxa in the vaginal microbiota, researchers identified a number of fungal OTUs, including

Candida, *Saccharomyces*, *Dothideomycetes*, and *Paecilomyces* species, in healthy subjects (225). More recently, researchers used ITS sequencing in a large study of healthy women. They found that *Candida* species were the most prevalent, being present in 64.5% of subjects and having a mean relative abundance of 36.9% (226). In addition, they identified OTUs mapping to *Cladosporium*, *Eurotium*, and *Alternaria* present at low abundances, although their analysis was complicated by a large number of unspecified OTUs, which had a summarized relative abundance of 38.6% (226).

In general, work on the human mycobiome has been minimal in comparison to interest in the bacteriome. Much is still unknown regarding the "core" or healthy members of the fungal microbiota at various body sites, although it is clear that *Candida* species are commonly found across multiple mucosal body sites while *Malassezia* dominates much of the skin. Interestingly, studies identified the presence of several potentially pathogenic fungal genera, including *Aspergillus*, *Fusarium*, and *Cryptococcus*, suggesting that, like *Candida*, these taxa may be commonly present in healthy individuals without immune dysfunction. Authors of the studies discussed here are in general agreement that more work is required and that a number of steps should be taken to improve comparability between studies and increase the level of detail that can be obtained from sequencing. Similar to calls in the field of the bacteriome, a unified process for sample collection, DNA extraction, amplicon generation, marker gene sequencing, and sequence analysis has been identified as being potentially helpful in making comparisons between different studies. Particularly with regard to the oral and gut mycobiota, additional steps to minimize the influence of food-associated, noncolonizing fungi, such as sampling from mucosa rather than feces or saliva, may help to accurately assess the truly resident fungal taxa. Additionally, improved scope, accuracy, and curation of fungal sequence databases will be important to increase the number of OTUs that can be mapped to specific taxa, to decrease the number of potentially spurious results matching sequencing artifacts, and to prevent the classification of the same sequences with taxonomic names pertaining to different sexual states. In pursuit of this goal, Tang et al. recently developed the Targeted Host-Associated Fungi (THF) database, which has been curated and optimized for identification of human and murine gastrointestinal fungi using ITS1 sequencing, in an effort to improve future surveys of the mycobiome (227).

Given that fungi colonizing the human body cohabitate with the bacterial members of the microbiota, there is significant interest in how these species cooperate and compete in their shared niches. A significant proportion of existing work examines interactions between various bacterial species and *Candida* species, which have long been known to colonize the human host at multiple sites and to act as opportunistic pathogens under certain circumstances, making them a clear clinical and research priority. Existing research has revealed a diverse array of interactions, including competition, antagonism, growth enhancement, synergistic virulence, and others (Fig. 2). These interactions are frequently mediated by quorum-sensing molecules from both kingdoms and in some cases are dependent on the context or growth state of the organisms, including *in vivo* versus *in vitro*, in suspension versus in biofilm, or yeast cells versus hyphae.

Candida, Lactobacilli, and Colonization Resistance

Candida species are found as commensal organisms in the microbial communities of a large proportion of the human population, but their growth is typically limited by competition with other microorganisms and by the host immune system. When this dynamic, a form of colonization resistance, is disrupted, such as by antibiotic administration or immunosuppressive disorders or therapies, *Candida* species can bloom and cause local infections, including oropharyngeal, vulvovaginal, and cutaneous candidiasis or even the systemic infection candidemia (228). This is commonly characterized by a transition from yeast to hyphal growth, allowing the fungus to become invasive and damaging to tissues (228, 229). The host microbiota plays an important role in keeping this opportunistic pathogen in check, competing with it and inhibiting its expansion in

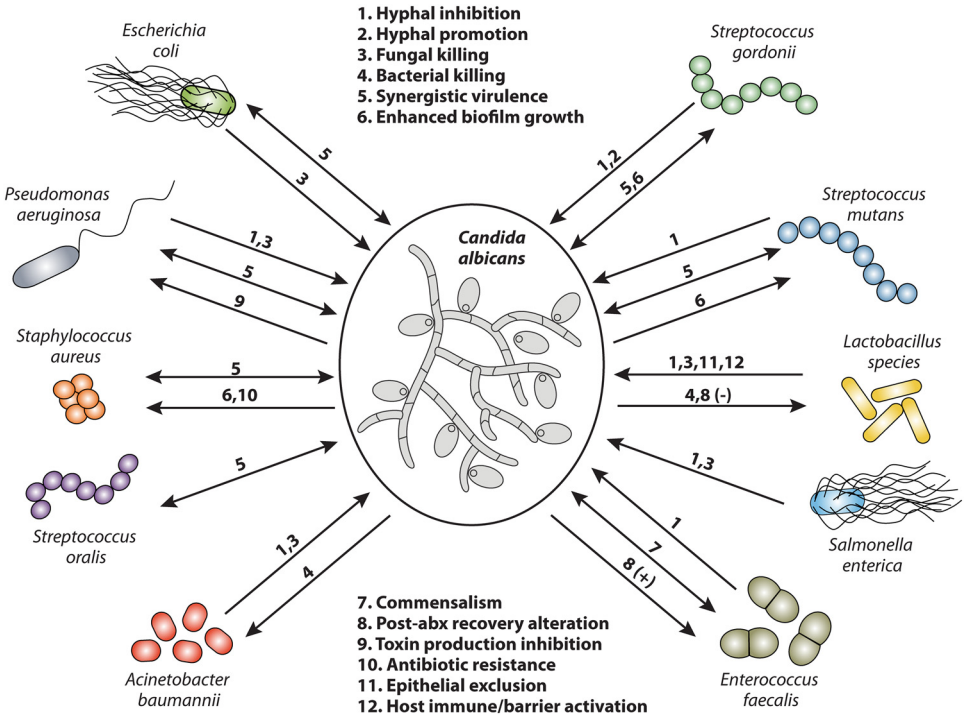


FIG 2 Outline of significant interactions between various human-associated bacteria and the fungus *Candida albicans*, ranging from cooperation to antagonism. abx, antibiotics.

a healthy microbiome. For example, laboratory rodents are not typically colonized by *Candida albicans*, but they become susceptible if raised under germfree conditions where they have no resident microbial community or if treated with broad-spectrum antibiotics that reduce the density and diversity of their native bacterial flora (203, 230).

While colonization resistance is mediated by a range of species inhabiting the host, bacteria of the genus *Lactobacillus* are particularly known for their antagonistic relationship with *Candida* species, especially in the vaginal microbiota, where *Lactobacillus* species frequently dominate (Fig. 2) (196, 231–233). Early studies showed that *Lactobacillus* was capable of displacing *Candida* from stomach epithelia in a murine model (234), and more recent work has shown that certain *Lactobacillus* species can interfere with *Candida* adhesion to vaginal epithelial cells via exclusion, competition, and displacement (235, 236). Some of this effect may be mediated by exopolysaccharide produced by some *Lactobacillus* species, which is structurally similar to that of *C. albicans* and is itself sufficient to antagonize *Candida* colonization via exclusion (235).

On a molecular level, there are a number of mechanisms by which lactobacilli can negatively impact the growth of *Candida*. A range of *Lactobacillus* species and their supernatants can inhibit growth, hyphal morphogenesis, and biofilm development (237). *Lactobacillus rhamnosus* can antagonize virulence factor production, biofilm capacity, and antifungal tolerance in a mixed biofilm with *C. albicans*, while lactobacilli more generally can compete with *Candida* for nutrients, particularly glucose (238, 239). Some species of *Lactobacillus* produce molecules toxic to *Candida*, including bacteriocins, biosurfactants, and hydrogen peroxide, which may help to antagonize *Candida* growth in shared environments (240–242). Additionally, while their name derives from their production of lactic acid, many lactobacilli also produce SCFA such as butyrate or acetate. This may contribute to the acidification of their environment, dampening the ability of *Candida* to grow or to undergo transition to the invasive hyphal phenotype (243); in addition, lactic, butyric, and acetic acids have been shown to have direct toxicity against *C. albicans* (244–246).

Lactobacilli may also interact with the host to improve defenses against *Candida* overgrowth or inflammatory damage. *Lactobacillus* species may benefit epithelial bar-

rier integrity, including via impacts on mucus production and modification and on intercellular junctions (179, 247–251). They or the SCFA that they produce may also stimulate the production of antimicrobial peptides lethal to *Candida*, such as defensins or cathelicidin (235), as well as modulate the expression or activity of mucosal Toll-like receptors (TLRs) (252). Finally, lactobacilli may help to reduce inflammation resulting from *Candida* activity, as SCFA are known for their anti-inflammatory properties, and some species of *Lactobacillus* have been shown to promote regulatory T-cell differentiation (253).

It should be noted that many of these mechanisms are shared by other members of the native microbiota, and other bacteria contribute to colonization resistance, especially in the gut, where *Lactobacillus* makes up a relatively small proportion of the total flora (254). For example, SCFA are produced by other gut microflora, and species such as *B. thetaiotaomicron* and *Blautia producta* have been shown to confer colonization resistance to *C. albicans* in the mouse gut (255, 256). Additionally, the ability to antagonize *Candida* growth and colonization is likely species or even strain specific; some *Lactobacillus* species have demonstrated strong anti-*Candida* activity in a number of studies, while others have shown little or a lesser effect (237, 240, 242). As such, there has been significant research interest in identifying potentially probiotic strains of *Lactobacillus*, commonly including the species *L. plantarum*, *L. crispatus*, and *L. rhamnosus*, that may be useful in promoting resistance to *Candida* overgrowth in vulnerable populations (257–262).

However, the interaction between these two groups may not be entirely one-sided, and *Candida* may also be able to antagonize the growth of *Lactobacillus* in mixed communities. Most directly, one group found that *C. albicans* produces a quorum-sensing molecule, farnesol, that may interfere with membrane integrity in *L. fermentum* and potentially other lactobacilli (263). Another group analyzed the ability of multiple *C. albicans* secreted aspartate proteases (SAP) to generate antimicrobial peptides from hemoglobin, or hemocidins (264). Many of the tested SAP generated a wide range of these peptides, and these hemocidins demonstrated significant antimicrobial activity against *L. acidophilus*, a component of the human vaginal microflora (264). Given the presence of hemocidins as a major component of human menstrual discharge, those authors speculate that *Candida* may be able to regulate the bacterial composition of the microbiota in this niche via the production of these antimicrobial peptides from host hemoglobin (264, 265). In the gut, researchers found that inhibition of dectin-1 signaling, a C-type lectin pathway through which the host recognizes beta-glucans (components of the fungal cell wall), led to an expansion of *L. murinus* in a mouse model (253). Furthermore, they demonstrated that the lack of dectin-1 signaling led to a significant reduction in intestinal levels of antimicrobial peptides that suppress *L. murinus* growth. While researchers could not detect viable fungi in the specific-pathogen-free mouse hosts and speculated that dectin-1 was activated by food beta-glucan ligands, this study raises the possibility that gut-resident *Candida* or other fungi may provoke host responses that antagonize lactobacilli (253).

Researchers have also found that colonization with *C. albicans* after antibiotic administration altered the recovery of the digestive tract bacterial population in mice, enhancing the recovery of *Bacteroidetes* and biasing the recovery of lactic acid bacteria toward *Enterococcus faecalis* instead of *Lactobacillus* species (266, 267). Importantly, this could occur in the context of nonpathogenic colonization, indicating that even in the absence of overt invasive disease, the presence of *Candida* may be able to influence the bacterial makeup of the microbiome. Interestingly, *E. faecalis* has previously been linked with *Candida*, as they are frequently isolated together from nosocomial infections and *E. faecalis* may even be enriched in clinical samples containing *Candida* species (268–271). Additionally, researchers found that in a nematode model of polymicrobial infections, coinfection with *C. albicans* and *E. faecalis* led to reduced virulence, increased host survival, and decreased hyphal morphogenesis, suggesting that the two may together promote a commensal rather than an invasive lifestyle (272). The reduction in hypha formation was partially mediated by the *E. faecalis* quorum-sensing

virulence regulator Fsr, one of many examples of quorum-sensing systems involved in mediating fungus-bacterium interactions; the bacteriocin EntV also appears to play an important role, reducing hyphal morphogenesis in both *in vitro* and *in vivo* models (272, 273).

Competition and Antagonism between Fungal and Bacterial Pathogens

While host-associated *Candida* will most frequently encounter the commensal microflora, as discussed above, it may also interact with bacterial pathogens. Given the potential for coinfections, there has been significant research interest in understanding the relationship between *Candida* and such bacteria. In many cases, including interactions with *Pseudomonas aeruginosa*, *E. coli*, *Acinetobacter baumannii*, and *S. enterica*, there is evidence of antagonistic relationships, particularly in a coculture setting (274). Frequently, the antagonism is mutual, with both bacteria and fungi inhibiting the growth or function of the other.

P. aeruginosa, a Gram-negative opportunistic pathogen of significant clinical importance due to high prevalence of antibiotic resistance and frequent implication in nosocomial infections, has multiple mechanisms of antagonizing *C. albicans* when the two are grown together (Fig. 2). For example, *P. aeruginosa* inhibits *Candida* hyphal morphogenesis *in vitro* via secretion of a homoserine lactone (HSL) quorum-sensing molecule. HSL resembles the *Candida* quorum-sensing molecule farnesol, which is used by the fungus to limit its own hyphal growth at high cell densities and has been shown to negatively impact membrane integrity in some bacterial species (263, 275, 276). A similar effect has been observed in another Gram-negative pathogen, *Burkholderia cenocepacia*, which also produces a farnesol-like signaling molecule that inhibits hyphal morphogenesis in *Candida* (277). Additionally, *P. aeruginosa* forms biofilms on *C. albicans* hyphae, which can lead to hyphal death through bacterial production of the virulence factors phospholipase C and phenazines (278, 279). Even at lower, nonlethal concentrations, phenazines can inhibit hyphal morphogenesis and biofilm formation (280). The farnesol produced by *C. albicans*, in turn, interferes with the production of the *Pseudomonas* quinolone signal (PQS) that regulates the production of phenazines such as pyocyanin, which may serve to help protect *C. albicans* from toxic effects of *P. aeruginosa* (281). Contrarily, the same group showed that farnesol could actually lead to the activation of downstream genes such as phenazines in strains of *P. aeruginosa* lacking the ability to produce PQS (282). Finally, *P. aeruginosa* may also be able to inhibit the growth of other fungi, including *Aspergillus fumigatus*, *Scedosporium aurantiacum*, and *Lomentospora prolificans*, although whether this occurs via the same mechanisms as its interactions with *Candida* is unclear (283, 284).

Similarly, there is evidence that *E. coli*, which is typically a human commensal but can cause disease with the acquisition of certain virulence factors, can negatively impact the growth of *C. albicans* (Fig. 2). Early studies indicated that *E. coli* could kill *C. albicans* when cocultured, an effect that was not observed when using heat-killed *E. coli* (285). Similarly, some clinical isolates of *E. coli* could inhibit the growth of *C. albicans* both in culture and in a gnotobiotic mouse model, and this effect could be traced to a diffusible factor produced by the inhibitory strains (286). More recently, Cabral et al. found that *E. coli* could kill *C. albicans* through a secreted, heat-labile factor dependent on low magnesium levels (287). The impacts of *C. albicans* on *E. coli* in coculture experiments are less clear. Some evidence indicates that *C. albicans* can enhance the growth of a commensal *E. coli* strain in culture via an iron-dependent interaction or that growth in a mixed biofilm can increase bacterial ofloxacin tolerance through interactions of *E. coli* with fungal beta-1,3-glucan (288, 289). Alternatively, *C. albicans* may negatively impact the short-term growth of *E. coli* in a biofilm, while farnesol produced by the fungus sensitizes *E. coli* to the antibiotic polymyxin B (263, 290).

The relationship between *A. baumannii*, a common cause of antibiotic-resistant nosocomial infections, and *C. albicans* is antagonistic in both *in vitro* and *in vivo* models (Fig. 2). *A. baumannii* has been shown to preferentially associate with the hyphal form of *C. albicans* and to demonstrate significant killing of hyphae but not yeast cells (291).

As a result, *A. baumannii* was able to decrease the lethality of *C. albicans* infection in a nematode model, as well as biofilm formation on abiotic surfaces (291). It was later shown that hyphal adherence and killing were mediated by both the bacterial outer membrane protein OmpA and heat-labile secreted proteins (292). However, *C. albicans* is not defenseless in this interaction, and farnesol produced by *C. albicans* can inhibit the growth of *A. baumannii* (291). Farnesol interferes with a number of cellular functions in *A. baumannii*, including membrane integrity, cell division, biofilm formation, and motility (293). It also induced an upregulation of efflux pumps and OmpA, suggesting possible defense mechanisms of *A. baumannii* against *C. albicans* (293). Interestingly, recent work has revealed that *A. baumannii* uses OmpA to bind to the *C. albicans* protein Hyr1p and that antibodies raised against Hyr1p recognize several *A. baumannii* surface antigens and are protective against infection in a mouse model (294).

S. enterica serovar Typhimurium, a pathogen responsible for the eponymous diarrheal disease salmonellosis, also appears to be able to antagonize *C. albicans*, particularly in the hyphal form (Fig. 2). A nematode model demonstrated that during coinfection along with *S. enterica*, filamentation of *C. albicans* was reduced in a dose-dependent fashion (295). A similar effect could be achieved by growing *Candida*-infected worms in filtered stationary-phase *S. enterica* supernatants, implicating a secreted and growth-dependent molecule. Additionally, in a coculture model, *S. enterica* reduced the viability of fungal cells in planktonic culture, particularly at 37°C (human body temperature), and inhibited *Candida* biofilm formation in a dose-dependent manner (295). The bacteria were able to kill both yeast and hyphal cells, although hyphal killing was more pronounced. Later work implicated the *S. enterica* type III secretion system (T3SS) proteins SopB (an effector protein) and SipB (a translocase) in the killing of *C. albicans*, particularly hyphal cells (296). The bacteria responded to the presence of *C. albicans* by upregulating the production of *sopB* transcripts, while the inactivation of either gene led to significant reductions in *S. enterica*-mediated killing (296). It is likely that there are other mechanisms of *S. enterica* interfering with *C. albicans* growth, however, given that T3SS killing occurs via direct contact, while the previous study found that the bacterial supernatant could inhibit hyphal growth (295).

In some cases, fungi may be able to play a protective role against bacterial pathogens. For example, recent work demonstrated that the yeast *Malassezia globosa* secretes an aspartyl protease that cleaves a *Staphylococcus aureus* virulence factor (protein A) important in biofilm formation and immune evasion, potentially protecting the skin environment from bacterial pathogenesis (297). Additionally, gut fungi may be protective against *C. difficile*, an opportunistic pathogen that causes severe and debilitating diarrhea and frequently becomes a problem after broad-spectrum antibiotic treatment depletes competing bacterial flora and allows the pathogen to bloom. There is evidence that the yeast *Saccharomyces boulardii* may be able to both prevent and mitigate this bacterial infection during antibiotic treatment (298–300). Mechanistically, *S. boulardii* can increase the intestinal production of anti-*C. difficile*-toxoid IgA, reduce toxin binding by producing a protease that degrades *C. difficile* toxins, and promote anti-inflammatory pathways in the host (301–304). As such, *S. boulardii* is of interest as a potential probiotic organism to prevent *C. difficile* infection in susceptible populations or to treat recurrent, refractory existing infections (299, 305–307).

Synergistic Virulence in Mixed Fungal-Bacterial Infections

While there are numerous examples of antagonism between fungi and bacteria in culture models, *in vivo* work reveals that coinfection can frequently lead to synergistic virulence and worse outcomes for the host, even when it comes to some of those same antagonistic species (274). For example, in contrast to their relationship outside the host, infection models frequently demonstrate enhanced virulence occurring due to coinfection with *C. albicans* and *P. aeruginosa* (Fig. 2). Hospitalized patients with *Candida* colonization of the respiratory tract were more susceptible to pseudomonal

ventilator-associated pneumonia, and patients colonized with *Candida* treated with antifungals had a decreased risk of pseudomonal pneumonia (308, 309). In murine models, rats given *P. aeruginosa* developed pneumonia only in the presence of *C. albicans*, while a mouse burn injury model demonstrated that infection with *P. aeruginosa* preceding infection with *C. albicans* led to significantly increased mortality compared to infection with either microbe alone (310, 311). Recently, a zebrafish swim bladder infection model was used to demonstrate the synergistic virulence of *P. aeruginosa* and *C. albicans*, with significant mortality which could be attributed to enhanced *C. albicans* epithelial invasion (312). Importantly, the two organisms may still exert antagonistic effects in the *in vivo* environment, but interactions with the host may be responsible for synergistic impacts of coinfection. Additionally, the context and type of infection may affect the relationship, with a mouse gut coinfection model indicating that *C. albicans* in fact has a protective effect against *P. aeruginosa* virulence by suppression of the siderophores pyoverdine and pyochelin (313).

Similarly, despite antagonism in culture, both *in vivo* and *in vitro* infection models of *C. albicans* and *E. coli* show increased virulence and mortality relative to either infection alone (Fig. 2). Intravenous infection by *C. albicans* followed by either *E. coli* or just its LPS led to increased mortality in a mouse model (314). Similarly, a synergistic effect is seen when mortality of intraperitoneal coinfection is compared to mortality of either microbe alone (315). LPS appears to be critical for this effect; multiple studies have indicated that LPS alone is sufficient to induce increased mortality during both intravenous and intraperitoneal *C. albicans* infections, in addition to showing that administration of anti-LPS antibody reduced mortality, endotoxemia, and bacteremia during coinfections of *C. albicans* with either *E. coli* or just its LPS (314, 316). The involvement of LPS suggests a role for the host immune response in this interaction. *In vitro*, a Caco-2 cell culture model demonstrated that coculture with *C. albicans* enhanced epithelial translocation of *E. coli* cells, even in the presence of secretory IgA (317). Similarly, coinfection of Caco-2 cells with *C. albicans* and enterohemorrhagic *E. coli* led to faster pathogen invasion, increased *C. albicans* virulence and hypha-associated gene expression, and greater severity of damage to cells (318).

C. albicans also demonstrates synergistic virulence in mixed infections with *Staphylococcus aureus* as well as a beneficial relationship in mixed-culture models (Fig. 2) (231). While *S. aureus* is ineffective at forming biofilms alone, *in vitro* it can form polymicrobial biofilms with *C. albicans*, specifically binding with a high affinity to hyphae (319, 320). Furthermore, in such a mixed-species biofilm, *S. aureus* demonstrates increased antimicrobial resistance, including resistance to vancomycin. Multiple studies have shown that this is at least partially due to physical protection of *S. aureus* by the extracellular matrix of the biofilm (319, 321); however, recent work also implicates low doses of the *Candida* quorum-sensing molecule farnesol in the development of antibiotic resistance in *S. aureus* due to an increase in the expression of efflux pumps (322). Importantly, these effects are context-dependent, as higher doses of farnesol can instead inhibit biofilm growth of *S. aureus* and sensitize it to antibiotics (320). On the *in vivo* side, mouse models also demonstrate that *C. albicans* can enhance the establishment of infection with *S. aureus*. Oral candidiasis facilitated both oral establishment and systemic dissemination of *S. aureus*, which could be impeded by antifungal therapy; furthermore, *C. albicans* enhanced the establishment of infection with *S. aureus* even when the two microbes were injected at different sites in a mouse model, suggesting the influence of the host immune response (323–325). Further work demonstrates that the two can exhibit synergistic virulence in a peritonitis model and lead to increased mortality compared to single infections (326). Given the frequent coisolation of *S. aureus* and *C. albicans* from human infections, ranging from burn wounds to lung infections, candidemia, and infections of implanted medical devices, the interaction between the two species is of significant clinical concern.

Candida and Oral *Streptococcus* Species: Multiple Mechanisms of Mutualism

The relationships between *Candida* and *Streptococcus* species in the oral cavity are

of particular clinical and research interest, as both colonize much of the human population, and their interactions may contribute to the pathogenicity of dental plaque biofilms (232, 327). *Candida* typically colonizes mucosal surfaces in the oral cavity but has been shown to associate with bacterial species, frequently *Streptococcus*, in oral biofilms on both mucosal and dental surfaces (328–330). Several studies indicate that *Candida* and *Streptococcus* species can have synergistic relationships in biofilm formation, although specific interactions may vary between the specific *Streptococcus* species involved (Fig. 2). On a global level, 16S rRNA and ITS gene sequencing of the oral microbiota showed that adults with higher levels of *Candida* had increased levels of saccharolytic, acidogenic bacteria, including *Streptococcus* species (331). In general, *Streptococcus* species may produce nutrients such as lactate and glucose that can be used by *Candida*, while *Candida* can relieve oxygen tension to allow growth of *Streptococcus* (231, 332).

In one example, mixed biofilms of *C. albicans* and *Streptococcus gordonii* on saliva-coated surfaces showed increased biomass and development of fungal hyphae compared to *Candida*-only biofilms (Fig. 2) (333). Mechanistically, while *Candida* uses the quorum-sensing molecule farnesol to repress its own hyphal morphogenesis at high concentrations, *S. gordonii* was able to relieve farnesol-induced hyphal repression, a capability that was dependent on the interspecies quorum-sensing molecule autoinducer 2 (AI-2) (333). Interestingly, the AI-2 molecule produced by another oral bacterial species, *Aggregatibacter actinomycetemcomitans*, has the opposite effect and inhibits hyphal growth and therefore biofilm formation of *C. albicans* (334). Additionally, while growth in a mixed biofilm increased total biomass, the quorum-sensing molecule competence-stimulating peptide (CSP) produced by *S. gordonii* favored the growth of *C. albicans* in a planktonic state rather than in a biofilm (335); the use of both AI-2 and CSP may allow the bacteria to modulate the amount of fungi in mixed communities by promoting either hyphal growth and planktonic dispersal from the biofilm, respectively.

The oral bacterium *Streptococcus mutans* is also capable of growing in mixed communities with *C. albicans*, with beneficial effects on its growth (336). This relationship appears largely dependent upon the presence of sucrose; while *S. mutans* binds poorly to *C. albicans* itself, it produces glucosyltransferase enzymes that bind to the surface of the fungi and produce glucans from sucrose. This creates a polysaccharide-rich extracellular matrix to which the bacteria themselves can bind (337, 338). There is evidence that *C. albicans* itself may promote this interaction; bacterial-fungal conditioned media increased the growth of *S. mutans* in a biofilm, specifically upregulating glucosyltransferase activity to enhance microcolony formation (336). It was later identified that farnesol achieved this effect at low concentrations, despite inhibiting the growth of *S. mutans*, and other bacterial species, at high concentrations (336).

However, *S. mutans* can repress hyphal morphogenesis in the yeast, which has been attributed to multiple mechanisms (Fig. 2). *S. mutans* CSP repressed hyphal growth, similarly to *S. gordonii*, while the bacterium also produces a fatty acid signaling molecule, dubbed *Streptococcus* diffusible signal factor or SDSF, that has a similar effect (339, 340). Interestingly, this effect may be overcome in a more complex community, as *C. albicans* showed enhanced hyphal formation and tissue invasion when grown with a combination of *S. mutans*, *S. sanguinis*, *Actinomyces viscosus*, and *Actinomyces odontolyticus* (341). Additionally, it was shown that a mixed biofilm with *C. albicans* strongly upregulated *S. mutans* quorum-sensing gene networks, through increased levels of the hexapeptide pheromone *comX*-inducing peptide (XIP); those authors speculate that proteases produced by *C. albicans* degrade proteins to produce XIP, thereby triggering the activation of the bacterial quorum-sensing networks (342).

S. mutans has long been known as an etiological agent of early childhood dental caries, and more recent work has found that *C. albicans* is also frequently found in carious lesions. Therefore, there is interest in the potential cariogenic synergy of these two organisms (343, 344). However, the effects of mixed growth on caries formation remains somewhat unclear. For example, it was shown that mixed-species biofilms have increased lactic acid production *in vitro* but that *C. albicans* actively increases the pH of

the biofilm, which could theoretically reduce cariogenic potential (345). Contrarily, in an *in vivo* model, coinfection with *S. mutans* and *C. albicans* in a rat model led to significant increases in the viable populations of both organisms in plaque biofilms as well as increased and synergistic severity of dental caries on the smooth sides of the teeth (338). Finally, another study found that while both *S. mutans* and *C. albicans* were capable of generating occlusal caries (those at the interface between the upper and lower teeth) in a rat model, there was no enhancement of this effect when both organisms were inoculated together (346).

Streptococcus oralis has been more definitively implicated in pathogenic interactions with *C. albicans* in the oral cavity (Fig. 2). Both organisms may enhance the growth of the other in mixed biofilms on both abiotic and mucosal surfaces, and dual-species biofilms conferred a greater ability to invade both oral and esophageal mucosal tissue models on *C. albicans* (347–349). *In vivo*, the two organisms had a synergistic effect on infection in a mouse model; specifically, *C. albicans* promoted colonization with *S. oralis*, while coinfection promoted deep-organ dissemination of *C. albicans* and increased levels of oral candidiasis (350). A follow-up study examined the potential mechanism behind this effect and showed that the two organisms synergistically stimulate increases in the host protein calpain, which targets and cleaves the epithelial junction protein E-cadherin and allows for increased fungal paracellular invasion (351).

Overall, these observations indicate that several commonly occurring oral *Streptococcus* species have developed mutualistic relationships with *C. albicans*, resulting in enhanced biofilm formation, growth, or infective potential for one or both species. *In vivo*, it is likely that multiple species interact in more-intricate communities, with the production of multiple sets of quorum-sensing molecules, metabolites, and nutrients that may have more-complex interactions and implications for oral health.

Fungus-Bacterium Interactions in the Microbiome

Several studies of the human mycobiome have been performed in parallel with analyses of the resident bacteria, providing an opportunity for correlating the presence and abundance of fungal and bacterial taxa. One group was able to identify a number of such correlations in the gut microbiome, including strong negative correlations between *Bacteroides* and *Candida* and strong positive correlations between *Syntrophococcus* and *Pichia*, *Anaerostipes* and *Fusarium*, and *Bryantella* and *Fusarium* (204). Additionally, *Candida* and *Saccharomyces* were both positively correlated with the archaeon *Methanobrevibacter* and negatively correlated with the archaeon *Nitrososphaera* (204). A similar analysis of fungus-bacterium correlations in the gut microbiome found negative correlations between *Penicillium* and *Faecalibacterium* and between *Cladosporium* and *Ruminococcaceae* and a positive correlation between *Botrytis* and *Rikenellaceae* (206). While interesting, the potential implications of these associations have not been identified. Hoffman et al. suggested the existence of syntrophic "guilds" of bacteria, archaea, and fungi, with members cooperatively producing metabolites for others to use, but further work is required to experimentally assess the functional relationships between taxa across kingdoms (204).

Some work has also investigated bacterial-fungal networks in the oral microbiome. Increased levels of *Candida* have been associated with reduced bacterial diversity, with increased *Bacillus* and reduced *Fusobacteria*, *Flavobacteria*, and *Bacteroidia* abundances (331). As mentioned above, these microbiomes became dominated by saccharolytic and acidogenic bacteria, including *Streptococcus* species; this may be linked to the increased cariogenic potential of these communities (331). Additionally, an *in vitro* model suggested that the presence of *Candida* may have impacts on the proliferation of different species in an oral microbial community. Specifically, researchers found that inoculating saliva samples with *C. albicans* allowed the growth of anaerobic bacteria, including *Veillonella*, *Fusobacterium*, *Prevotella*, and *Leptotrichia*, even under apparently aerobic culture conditions, while samples that were not inoculated with the yeast were dominated by strictly or facultatively aerobic species (352). The authors of that study speculate that the rapid oxygen consumption by *C. albicans* may create microanaerobic

niches for such bacterial genera to grow and that its presence in salivary biofilms may have important implications for microbial composition.

Other Unicellular Eukaryotes in the Microbiome

While fungi are the most common unicellular eukaryotes colonizing the body, organisms such as amoebozoans, trypanosomatids, apicomplexans, metamonads, and heterokonts can also infect human hosts (353). While there is comparatively less work exploring this topic, recent research has revealed that some of these organisms can interact with bacterial commensals or pathogens in a variety of ways. Infection with such organisms can itself alter the resident microbiota, the preexisting commensal community can alter the course of infection with these organisms, or, in some cases, both can occur (354). Here, we discuss the interactions of the microbiota at multiple body sites with the heterokont genus *Blastocystis*; the amoebozoan genus *Entamoeba*; the metamonad genera *Giardia* and *Trichomonas*; the apicomplexan genera *Plasmodium*, *Toxoplasma*, and *Cryptosporidium*; and the trypanosomatid genus *Leishmania*.

According to culture-independent surveys of the human gut, *Blastocystis* species are the most common nonfungal eukaryotes, with significant carriage rates even in industrialized nations (353, 355, 356). In a rural Mexican community, carriage of *Blastocystis* was found to be associated with increased bacterial alpha diversity and significant differences in bacterial community composition, including reductions in the abundance of *Prevotella copri* and increases in the abundances of several *Clostridia* lineages, including *Ruminococcus bromii*. Additionally, colonization was associated with increased levels of several fungal taxa as well as metabolic differences and reduced levels of intestinal inflammation markers (356). Similarly, another group studying *Blastocystis* in a French cohort found increased alpha diversity and enrichment in *Ruminococcaceae* associated with *Blastocystis* colonization. However, they also found that the *Prevotellaceae* abundance was increased rather than decreased in colonized individuals (357). Supporting this, a study in Denmark found that colonization with *Blastocystis* was associated with enrichments in *Prevotella*, alongside reductions in *Bacteroides* abundances (358), and a metastudy of subjects from multiple continents found that *Prevotella copri* was associated with *Blastocystis* colonization and that *Bacteroides* species and *Ruminococcus gnavus* were associated with uncolonized subjects (359). Overall, the specific impacts of *Blastocystis* colonization are somewhat unclear and may depend on the sociogeographic context and particular subtype of *Blastocystis* used (356).

Entamoeba histolytica causes the potentially lethal illness amoebic dysentery, a significant health issue in some parts of the world, although it has long been recognized that exposures are frequently asymptomatic; on the contrary, the related species *Entamoeba dispar* is nonpathogenic (360, 361). *E. histolytica* infects the gut and actually preys on the gut microbiota, although they are not strictly necessary for its growth, and its association with the gut microbiota may enhance its pathogenicity and protect it from oxidative stress (362–364). Both amoeba-induced dysentery and asymptomatic colonization with *Entamoeba* species have been associated with alterations to the gut microbiota. Quantitative PCR (qPCR) analysis revealed that amoebic dysentery reduced the abundance of numerous taxa while increasing the abundance of *Bifidobacterium*, while 16S rRNA gene sequencing demonstrated that asymptomatic colonization was associated with increased alpha diversity and increased abundances of several taxa but significant reductions in the abundance of *Prevotella* species, particularly *P. copri* (365, 366). Interestingly, a study of Bangladeshi children found that among those found to be colonized with *E. histolytica*, those that experienced symptomatic disease had higher levels of *P. copri* detected by qPCR, suggesting that higher levels of this taxon may predispose individuals to developing symptoms of amoebic infection (367).

On the other hand, the same group found that some gut microbes may be protective against *E. histolytica* in mouse models. First, mice treated with antibiotics to deplete their bacterial microbiota were more susceptible to *E. histolytica* infection and displayed a deficit in neutrophil recruitment to the gut (368). More specifically, mice colonized with SFB, which closely associate with the gut epithelium and provoke a T_H17

response, were protected from amoebic colonization and had an increased neutrophil presence in the gut. Intriguingly, this protection appears to be at least partially due to changes induced in bone marrow dendritic cells, as adoptive transfers of these cells from SFB-positive (SFB^+) to SFB-negative (SFB^-) mice was sufficient to confer protection, and gut-marrow signaling may be based on SFB-induced increases in levels of serum amyloid A (369).

Gut bacteria have also been studied in the context of infection with *Giardia*, a genus of metamonads that primarily colonizes the small intestine. While asymptomatic in many, it can cause acute diarrheal illness and recently has also been associated with the development of postdiarrheal irritable bowel syndrome (IBS). An accumulating body of work demonstrates bidirectional impacts between *Giardia* infection and the composition of the gut microbiota (370). For example, it was recognized that susceptibility to *Giardia lamblia* (also known as *G. duodenalis* or *G. intestinalis*) infection in mouse models was dependent on the source of the mouse, with Taconic Farms mice being resistant but Jackson Laboratories mice being susceptible. Antibiotic treatment eliminated resistance, but cohousing of the two groups conferred resistance to all mice, implicating the composition of the gut microbiota in this phenotype (371).

However, gut bacteria may contribute to symptom development, as germfree mice can be colonized by *Giardia* but suffer less intestinal pathology than conventional mice (372, 373). This may be due to a dampened host response, as germfree animals produce fewer *Giardia*-specific antibodies and have less immune cell accumulation in the lamina propria than their conventional counterparts (372). Similarly, antibiotic-treated animals have fewer activated $CD8^+$ T cells in the lamina propria during infection (374). Furthermore, *G. lamblia* has been associated with small intestinal bacterial overgrowth during infection as well as decreased epithelial tight junction integrity, increased permeability and bacterial adhesion, and mucosal inflammation even after parasite clearance; this suggests a long-term disturbance of intestinal homeostasis due to infection, which could potentially contribute to the development of postdiarrheal IBS (375–377). Work in a *Caenorhabditis elegans* model has also suggested that exposure to *G. lamblia* may induce functional changes in commensal microbes, promoting intestinal colonization by bacteria and leading to lethality not observed during individual inoculations of either bacteria or parasites (378).

To more specifically profile the changes observed in the bacterial community, Barash et al. studied the effects of *G. lamblia* infection on the bacterial composition along the gastrointestinal tract in antibiotic-treated and naive mice (379). They found that antibiotic-treated mice were significantly more susceptible to *Giardia* colonization, displaying increased parasite burdens even at 2 weeks postinfection, and antibiotic-treated mice had more-widespread and longer-lasting community disruptions associated with *Giardia* infection; these included depletions of *Clostridiaceae* (*Firmicutes*) and enrichments of *Moraxellaceae* and *Rhodocyclaceae* (both *Proteobacteria*), particularly in the proximal small intestine. However, even untreated mice suffered disruptions to the composition of their gut bacteria (379).

However, there is some research suggesting that *Giardia* infection may actually reduce the incidence of diarrheal disease in areas with frequent gastrointestinal pathogen exposure, although the evidence is mixed and may be dependent on the specific coinfection (380–384). In support of such an effect for bacterial pathogens, in a mouse model, *Giardia muris* coinfection significantly attenuated pathology from the bacterium *Citrobacter rodentium*, reducing weight loss, colitis, pathogen load, and bacterial attachment while increasing host antimicrobial peptide production. Furthermore, coinfection of human intestinal epithelial cell monolayers with *G. lamblia* and enteropathogenic *E. coli* led to enhanced antimicrobial peptide production, and *G. muris* reduced the survival of both *C. rodentium* and *E. coli* in a coculture model (385). *G. lamblia* also appears to be able to antagonize host inflammatory responses, possibly by different mechanisms based on the genetic assemblage of the parasite. One study demonstrated that *G. lamblia* assemblage A secretes a cysteine protease that degrades the neutrophil chemoattractant IL-8 (CXCL8), even in the presence of a direct, inflammatory bacterial

insult in the form of *S. enterica* serovar Typhimurium (386, 387). On the other hand, a study reported that *G. lamblia* assemblage B instead appeared to dampen inflammatory responses to enteroaggregative *E. coli* during protein malnutrition, reducing myeloid cell activation despite an increased number of these cells in the ileum (388).

Not all metamonads interact with bacteria in the gut, however, and there is evidence that the human genital tract pathogen *Trichomonas vaginalis* may have a mutually antagonistic interaction with *Lactobacillus* species in the vaginal microbiota. Epidemiologically, some recent studies have demonstrated a relationship between trichomoniasis and non-*Lactobacillus*-dominated vaginal microbiota, which can be associated with the disease bacterial vaginosis but also occurs asymptomatically in a subset of women (75, 389–393). Experimentally, early work recognized that *T. vaginalis* could have a negative impact on the growth of *Lactobacillus acidophilus* in coculture experiments (394). More recently, others have found that *T. vaginalis* can reduce the numbers of lactobacilli but not other vaginal bacteria associated with epithelial cells (395), while a number of *Lactobacillus* species were seen to inhibit adhesion of *T. vaginalis* to epithelial cells and even promote displacement of the parasite in a contact-dependent manner (396, 397). Additionally, *T. vaginalis* appears to have an association with the vaginosis-associated bacterium *Mycoplasma hominis*, which can be taken up and survive within cytoplasmic vacuoles. Some *T. vaginalis* isolates are stably associated with the bacterium, and such isolates may increase the local inflammatory response, potentially increasing disease severity (398–402).

There is also evidence of interactions between apicomplexans and the microbiota. For example, *Cryptosporidium parvum*, an intestinal parasite, has a differential infective capacity in mice based on the presence of the microbiome; conventional mice are resistant to *C. parvum* colonization for several weeks, while germfree mice become heavily infected much more quickly (403, 404). However, mice treated with antibiotics to deplete their gut bacteria remained resistant to infection, suggesting a more complex mechanism than colonization resistance by competition (404). As the increased susceptibility in germfree mice occurs in both immunocompetent and severe combined immunodeficiency models, which lack an adaptive immune system, the gut microbiota may confer resistance through nonspecific immune mechanisms (403).

The gut microflora also appears to have a role in mediating the host response to infection with another apicomplexan parasite, *Toxoplasma gondii*. This organism initially infects the small intestine, invading the epithelium to undergo differentiation from infective sporozoites to mobile tachyzoites. This epithelial damage allows increased bacterial penetration into the epithelium and interaction with the immune system, which can have both positive and negative consequences (354, 405). On the one hand, gut bacteria may play a supportive role in the mucosal immune response to *T. gondii*. In mice, the recognition of and immune response to *T. gondii* canonically occur through the action of dendritic cell Toll-like receptor 11 (TLR-11) and subsequent production of IL-12 (406). However, stimulation of other TLRs by the gut microbiota can also lead to IL-12 production, even compensating for the lack of TLR-11 in knockout mice, during oral *T. gondii* infection. This does not occur during systemic infection, in which the immune response is entirely TLR-11-dependent, suggesting that local interactions such as gut epithelium damage by the parasite are necessary for microbial IL-12 stimulation to occur. Gut microbes may thus serve as a molecular adjuvant of the mucosal immune response and may perhaps be even more important during human infections, given the lack of a functional TLR-11 homologue (406).

On the other hand, the gut flora can exacerbate intestinal inflammation and lead to significant ileitis and even death in mouse models (407, 408). Multiple groups have found that *T. gondii* infection results in inflammation only in the presence of the gut microbiota, as germfree or antibiotic-treated mice do not suffer these effects (407, 408). In particular, it appears that infection with *T. gondii* selectively expands *Proteobacteria*, particularly *Enterobacteriaceae*, which leads to the development of intestinal inflammation and pathology in this context (407, 408). There may be a positive-feedback loop exacerbating both inflammation and *Enterobacteriaceae* overgrowth; *T. gondii* infection

in the presence of *Enterobacteriaceae* leads to intestinal damage and Paneth cell death, while the loss of the antimicrobial peptides produced by these cells may contribute to *Enterobacteriaceae* overgrowth (407). Additionally, some work suggests that *T. gondii* infection may lead to a loss of immunological tolerance to commensals, possibly due to increased mucosal exposure to gut microbes (409). Even after infection clearance and epithelial healing, mice had increased populations of apparently commensal-responsive $CD4^+$ T cells. Furthermore, transferring bacterium-specific splenic T cells to a *T. gondii*-infected mouse results in significant proliferation and differentiation into effector cells, while this does not occur in uninfected hosts, demonstrating an increase in the frequency of immune cells responsive to the commensal microbiota during infection (409).

The host gut microbiota may also impact the severity and transmission of another apicomplexan-related disease, malaria, caused by parasites of the genus *Plasmodium* (410). Studies in mice have demonstrated that animals from different vendors, and therefore with different gut microbial compositions, were differentially susceptible to infection, with varied parasite burdens and mortality outcomes. Furthermore, this resistance or susceptibility could be transferred to germfree mice via cecal transplants (411). This resistance appeared to be related to an elevated humoral immune response, and resistance was associated with increased abundances of *Lactobacillaceae*, *Bifidobacteriaceae*, and *Clostridiaceae* while susceptibility was associated with higher levels of *Bacteroidaceae*, *Prevotellaceae*, and *Sutterellaceae* (411). Similarly, a study of the fecal bacterial microbiota of a Malian cohort found that a particular microbial assemblage, which included higher levels of *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* and lower levels of *Prevotellaceae* and various *Clostridia* taxa, was associated with a reduced incidence of malaria (412). Furthermore, *Plasmodium* sporozoites possess certain surface glycans shared by some species of *Enterobacteriaceae*, and studies in both humans and mice suggest that antibodies against these glycans may be cross-protective against infection with *Plasmodium* (413).

Plasmodium infection may also cause alterations to the gut microbiota, and humans suffering from malaria may experience gastrointestinal symptoms (414–416). Work in a mouse model found that infection with *Plasmodium berghei* led to intestinal pathology in addition to dysbiosis, characterized by reductions in *Firmicutes* and increases in *Proteobacteria* and *Verrucomicrobia* abundances. In particular, *Lactobacillaceae* abundances were reduced during infection, while *Verrucomicrobiaceae* and *Enterobacteriaceae* abundances were increased; interestingly, alterations to the gut community could be observed before the onset of gastrointestinal pathologies (416). Additionally, infection of mice with *Plasmodium yoelii* leads to a reduction of colonization resistance against *S. enterica* (417). The microbiota may also play a role in the susceptibility of the mosquito host to carriage of the parasite. For example, the ability of *Plasmodium* to colonize and mature within its *Anopheles* mosquito vector is at least partially dependent on the midgut bacteria, via mechanisms including direct antiparasite antagonism and the ability to influence the insect's immune system. In particular, the bacterium *Serratia marcescens* and some species of *Enterobacter* have been implicated in reducing parasite colonization (418–422).

Interactions between bacterial microbiota and unicellular eukaryotes may also take place in skin communities; several studies have suggested that cutaneous leishmaniasis, caused by trypanosomatids of the genus *Leishmania*, may interact with skin bacteria (422). Infection with *Leishmania braziliensis* in humans leads to dysbiosis of the skin bacterial microbiota, with reductions in the diversity and overgrowth of the genera *Staphylococcus* and/or *Streptococcus*; similar changes can be observed in a mouse model using *Leishmania major* ear infection (423). Interestingly, the specific changes observed may be dependent on symptom severity, with resolvable infections leading to *Staphylococcus* overgrowth and nonhealing lesions leading first to *Staphylococcus* but ultimately to *Streptococcus* dominance. Furthermore, parasite-associated dysbiosis could be transferred to naive mice by cohousing, and if these dysbiotic mice were then exposed to *L. major*, they displayed greater lesion severity and higher levels of

inflammatory markers than mice with normal skin microbiota (423). The gut bacterial microbiota may also be affected during *Leishmania* infection, with a study demonstrating decreases in *Gammaproteobacteria* abundances in both healing and nonhealing mouse models, although such an effect requires more research (424).

The outcome of *Leishmania* infection in germfree animals is unclear, potentially dependent on the route of infection, parasite inoculation dose, or mouse strain. In one case using subcutaneous footpad infection, it was shown that germfree mice were able to mount an antiparasite response similar to that of conventional animals but that they failed to heal lesions and were more densely colonized than conventional mice or mice conventionalized postinfection (425, 426). However, in a study using an intradermal ear infection model, germfree mice were more permissive to growth of *L. major* but had lesions that were smaller and less necrotic (427). Similarly, subcutaneous tail and ear infection models with *Leishmania amazonensis* demonstrated that germfree mice had smaller lesions than those of conventional mice (422, 428). Additionally, the microbiota of the *Leishmania infantum* vector *Lutzomyia longipalpis* (sand fly) appears to be critical for the development of the parasite in the insect midgut; infection of *L. longipalpis* results in disturbance and loss of diversity in the bacterial microbiota, while depletion of the gut microbiota with antibiotics impeded the parasite's ability to replicate and develop in the fly midgut (429).

Summary

Fungi may make up a relatively small proportion of the human microbiota, but their relationships with their bacterial neighbors and the host are not insignificant. Advances in sequencing technology have allowed a much greater understanding of the diversity of human-associated fungi than was possible with culture-dependent methods, and while fungal marker gene databases lag behind those for bacteria, progress is being made. It is becoming increasingly clear that fungi persistently colonize a range of body sites, form a variety of antagonistic or cooperative relationships with bacterial species, and can affect the course of disease during coinfections.

In particular, the ubiquitous human-associated fungus *C. albicans* demonstrates a range of interactions with bacteria. There are a number of examples of cooperative interactions between *C. albicans* and bacterial species, particularly in the oral environment. However, there are just as many examples of antagonism, including competition in shared niches and direct mechanisms of killing. Importantly, there are multiple examples in which bacterial species have an antagonistic relationship with *C. albicans* in an *in vitro* setting but demonstrate synergistic virulence in *in vivo* models, highlighting the important role that the host and possibly other species of bacteria and fungi may play in modulating some of these interactions.

Much of the interaction between bacteria and fungi is mediated by quorum-sensing molecules. Farnesol, a *C. albicans* quorum sensor that inhibits hyphal morphogenesis, is particularly important and has various effects depending on the concentration and context; it has fairly strong antimicrobial effects on multiple bacterial species at high doses but can have positive effects on bacterial fitness at low doses, including enhancing biofilm growth and increasing antibiotic resistance. Bacterial species themselves use a host of quorum sensors, including AI-2, CSP, Fsr, and farnesol mimics such as HSL, to regulate hyphal growth of *C. albicans* in mixed communities; in at least one case, *C. albicans* may actually induce a quorum-sensing system, specifically the XIP system in *S. mutans*. Additionally, a number of bacterial species have developed strategies to kill or suppress *C. albicans*, particularly in the hyphal form.

Moving forward, there are several directions to pursue. On a technical level, expansion and improved curation of fungal databases will be required to continue making progress, particularly in the arena of metagenomics and metatranscriptomics. This is particularly important to help overcome some of the limits of marker gene sequencing, which lacks detail and the ability to identify strain-specific differences but has been used in most studies to date. As has been found in bacteriome work, while descriptions of resident taxa are informative, this approach cannot accurately account for the

contributions of specific genes, regulatory pathways, and/or metabolites to the gut community. Additionally, when studying relationships between fungi and bacterial pathogens, work comparing *in vitro* and *in vivo* models will be important to account for the impacts of the host and other microbiota, which may have a significant influence on the interaction. Finally, while next-generation sequencing studies have revealed a number of potential relationships between fungal and bacterial taxa in the gut microbiome, the biological implications of such relationships generally have yet to be revealed. Performing interventional studies to identify the impacts of diet or other factors, in parallel with in-depth metagenomics, metatranscriptomics, and/or metabolomics, may help to reveal the potential relevance of such observations and uncover functional networks of both fungal and bacterial species in the microbiome.

In addition to fungal colonization, human can be infected with a diverse array of other unicellular eukaryotes, which frequently demonstrate a parasitic relationship with their hosts. While the exact associations vary by organism, there is evidence for interactions between unicellular eukaryotes and the microbiota of the skin, gut, and genital tract. Infection with these organisms can alter the composition or function of the resident microbiota, commensal microbes may provide protection against infection, or resident microbes can contribute to the pathology of infection; in many cases, these interactions involve modulations of host immunity to exacerbate or protect against pathological responses. Furthermore, the microbiota of insect vectors can also interact with the parasites that they carry, either promoting or inhibiting development, which can have important implications for the spread and transmission of these diseases. Continuing work in this arena will benefit from the identification of specific microbes that confer protective effects, comparisons of results from mouse models with data from human subjects, and further elucidation of mechanisms via which unicellular eukaryotes, the microbiota, and the host interact.

HELMINTHS

In research on the residents of the human gut, the relationship of macroflora such as helminths to their microbial neighbors has been understudied. The term "helminth" refers to a number of multicellular, parasitic worms that are responsible for a wide variety of human diseases collectively known as helminthiases (430, 431). While many have been largely eradicated from developed nations, they are a significant burden in other parts of the world and cause hundreds of millions of cases of neglected tropical diseases, including schistosomiasis, hookworm infection, elephantiasis, and tapeworm infection, among others (430, 432–434). While commonly grouped together due to gross morphological similarities, helminths actually form a polyphyletic group and have a wide range of life cycles, reproductive strategies, infection locations, transmission routes, and associated pathologies (431). Despite these differences, they are all extracellular pathogens and can therefore elicit similar immune responses in the human host. In particular, it has been suggested as part of the "old friends" hypothesis that the increased incidence of autoimmune and allergic diseases in the developed world is partially due to the successful eradication of helminth infections, leading to the loss of a coevolved immunomodulatory influence (Fig. 3) (102–105, 107, 435–437). Currently, there is even some research into the medicinal properties of worm infection for some inflammatory autoimmune diseases (438–440).

Helminths: Multicellular Parasites

There are three major groups of helminths that infect humans (Fig. 1); two of these groups fall into the phylum Platyhelminthes, commonly known as flatworms for their appearance (431, 433, 441, 442). First, there are trematodes, members of the class Trematoda, which are parasitic flatworms referred to as "flukes." They can be broken up into two practical groups: *Schistosoma* species are blood flukes that primarily reside in the vasculature around the gut (*S. mansoni* and *S. japonicum*) or bladder (*S. haematobium*) in the human host, while tissue flukes, including *Fasciola hepatica*, *Paragonimus westermani*, and *Echinostoma* species, infect the liver, lungs, or intestines (443–445).

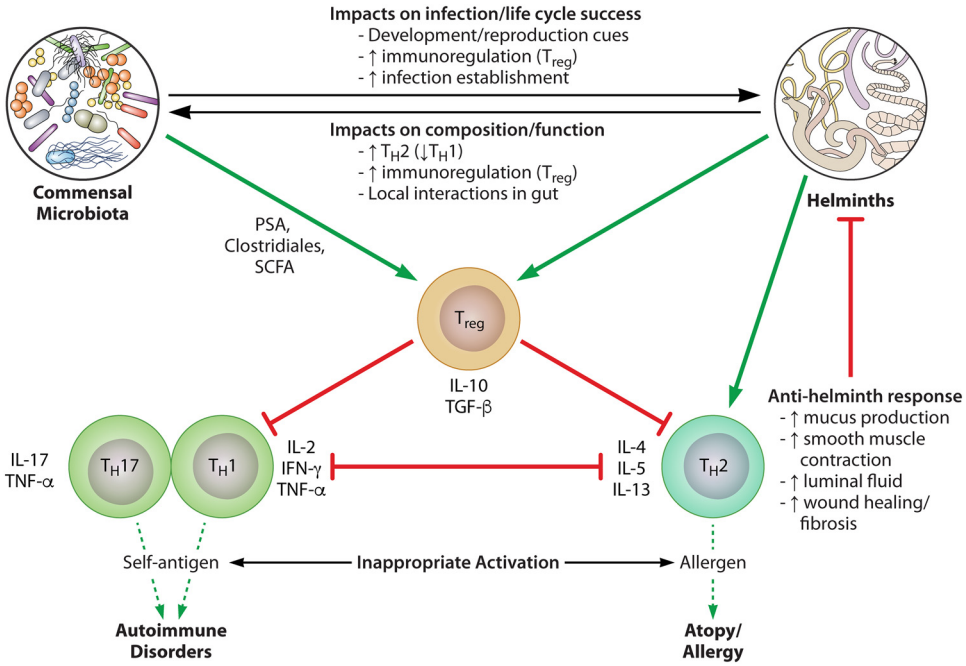


FIG 3 Outline of key interactions between helminths, the bacterial gut microbiota, and the host immune system, including the "old friends" hypothesis that immunomodulatory influences of commensal bacteria and helminths can promote a tolerance phenotype and reduce the risk of immune-mediated autoimmune or allergic disorders. TNF-α, tumor necrosis factor alpha.

Their life cycle includes a primary vertebrate host, where sexual reproduction occurs, and one or more intermediate mollusk hosts, where they reproduce asexually (431, 446). The second group of helminths implicated in human disease are the cestodes (*Taenia* species, *Hymenolepis* species, and *Diphyllobothrium* species), which are parasitic flatworms of the class Cestoda in the phylum Platyhelminthes. Cestodes are more commonly known as tapeworms and generally infect the gastrointestinal tract of vertebrate species (447). They frequently have a two-phase life cycle: an intermediate host consumes eggs passed from the primary host, hatched larvae penetrate into the muscle, the primary host eats such contaminated meat, and the larvae reach maturity in the gut and begin producing eggs (431, 433, 441, 442).

The largest group of clinically relevant helminths are the nematodes, or roundworms. Collectively, they comprise the phylum Nematoda and include a wide and diverse range of species with various habitats, reproductive strategies, and life cycles. Many of these organisms parasitize vertebrates, and several dozen infect humans (431, 433, 441, 442). Filarial worms, which include the causative agents of elephantiasis (*Wucheria bancrofti*, *Brugia malayi*, and *Brugia timori*) and river blindness (*Onchocerca volvulus*), use arthropods as intermediate hosts and are transmitted via insect bites (448). As such, they do not spend time in the gastrointestinal tract as a normal part of their life cycle, instead living in the lymphatic system and subcutaneous tissues (449). Most other pathogenic nematodes are categorized as intestinal helminths, as they are transmitted via ingestion of contaminated food or water and spend at least part of their life in the human host in the gut. Examples include pinworms (*Enterobius vermicularis*), whipworms (*Trichuris trichiura*), and Guinea worms (*Dracunculus medinensis*) as well as *Ascaris* and *Trichinella* species (433, 441, 442, 450). Hookworms (*Necator americanus* and *Ancyclostoma duodenale*) and threadworms (*Strongyloides stercoralis*) are also partially gut-resident, although these are transmitted through skin contact with contaminated soil (433, 441, 442).

Importantly, as many helminths spend a significant portion of their life cycles in the gastrointestinal tract of the human host, there is interest in their potential interactions

with the native gut microbiota (Fig. 3). For gut-resident helminths, there is significant evidence that the presence of the intestinal microflora is important for successful infection, and some work has even implicated specific members of the microbiome. Conversely, there is also a body of work investigating the impacts that helminth infections have on the gut microflora of human and animal hosts; local interactions or systemic immunological responses to worm infection have the potential to influence the diversity, makeup, and function of the native gut community.

Ancient Enemies or Old Friends?

As mentioned above, helminth infection is thought to be an important part of the old friends hypothesis (102, 103, 105, 107, 435–437). There is evidence that over the thousands of years of coevolution with their human hosts, many helminths have adapted to modulate the immune system, thereby allowing them to persist in chronic infections. At the same time, the human immune system evolved in the context of these chronic infections and may have adapted to use helminthic immunomodulatory cues to regulate itself and prevent inappropriate activation and damage (103, 104). As modern living conditions have become more hygienic, with cleaner food and water supplies, exposure to helminths has become rare in developed nations. Given the negative impacts of many helminth infections, this has been generally beneficial; however, it is theorized that the lack of helminth infections may be associated with deficiencies in immune system regulation, particularly in the arena of immunological tolerance (451). Accordingly, individuals living in developed nations become more likely to develop disorders involving inappropriate immunological reactions to harmless or self antigens, including allergies, asthma, IBD, type 1 diabetes, and multiple sclerosis (Fig. 3) (437, 452, 453). On a more specific level, observational human studies have demonstrated an inverse relationship between helminth infection and markers of both atopy and autoimmune activity (454–457). For example, helminth infection was associated with reduced dust mite skin test reactivity in Gabonese schoolchildren, while a randomized controlled trial demonstrated that antihelminthic treatment reversed this trend, and the intensity of schistosome infection was negatively associated with antinuclear autoantibodies in a Zimbabwean population (456, 457).

It is believed that such effects occur through helminth-induced modulation of the host immune system. A striking feature of helminth infection in general is the activation of type 2 immunity, the host response against extracellular pathogens and allergens, which is characterized by the differentiation of T_H2 $CD4^+$ T cells and the production of the interleukins IL-4, IL-5, and IL-13 (433, 458–460). Practically, this response results in enhanced epithelial mucus production, smooth muscle contraction, and fibrosis of granulomas, which can help to kill, expel, or seal off the parasite or its eggs and promote rapid healing of parasite-induced damage (Fig. 3) (458, 460). Additionally, activation of the T_H2 response downregulates the proinflammatory T_H1 and T_H17 pathways, which are commonly associated with viral, bacterial, and autoimmune diseases but can also contribute to pathological inflammation and tissue damage during some helminth infections (458, 460). However, a range of helminth species have developed mechanisms to dampen immune responses against them and thereby promote their own persistence, including activation of T_{reg} cells, regulatory B cells, tolerogenic dendritic cells, and alternatively activated macrophages, as well as inhibiting host responsiveness to the parasite's own antigens; in particular, production of the regulatory cytokines IL-10 and transforming growth factor β (TGF-β) is implicated in the control of both proinflammatory T_H1/T_H17 pathologies and allergic T_H2-driven responses (Fig. 3) (430, 458–471). These changes allow some helminths to persist for years in the human host and may be protective against allergic and autoimmune disorders. Additionally, the immune system itself may have evolved to tolerate such immunomodulation rather than risking serious organ damage through a sustained immune response (104, 430, 458); for example, several studies of chronic filariasis and schistosomiasis indicate that those with greater disease pathology tend to have stronger

immune responses, while those that maintain chronic, low-level infection demonstrate immune hyporesponsiveness (472–477).

Due to these broad immunological changes, deliberate helminth infection is being investigated as a treatment for several autoimmune diseases. Helminths, given their association with T_H2 polarization, promotion of an immunoregulatory state, and immune hyporesponsiveness, may be able to downregulate the self-inflammatory responses involved in autoimmune pathology. Furthermore, promising early observations indicating disease remission in naturally infected multiple sclerosis patients, which was reversed after antihelminthic treatment, have encouraged research interest in this area (478). There are several helminth species being investigated for this purpose, including *Trichuris suis* (pig whipworm) and *N. americanus* (hookworm) (479). A number of studies in animal models of autoimmune disorders have lent support to this approach (470, 480–483); human trials for several autoimmune disorders are ongoing and thus far have had mixed results (438, 484–487). However, helminth-associated immunomodulation can also negatively impact the immune response to secondary microbial infections. Studies have demonstrated potential helminth-related defects in response to an array of vaccines and infections, including tetanus, toxoplasmosis, tuberculosis, cholera, malaria, and infections by several viruses (488–500). In one example, researchers demonstrated that mice infected with the gastrointestinal nematode *Heligmosomoides polygyrus* or eggs of the trematode *Schistosoma mansoni* experience increased viral replication and even reactivation of latent herpesvirus via T_H2 cytokine responses, particularly IL-4 (501).

Importantly, the immune regulation mediated by helminths may impact or be impacted by the gut microflora, which occupy the same physical niche as many worms. Humans, gut commensals, and helminths have coevolved for millennia, likely leading to the development of bi- and tripartite interactions between host, bacteria, and worms. Here, we discuss evidence that host-associated microbes may impact helminth infection success, that helminth colonization may impact the diversity and composition of the gut microbiome, and that both can have impacts on immune function.

Helminths Can Modify the Native Microflora

Perhaps unsurprisingly given the range of immunological impacts that helminths can have on their hosts, there is significant evidence that helminth infection is capable of altering the composition and function of the gut microbiota. While specific mechanisms remain, in many cases, unelucidated, it is likely that direct effects exerted by the helminths themselves, local immune responses to worms, general immunological shifts induced by infection, and other mechanisms may coincide to alter gastrointestinal conditions and allow for the proliferation of alternate gut microflora. However, there is little consensus on specific effects such as taxonomic shifts, given that studies have been conducted on a wide array of host and helminth species, and therefore, there is significant breadth but little depth to the field at the moment (502–504). Despite this, it seems clear that infections with a variety of helminths have the capacity to alter the composition of the host microbiome.

In an example of a potentially common impact of different helminth species, infections of rodents with the nematodes *H. polygyrus* (formerly *Nematospiroides dubius*), *Trichuris muris*, *Nippostrongylus brasiliensis*, and *Trichinella spiralis* may all promote enrichment in the *Lactobacillaceae* lineage of gut bacteria, particularly in the ileum, where the parasites reside. In one case, researchers analyzed the microbiota of healthy mice during *H. polygyrus* infection, finding an enrichment in ileal *Lactobacillaceae* across multiple experiments as well as an increase in the number of bacteria overall (505). Interestingly, this group also demonstrated that while the larvae of this helminth have a distinct microbial community, adult nematodes themselves come to harbor microbiota closely resembling that of their host ileal environment (505). In another study of *H. polygyrus* infection, it was shown that the abundance of *Lactobacillus* species in the mouse small intestine correlated positively with infection (506). Specifically, researchers found that the abundance of *Lactobacillus* increased during infection of the *H.*

polygyrus-susceptible C57BL/6 strain of mice but not in the resistant BALB/c strain, where the helminth cannot take hold (506).

Similarly, multiple studies of mice infected with *T. muris* showed an association between infection and levels of this microbial lineage. Holm et al. found a significant enrichment in *Lactobacillaceae*, while a contemporaneous study by Houlden et al. found increased levels of *Lactobacillales* in infected animals; both studies also found reductions in diversity resulting from chronic *T. muris* infection (507, 508). Increases in levels of ileal *Lactobacillaceae* have also been observed in mice infected with *N. brasiliensis*, alongside increases in *Coriobacteriaceae* and decreases in levels of *Peptostreptococcaceae*, *Clostridiaceae*, *Turicibacteraceae*, and IL-17-inducing SFB (509). Similarly, mice infected with *Trichinella spiralis* displayed increased *Lactobacillaceae* and decreased *Turicibacteraceae* and *Clostridiaceae* abundances in the ileum as well as increased proportions of *Ruminococcaceae* and *Lachnospiraceae* in the colon (492). However, not all models show this trend in response to nematode infection; *Trichostrongylus retortaeformis* infection of rabbits did not lead to changes in *Lactobacillaceae* but resulted in decreased diversity, increased abundances of *Leptonema* and *Desulfocella*, and decreased abundances of *Ruminococcus* and *Bacteroides* (510). Beyond altering the intestinal bacterial populations, murine infection with gastrointestinal nematodes may have impacts on the virulence of bacterial pathogens. While they did not directly assess changes in the resident bacterial microbiota, Reynolds et al. demonstrated that *H. polygyrus* infection enhances colonization, pathogenicity, and invasion by *S. enterica* by altering the metabolic profile of the small intestine (511).

In addition to rodents, several studies have examined the impacts of infection with various nematodes on the microbiota of the abomasum (fourth stomach) of ruminant species. Infection with several related helminths has been shown to increase the abomasal pH due to reduced gastric acid production, and this pH shift was associated with an enrichment in anaerobic bacteria in the abomasa of sheep infected with *Ostertagia circumcincta* (512). Later, the same group investigated the impacts of infection with another related nematode, *Haemonchus contortus*, on the microbiome of the goat abomasum, finding significant differences in the beta diversities of the two groups and that 19% of OTUs were different between infected and uninfected goats (513). Specifically, infection was associated with increases in the abundances of several *Veillonellaceae* OTUs, decreases in the abundances of several *Lachnospiraceae* OTUs, and a mix of increases and decreases in the abundances of *Bacteroidales* OTUs. Additionally, functional potential analysis, using the 16S rRNA gene composition to predict the metagenomes present, revealed differential abundances of six gene families and 9 KEGG pathways between the two groups, indicating possible modifications of microbiome function (513).

The same group also demonstrated changes in the porcine microbiome after infection with the pig whipworm, *T. suis*, with these alterations persisting even after parasite clearance (514). Pigs were infected with worms for 53 days and then separated into worm-free pigs that had cleared infection and pigs still carrying significant worm burdens. The abundances of 48 out of 372 genera identified in the proximal colon microbiota of the pigs were significantly altered by infection, whether infection persisted or was cleared, suggesting long-lasting impacts from initial infection (514). Interestingly, the abundance of *Campylobacter* varied by worm status: compared with parasite-naive pigs, worm-heavy pigs harbored more *Campylobacter* bacteria, while pigs which had cleared infection harbored less (514). In another study from the same laboratory, researchers showed that a shorter infection with *T. suis* for 21 days was also sufficient to induce significant changes in the gut microflora, impacting approximately 10% of genera present in control pigs (515). Specifically, researchers observed significant increases in the abundances of *Mucispirillum*, *Paraprevotella*, and *Desulfovibrio* and decreases in the abundances of several *Clostridiales*, including *Ruminococcus*, *Blautia*, and *Dorea* (515). Additionally, metagenomics indicated that infected pigs showed an reduction in the abundance of KEGG pathways involved in carbohydrate metabolism and amino acid metabolism (515). Finally, a study of ponies infected by strongyles

(nematodes of the families Strongylinae and Cyathostominae) also found reductions in the abundances of *Clostridiales* associated with infection; susceptible ponies that developed more-intense infections demonstrated reductions in the abundances of the commensal *Clostridiales Ruminococcus*, *Lachnospiraceae*, and *Clostridium* XlVa and increases in abundances of *Pseudomonas*, *Campylobacter*, and *Bacillus* relative to resistant ponies that developed less-intense infections (516).

Several studies have used animal models of IBD to investigate the impacts of helminth infection in the context of inflammation. For example, one study investigated the impact of helminth therapy in the nonhuman primate *Macaca mulatta* (rhesus macaque) with chronic idiopathic diarrhea (CID), a condition similar to human ulcerative colitis (517). Researchers treated five juvenile macaques with CID with the whipworm *T. trichiura* and found improved fecal consistency and weight gain in four subjects, despite a lack of establishment of patent infection. Analysis of the bacteria attached to the intestinal mucosa revealed a decrease in the absolute amount of bacterial attachment, an increase in Shannon diversity, and significantly different measures of beta diversity in posttreatment macaques; specifically, those researchers observed a significant increase in the abundance of the phylum *Tenericutes* in response to helminth treatment. Generally, they speculate that helminth treatment increased T_H2 responses, including mucus production and epithelial cell turnover, decreasing the attachment of immunostimulatory bacteria to the mucosa and improving symptoms (517). Similarly, researchers examined the impact of infecting mice lacking the IBD susceptibility gene *nod2* with either *H. polygyrus* or *T. muris* (518). *nod2*$^{-/-}$ mice typically display small intestinal abnormalities, including goblet cell defects and colonization by the bacterium *Bacteroides vulgatus*; after colonization with helminths, however, the goblet cell defect was corrected, and levels of *B. vulgatus* fell dramatically. The inhibition of *B. vulgatus* was associated with an increase in the abundance of the family *Lachnospiraceae* in the class *Clostridiales* and could be transferred to helminth-free *nod2*$^{-/-}$ mice by cohousing. Those authors suggest that the increased mucus production occurring during helminth infection promotes the growth of *Clostridiales* over *B. vulgatus* (518).

While the majority of studies in animal models have been conducted using nematode parasites, there are some studies using other types of helminths. For example, while colonization of rats with the tapeworm *Hymenolepis diminuta* did not alter alpha or beta diversity, there were distinct differences in the cecal community composition affecting approximately 20% of the microbiome (519). Specifically, there were significant decreases in *Turicibacter* and significant increases in *Peptostreptococcaceae* abundances. They also exposed half of the animals to LPS challenge several days before analysis to assess the impacts of inflammatory challenge in the context of helminth infection. Interestingly, beta diversity was altered between LPS groups in uninfected animals, but there was no impact of LPS in helminth-infected rats (519). Further analysis showed that most of the changes observed resulted from shifts internal to the major phylum *Firmicutes*, with increases in the abundances of the class *Clostridia* and decreases in the abundances of the class *Bacilli*. Importantly, while the worm resides in the small intestine, these effects were observed in the cecum, indicating the potential for more than simply local effects of infection (519). Another group analyzed the effects of infection with the trematode liver fluke *Opisthorchis viverrini* on the microbiome of Syrian hamsters, a model for the bile duct cancer that this helminth can induce (520). That group found that hamsters infected with *O. viverrini* had an increased diversity of the gut microbiome, with increases in the abundances of *Ruminococcaceae*, *Lachnospiraceae*, and *Lactobacillus* and decreases in the abundances of *Porphyromonadaceae*, *Erysipelotrichaceae*, and *Eubacteriaceae* (520). Finally, a recent study using a mouse model of *S. mansoni* infection found significant reductions in alpha diversity associated with infection; additionally, researchers observed a significant expansion of the *Verrucomicrobia* member *A. muciniphila*, in addition to increases in the abundances of members of the *Bacteroidales*, *Coriobacteriales*, and certain *Clostridiales* and decreases in the abundances of *Erysipelotrichia* and other *Clostridiales* (521). Importantly, the

microbiota was assessed both before and after the beginning of egg production (28 and 50 days postinfection, respectively), which demonstrated progressive differences in microbiome composition that specifically accompanied the onset of egg production and associated intestinal damage and inflammation (521).

There have also been some studies in humans, although these have generally been observational. One study used 16S rRNA gene sequencing to examine the gut microbiomes of rural Malaysians infected with one or more gastrointestinal helminth species (*Ascaris* species, *Trichuris* species, and hookworm) and compared them to those of uninfected controls, finding that helminth infection was associated with greater diversity of the fecal microbiome (522). Analysis of changes in the community's predicted functional potential indicated an enrichment in the abundance of a number of metabolic pathways, including those involved in nucleotides and amino acids. Further analysis revealed that *Trichuris* infection specifically was strongly associated with enrichment in *Paraprevotellaceae*, and functional potential analysis indicated an enrichment in pathways associated with nucleotide metabolism, cell growth, and cell death (522). A later study by the same group studied a similar population before and after antihelminthic treatment and found that reductions in parasite burden were associated with decreases in *Clostridiales* and increases in *Bacteroidales* abundances, which supported their findings of helminth infection in *nod2*$^{-/-}$ mice discussed above (518).

In a similar study, another group analyzed the impacts of infection with *T. trichiura* alone or in combination with *Ascaris lumbricoides* on the gut microbiome of Ecuadorean children. For children infected with *T. trichiura* alone and a subset of uninfected children, a follow-up was performed after curative antihelminthic treatment (523). Those researchers found no significant differences in microbial composition in children infected with *T. trichiura* alone and no differences after antihelminthic treatment. However, they observed a decreased proportion of *Clostridia* and reduced microbial diversity in children with mixed infections, suggesting a specific effect of *A. lumbricoides* infection on the microbiome (523). However, in the course of a larger study, Rosa et al. reanalyzed this data set using their own pipeline; they found associations between helminth infection and differences in various taxa, including increases in abundances of *Eubacteria*, *Streptococcus*, and the order *Lactobacillales* (524). In addition, they performed a study investigating the impacts of infection with *A. lumbricoides*, *N. americanus*, and *T. trichiura* in populations in both Liberia and Indonesia; a double-blind study of the impacts of antihelminthic treatment was performed on the Indonesian population to study the impact of worm clearance after 2 years (524). They found a number of associations with specific helminth infections as well as several taxa that were associated with helminth infection generally in both populations; for example, *Lachnospiraceae incertae sedis* were consistently negatively associated with helminths, while *Desulfovibrionaceae*, *Olsenella*, and *Enterococcus* were among taxa positively associated with infection. When studying the impacts of antihelminthic treatment after 2 years, they found that dewormed microbiota were more similar to infected than uninfected microbiota, suggesting that there may be lingering effects from helminth colonization (524).

Another group studied the impacts of gastrointestinal nematodes on the fecal microbiota of a Sri Lankan population; specifically, the authors examined individuals with current infection with *A. lumbricoides*, *N. americanus*, *A. duodenale*, and/or *T. trichiura* compared to those who were uninfected and those who had recently received antihelminthic treatment (525). While there was no observed difference in the alpha diversity of the communities, the authors found an increase in the beta diversity of the infected and treated groups. Additionally, there were some associations of specific taxa with infection status: infected subjects demonstrated increased abundances of *Lactococcus*, *Akkermansia*, and *Verrucomicrobiaceae*; uninfected subjects demonstrated an increased abundance of *Leuconostocaceae*; and treated subjects demonstrated an increased abundance of *Bacteroides* (525). Predictions of the functional potential of the communities indicated that helminth-infected subjects had downregulated ether lipid metabolism and apoptosis pathways and upregulated biotin metabolism pathways

(525). Members of this group have also worked on several studies of experimental infection with *N. americanus* in human subjects, generally finding that infection was associated with minor increases in the microbial richness of the fecal and duodenal microbiota, particularly in the context of gluten challenge in subjects with celiac disease, but not with significant impacts on the community structure or abundance of specific taxa (526–528).

Other studies have studied the impacts of human infection with *Schistosoma* species on the composition of the gut microbiome. Such effects are particularly interesting, as this organism does not reside directly in the gut but resides in the vascular system. One study examined the impacts of *S. haematobium*, which resides in the urogenital vasculature and extrudes eggs into the bladder, on the fecal microbiomes of children in rural Zimbabwe, where the parasite is endemic (529). Those authors found 21 OTUs that varied with infection status, although only 5 were robust to multiple-hypothesis testing, all belonging to the genus *Prevotella*. Additionally, as all participants were treated with curative praziquantel at the time of sample collection, the authors performed follow-up at 12 weeks and found that treatment did not significantly impact the microbiomes of previously infected children at this time point (529). In a similar study, researchers studied the impact of infection with *S. mansoni*, which resides in the mesenteric vasculature and extrudes eggs into the gut, and praziquantel treatment on the microbiota of children in Cote d'Ivoire (530). They found that children with various levels of infection intensity were more likely to have an enrichment in the phylum *Proteobacteria* and specifically found an association between infection and the genus *Klebsiella*. In terms of praziquantel, treatment was associated with higher levels of the classes *Bacilli* and *Erysipelotrichia* after 24 h, and success of treatment was associated with higher levels of *Fusobacteriales* both before and after treatment (530).

In general, it seems clear that infection with a variety of both gut-resident and tissue-resident helminths can have impacts on the composition and function of the gut microbiota (Fig. 3). However, it is difficult to draw conclusions about specific changes, and in some cases, different groups have obtained contradictory results; for example, studies of humans naturally infected with gut-resident helminths have alternately found increased, decreased, and unchanged microbial diversity associated with infection (522, 523, 525). This may be due in part to the wide range of study conditions used. Across the studies, hosts include multiple animal models and various human populations, while helminths include species that inhabit the small intestine, large intestine, and vascular system. In addition, these studies utilize a multiplicity of analysis techniques, including different diagnosis strategies, sequencing platforms, 16S rRNA gene regions analyzed, databases used, and computational pipelines and analytics. Importantly, the majority of the human studies discussed here analyzed the fecal microbiota, frequently used as a proxy for the gut community due to the relative ease and lack of invasiveness of sample collection; however, it may miss changes that occur at the mucosal level, where gut-resident helminths are likely to reside, as well as differences between small and large intestinal communities. Despite such factors, some potential commonalities have arisen: several studies found an enrichment in *Lactobacillaceae* during infection of animal models with gut-resident nematodes, both human and animal studies found enrichment in potentially mucolytic (*Mucispirillum*, *Akkermansia*, and *Prevotella*) or sulfate-reducing (*Desulfovibrionaceae*, *Desulfocella*, and *Desulfovibrio*) bacteria, and several studies found evidence for microbiota disturbance persisting after helminth clearance.

Microbial Composition Can Alter the Course of Helminth Infection

Not only can helminths modify the native microbiota, but the presence or composition of the gut microflora may actually influence the course of infection with some helminths. Some of the first hints of such an effect came from studies of germfree animals. Experiments with germfree mice indicated that the presence of the gut microbiota has significant supportive effects on the course of infection with *H. polygyrus*. For example, infected conventional mice developed more adult worms, had

longer infections, and showed higher mortality rates than germfree mice. In fact, as the infection progressed, the difference in adult worms harbored by the two groups grew wider, suggesting that the presence of the microbiota was required for efficient adult worm survival. However, the germfree mice also developed eosinophilia, intestinal wall thickening, and pronounced nodule formation in the small intestine in comparison to conventional mice; this suggests a strong immune response that may have contributed to reduced worm survival, possibly due to a lack of immunoregulatory influence from gut microbiota (531).

Follow-up studies from the same group added gnotobiotic mice monocolonized with a single bacterial species for comparison with conventional and germfree mice. For example, Chang and Wescott performed a study in which they used an unspecified *Lactobacillus* species isolated from conventional mice to create a monoassociated group (532). These mice appeared to display an intermediate phenotype between conventional and germfree mice in terms of the development of adult *H. polygyrus* worms, nodule development, and parasite egg production. Additionally, increasing the length of time between monoassociation and infection favored parasite survival, with parasite numbers similar to those in germfree mice at 2 weeks but increased parasite numbers in monoassociated mice at 6 weeks (532). Similarly, researchers used the nematode *Trichinella spiralis* to infect mice monoassociated with *Bacillus mesentericus* or *Bacillus subtilis*, which do not colonize the small intestine where this parasite resides, or *P. aeruginosa*, which can colonize both the small and large intestines (533). Mice monoassociated with *P. aeruginosa* displayed an intermediate phenotype, with researchers recovering more adult worms than from germfree mice but fewer worms than from conventional mice. On the contrary, mice monoassociated with *Bacillus* species were similar to germfree mice and contained low numbers of adult worms, suggesting that proximity between a bacterial species and the parasite is required for interaction. Both monoassociated mice and germfree mice also displayed eosinophilia relative to conventional mice, indicating a similar immune response (533). Finally, this group also demonstrated that the gut microbiota favors the development of another intestinal nematode, *N. brasiliensis*; while its larvae appear to migrate through the lungs at equivalent rates in conventional, monocolonized, and germfree mice, significantly more adult worms were recovered from the gut of conventional animals (534). The conclusions of this series of experiments were further supported by a study performed by a different group using the nematode *Ascaridia galli* to infect conventional, germfree, and monoassociated chickens. As in the above-described studies, rates of parasite establishment and survival were highest in conventional animals, lowest in germfree animals, and intermediate in animals monoassociated with either *B. subtilis*, *Bacillus cereus*, or *Penicillium* (535). Together, data from these studies suggest that even the presence of a narrow gut microbial community is able to promote parasite development and survival.

One issue with such studies is that complete abolishment of the microbial community from birth has significant effects on immune system development (115, 117, 143, 536, 537), and thus it is difficult to parse out impacts of the microbes themselves relative to immunological defects in germfree animals. Therefore, more recent work has sought to analyze links between members of the gut microflora and helminths in the context of a healthy, diverse microbial community. For example, in addition to links suggesting that helminth infection can promote *Lactobacillus* in mouse models, there is also evidence that *Lactobacillus* can promote the establishment of infection with *H. polygyrus* (506). Specifically, administration of *Lactobacillus taiwanensis* to a typically resistant mouse strain increased the levels of regulatory T cells and nematode establishment frequencies. Together with this group's finding that *H. polygyrus* promotes *Lactobacillus* expansion, this indicates a self-reinforcing relationship in which the presence of *Lactobacillus* promotes *H. polygyrus* infection while the parasite increases the abundance of *Lactobacillus* (506). The authors of that study suggest that *Lactobacillus*-mediated T_{reg} expansion, potentially suppressing T_H2 immunity, may be the mechanism behind the increased susceptibility to *H. polygyrus* infection (506). In

support of this finding, another group found that oral administration of either live or dead *Lactobacillus* bacteria before infection increased susceptibility to another mouse parasite, *Trichuris muris*, and led to reductions of both T_H1 and T_H2 cytokines (538).

There are a few studies that suggest that some species of helminth depend on the host microbiota in executing key stages of their life cycles. For example, Hayes et al. demonstrated that the murine nematode whipworm *Trichuris muris* may rely on host microbiota as a developmental cue (539). First, *in vitro* experiments showed that *T. muris* eggs did not hatch unless in the presence of bacterial or yeast cells. To test this *in vivo*, that group experimentally reduced the number of bacteria in mice with enrofloxacin before administration of *T. muris* eggs, finding a decreased number of established infections and a strong T_H2 immune response (539). Additionally, they performed the same experiment on mice lacking an adaptive immune system and found a similar reduction in parasite establishment in the gut, suggesting that acquired immunity and the enhanced T_H2 response are not solely responsible for this effect. It is possible that the requirement for the presence of microflora for hatching may be an adaptation evolved to ensure that parasite eggs hatch in the bacterium-lined cecum and colon (539). Similarly, another group found an altered course of intestinal schistosomiasis upon depletion of the gut microflora (540). After administering antibiotics and antifungal drugs to mice infected with *S. mansoni*, which resides in the venules surrounding the gastrointestinal tract and excretes eggs into the gut lumen, that group found that gut inflammation, intestinal granuloma formation, and egg excretion were all reduced compared to untreated mice. Interestingly, liver pathology and worm fecundity (egg production) were unchanged, suggesting specific local effects of the gut microflora that promote successful egg translocation (540). Furthermore, researchers have found that immunoregulation induced by helminth infection is at least partially influenced by the intestinal microflora. Mice infected with *H. polygyrus* showed reduced allergic airway inflammation in response to a dust mite extract compared with uninfected controls, but this effect was not seen in mice that were first treated with antibiotics to reduce the numbers of gut bacteria despite a similar number of adult worms establishing infection (541). Importantly, the effect could be restored by reestablishing a normal microbiota, via either oral gavage of cecal contents from or cohousing with untreated mice (541).

Finally, while most of this work focuses on the relationship between the host microflora and infectious nematodes, there are also cases in which microbes associated with the parasites themselves are relevant to the course of disease. Specifically, filarial worms harbor endosymbiotic bacteria of the genus *Wolbachia*, and a significant part of the pathology caused by these parasites occurs due to the host immune response to *Wolbachia* rather than to the nematodes themselves (542–547). Additionally, most filarial worms are unable to survive without their *Wolbachia* symbionts, presenting a target for antibiotic therapy rather than antihelminthic drugs to eliminate infection (543, 544, 546).

Generally, this work suggests that the gut microflora can play a significant role in the establishment and maintenance of helminth infections (Fig. 3). Gut-resident or gut-proximal helminths appear to survive and reproduce more efficiently in the presence of a diverse community of gut bacteria, although monoassociation experiments demonstrate that even microflora lacking diversity can be utilized by parasites to promote infection. Given that humans have been infected with helminths for most of their existence and the lack of germfree hosts in nature, it is possible that helminths have coevolved to take advantage of the presence of the gut microflora for their successful development. Potential interactions include the use of bacterial signals as helminth developmental cues, bacterium-mediated T_{reg} activation allowing for effective colonization despite T_H2 polarization, or even the consumption of bacterium-produced metabolites.

Summary

The great variety of helminth and host species used in the studies described here

makes it difficult to draw specific conclusions about the effects of a given organism, but together, they paint a compelling picture of both bipartite and tripartite interactions between resident gut commensals, the host immune system, and helminth infections. The presence of gut microflora appears to support infection with certain helminths, including via direct interactions between the microbiota and helminths to promote life cycle progression or reproduction, immunomodulation to reduce the host response to helminth infection, and potentially other mechanisms. At the same time, infection with a variety of helminths appears to alter the composition and potentially the function of the gut microflora, although whether this occurs via local interactions between parasites and commensals, systemic immune changes favoring the proliferation of certain gut bacterial species, or a combination of the two remains unclear. There is some evidence that infection with helminths may impact the diversity of the gut microflora, although the directionality of this impact varied; however, whether or not overall diversity was altered, infection was frequently able to alter the abundances of a relatively large proportion of microbiome members.

These observations give rise to a number of interesting research directions. In terms of helminth infection prevention, there is evidence that microbiome depletion can reduce infection by or reproduction of certain helminth species. It would be useful to know how widespread this effect is across different clinically relevant helminths and to elucidate specific mechanisms, such as egg hatching in *T. muris* or egg excretion in *S. mansoni*, by which the course of infection is altered. Additionally, these effects have not yet been studied in humans, offering another point of study. There may also be specific bacterial taxa that interact with and promote infection with helminths, as seen in the mutually supportive relationship between *H. polygyrus* and *Lactobacillus* in mice. Identifying such interactions in other helminth infections could allow for the development of targeted interventions that might have lesser effects than microbiome abolishment, which can reduce microbial diversity and promote dysbiosis after recovery.

On the other side, the studies indicating the potential for helminths to alter the gut microbiome have implications for understanding the pathology of a number of neglected tropical diseases and for identifying potential side effects of helminth therapies for autoimmune disorders. For example, are helminths themselves directly responsible for the full extent of immunomodulation that occurs, or might they also modulate the gut microbiota to promote immunoregulatory bacteria? On a related note, does microflora alteration occur due to local interactions between gastrointestinal helminths and gut flora, as a consequence of a systemic immune alteration resulting from helminth infection, or both? While many of the immunological effects of helminth infection are well known, the exact mechanisms of the interactions between host, commensal, and helminth remain unclear. Studies of the impacts of non-gut-resident helminths, such as filarial worms and schistosomes, on the microbiota as well as profiling gut communities both proximal and distal to the site of gastrointestinal helminth infection may help to answer these questions.

Additionally, given that there is evidence from both human and animal studies that microbiome changes can persist even after parasite clearance, there is a question of what effect these changes might have on host health. If helminth infection alters the composition of the gut microflora, it leaves open the possibility of dysbiosis, inflammation, or opportunistic infections as potential sequelae, even after the parasite infection itself is treated. Especially given the interest in helminth therapy, it will be important to investigate and learn how to mitigate potential negative side effects of parasite-induced microbiome alterations; additionally, identifying specific helminth products or components that can promote beneficial immunomodulatory effects without requiring infection with live parasites is an important direction of research (548, 549). Finally, while many studies were able to identify changes in the composition and predicted function of the microbiota during helminth infection, full metagenomics and metatranscriptomics will be important in understanding the true functional differences in the microbiota, including nonbacterial members, under these conditions.

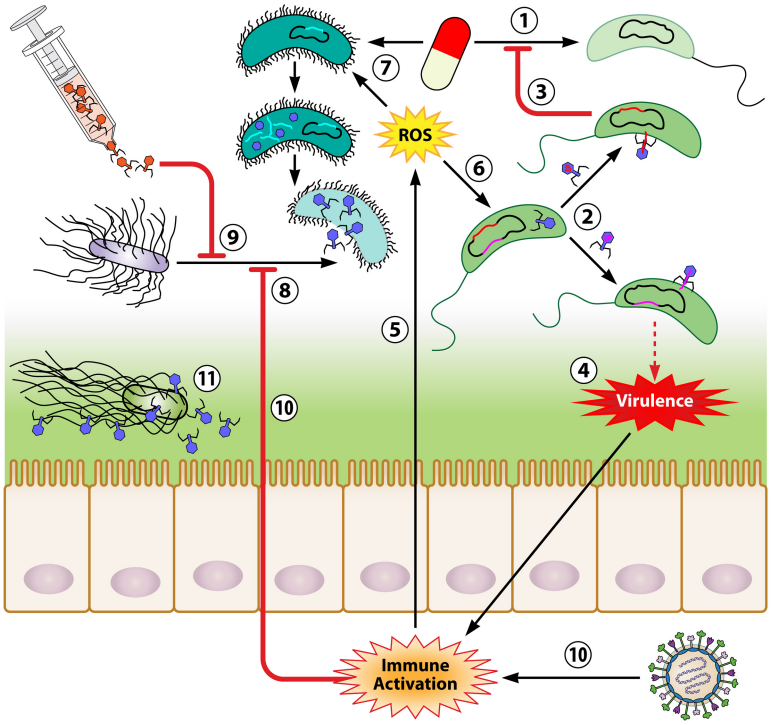
VIRUSES

There are an estimated 10^{31} viruses on earth, making them the most abundant and widespread biological entities on the planet (550, 551). Like bacteria, viruses are also ubiquitous members of the human ecosystem. The entire collective of eukaryotic and bacterial viruses (bacteriophages), including double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), and RNA viruses, has been termed the "virome." Significant effort has been devoted to characterizing this community as well as its role in shaping the microbiota. However, unlike for bacteria and fungi, there is no universal marker gene that can be utilized to study bacteriophage or viruses as a whole, so metagenomic approaches are frequently used. This review focuses mainly on work examining phage-microbiome interactions, as most of the viruses found in and on the human body have been determined to be phage based on metagenomic sequencing and transmission electron microscopy (552–556). Phage populations of numerous body sites, including the intestine, mouth, skin, respiratory tract, and urinary tract, have been profiled, although the intestinal virome has been the most widely characterized (552, 557–561). Additionally, in many cases, eukaryotic viruses exist as stable members of the human virome, and latent infections can play an important role in host health (562). Finally, RNA viruses isolated from human fecal samples were mostly plant RNA viruses, suggesting that the RNA virus portion of the intestine is derived from diet (563).

The Makeup of the Human Virome

The approximately 10^{15} phages that inhabit the human intestine are predominantly dsDNA viruses in the *Myoviridae*, *Podoviridae*, and *Siphoviridae* families of the order *Caudovirales* and ssDNA viruses of the family *Microviridae,* constituting an estimated 1,200 viral genotypes (Fig. 1) (552–554, 564, 565). The *Caudovirales* and *Microviridae* phages have an average genome size of 30 kb and exhibit both lytic and temperate lifestyles, although temperate phages dominate the human gut (552–554). The temperate lifestyle often leads to the integration of phages into bacterial hosts as either episomes or chromosomal prophages. An analysis of prophages in both Gram-positive and Gram-negative bacteria found prophages with lengths of 10 kb or more in 71% (40/56) of the bacteria examined (566). These prophages are replicated along with bacterial DNA and expand the genetic repertoire of the bacterium in which they reside, accounting for up to 20% of the bacterial genome (567). Multiple environmental stressors, including reactive oxygen species (ROS), reactive nitrogen species (RNS), UV radiation, or antibiotics, can lead to the induction of the prophage; this results in a lytic cycle, replication, and escape of the phage progeny and bacterial host death (568). Thus, phages in the human virome have the ability to modulate bacterial populations through a lytic lifestyle or can potentially provide a bacterium with novel traits while integrated as a prophage (Fig. 4). Widespread lysis of commensal bacteria through prophage induction could potentially facilitate colonization by pathogenic bacteria in formerly colonized niches (Fig. 4). Findings outlining the composition of phage communities have also revealed interesting trends in the establishment and steady-state dynamics of the human intestinal virome.

Intriguingly, a recent study identified a novel type of bacteriophage termed crAssphage, a name derived from the cross-assembly tool used to assemble the first 97-kb crAssphage genome from metagenome samples. crAssphages are thought to be the most prevalent human-associated virus and have been found to comprise up to 90% of reads from human fecal virome metagenomes (569). The bacterial host range of these phages is still not understood, but early investigation suggests that crAssphages may be associated with the bacterial phylum *Bacteroidetes*, and they are thought to potentially form a family within the order *Caudovirales* (570). Initial studies found that 80% of the predicted crAssphage proteins did not have matches in reference databases, but recent efforts have had more success in characterizing these proteins (570). crAssphages have been propagated *in vitro* and imaged for the first time, presenting a novel opportunity to study these phages in the laboratory setting (571). Guerin et al. further identified novel crAss-like phages from metagenomic data sets and proposed



1. Antibiotics kill sensitive bacteria
2. Phage-mediated HGT of antibiotic resistance/virulence genes
3. HGT leads to resistance
4. HGT leads to virulence, triggering immune activation
5. Immune activation produces ROS
6. ROS triggers HGT
7. ROS, antibiotics induce prophage
8. Pathogenic bacteria invade niche left by lysis of commensals
9. Phage therapy to kill pathogens
10. Latent viral infection causes immune activation, preventing bacterial infection
11. Phage-mediated immunity within mucus layer

FIG 4 Outline of prominent virome-bacterium interactions that shape the community dynamics and function of the host microbiota.

taxonomic assignments for these bacteriophages (571). While these phages remain understudied, their widespread abundance in human microbiota metagenomic data sets suggests that crAssphages may have an important role as a constituent of the human virome.

Much like the development of the bacterial community of the human microbiome, phages are partially inherited from the mother, and populations undergo a maturation process over the lifetime, forming a steady-state community of phages known as the "phageome." From birth to 2 years of age, the bacteriophage populations of infants transition from higher to lower diversity (572). During this time, there is also a shift in the relative abundance of phage populations, highlighted by a decrease in *Caudovirales* and an increase in *Microviridae* abundances (572). The changes in bacteriophage composition from infancy to adulthood are not well characterized and remain an area to be explored. In adulthood, phages of the families *Myoviridae*, *Podoviridae*, and *Siphoviridae* comprise a majority of the taxonomically identifiable viruses, while *Microviridae* make up a smaller but significant proportion (553, 554). Among healthy adults, the intestinal phageome exhibits high interpersonal variation, with individuals displaying distinct phage compositions. In contrast, in the same individuals, intrapersonal variation over time was comparably low. Ninety-five percent of viruses were retained over a 1-year study, similar to the gut bacterial population (28, 553). These findings suggest that humans harbor individual but highly stable intestinal phageomes.

The Human Virome in Gut Dysbiosis and Bacterial Coinfections

It is well established that the bacterial and even fungal members of the microbiome play a key role in human health and disease, but the role of the virome is less understood. Attempts to characterize the virome of healthy individuals have naturally led to studies of how this community changes during disease states, including the role of phages and eukaryotic viruses during bacterial infection and host microbiome dysbiosis. Studies have revealed that the human virome is altered during disease states and plays a role in bacterial pathogenicity but can also be protective against bacterial infection.

While the role of bacteria has been examined in the context of inflammatory gut dysbiosis, we are just beginning to learn about the interplay between conditions such as IBD and the virome (573). For example, Manrique et al. analyzed metagenomic data sets of intestinal bacteriophages from 62 healthy subjects. Their study identified 155 bacteriophages that were determined to be part of the "healthy gut phageome," although these represented only 4% of the estimated phage community. Of the 155 phages, 132 "common" bacteriophages were found in 20% to 50% of individuals, and a further 23 "core" bacteriophages were observed in >50% of subjects (565). Interestingly, this core phageome was depleted in subject groups with IBD, suggesting that the intestinal phageome may play a role in maintaining a healthy gut (565). However, this could also result from a reduction in bacterial host diversity common to IBD (574). Previously, studies of the enteric virome in IBD had observed that the gut mucosa of Crohn's disease patients harbored higher numbers of virus-like particles (VLPs) (2.9×10^9) of the order *Caudovirales* than healthy patients (1.2×10^8) (556). Further metagenomic evaluations of IBD patients confirmed this observation, finding that the disease state is characterized by greater interpersonal variation in virome composition along with an increased abundance and richness of *Caudovirales* bacteriophages but less overall diversity of the virome (575–577).

Additionally, metagenomic analysis of both control and colitis-induced mice has revealed responses of the virome to inflammation in a more controlled setting. In mice with colitis, there was an expansion of phages that infect potential pathogens such as *Proteobacteria*, which are known to expand during gut inflammation and IBD (577, 578). Phages of *Streptococcus* and *Alistipes* bacteria were more abundant in the colitis group, but interestingly, the levels of the bacterial hosts did not change with colitis (577). This suggests that increased levels of a phage are not always dependent on elevated host abundance; instead, lysogenic phages may be induced by inflammation leading to replication and excision of phage. One of the most significant findings from this study is the overlap in phage metagenomes between the mouse model and human studies, suggesting that this murine model is suitable for studying phage dynamics of human IBD (576, 577). Together, data from these studies suggest that inflammatory disease in the gut can lead to a defined shift away from a healthy virome, and IBD-specific shifts in phages may reflect the expansion of pathobionts associated with disease or the induction of conditions that favor a lytic lifestyle.

Human disease provides an opportunity to study not only how the virome shifts in response to a perturbation but also how viruses can play an active role in facilitating and exacerbating bacterial infections. For example, the highly unique environment of the cystic fibrosis (CF) lung drives selection and rapid evolution of bacterial pathogens and their phages through various ecological pressures. *P. aeruginosa* is the most common pathogen of the CF lung, and the density of this bacterium is positively correlated with densities of its phages (579); the phages play a role in the ability of *P. aeruginosa* to survive and thrive within the CF lung, highlighting the importance of the human phageome during bacterial infection (579). A subset of phages, known as LES phages due to their identification in the Liverpool epidemic strain of *P. aeruginosa*, have been shown to integrate as prophages and confer fitness advantages to their hosts. Isolation of a polylysogenic strain of *P. aeruginosa* (LESB58) from a CF patient allowed researchers to determine the fitness advantages associated with the various prophages

through competition assays in a rat lung infection model; by using signature-tagged mutagenesis of 3 of the prophages in the LESB58 genome, they found that a disruption of these prophage genes greatly reduced the competitiveness of strains *in vivo* (580). Moreover, growth of *P. aeruginosa* in the presence of lysogenic LES phages in an artificial sputum medium led to the development of mutations promoting fitness, frequently through prophage integration into genes such as those coding for type IV pilus machinery (581). This was in agreement with previous studies which showed that mutations in type IV pilus-associated genes increased the fitness of *P. aeruginosa* in the murine lung, potentially by providing immunity to superinfection by other phages that infect via this molecule (582).

Additionally, *P. aeruginosa* can utilize filamentous bacteriophages during the biofilm life cycle. For example, phages produced by the bacteria can associate with polymers to create a "liquid crystal" extracellular matrix that enhances adhesiveness and protection from desiccation and antibiotics (583). Counterintuitively, these phages can also contribute to the biofilm by inducing cell death in certain populations, specifically those at the center of microcolonies; this may serve as a form of directed cell death promoting the dispersal of small-colony-variant cells from the biofilm (584, 585). In fact, a strain of *P. aeruginosa* lacking the filamentous phage Pf4 formed abnormal and unstable biofilms, and mice infected with this strain survived longer that those infected with the wild-type counterpart with an intact phage (585). Phage production may be linked to the anaerobic growth mode of *P. aeruginosa* biofilms *in vivo*, as prophage genes were found to be strongly upregulated during anaerobic respiration of *P. aeruginosa* (586).

Phages can also play a role in facilitating the pathogenicity of ordinarily commensal bacteria by providing novel routes for infection. The bacterium *Streptococcus mitis* is typically an oral commensal in humans, but it can also cause infective endocarditis. Two proteins encoded by lysogenic phage SM1 play a direct role in the binding of *S. mitis* to platelets in the bloodstream, leading to endocarditis (587, 588). The phage proteins PblA and PblB attach to the cell wall of *S. mitis*, where they mediate binding of the bacteria to platelets; the enhanced platelet binding then increases the pathogenicity of *S. mitis* in an endocarditis mouse model (588). Viral metagenomic and 16S rRNA gene analyses have revealed that both the phage and its host are highly prevalent within the human oral cavity, and SM1 can be induced by dietary items such as soy sauce, leading to increased levels of PblA and PblB (589, 590).

Similarly, integrated prophages can confer traits that increase the virulence of many pathogenic bacteria. Pathogenic strains of *Escherichia*, *Bacillus*, *Pseudomonas*, and *Burkholderia* all contain greater numbers of prophages within their genomes than their benign relatives of the same species (591). In pathogenic *E. coli* strains, several phages, including Stx, Stx2, λ, and CP-933C, encode virulence factors and toxins (592–594). The human pathogen *Vibrio cholerae* derives its virulence from two phage components, CTX and TCP, which arise from the phages CTXφ and VPIφ (595–597). However, sometimes phages can confer traits to bacterial symbionts that are beneficial to the host. One striking example is the tripartite interaction between the eukaryotic pea aphid *Acyrthosiphon pisum*, its hemocoel-dwelling bacterial symbiont *Hamiltonella defensa*, and the bacteriophage APSE-3. This aphid is often parasitized by the wasp *Aphidius ervi*, which deposits eggs within the aphid hemocoel that kill the host when they pupate (598). However, *H. defensa* can prevent development of the wasp eggs, sparing the aphid host, but only when it contains the APSE-3 prophage, which contains the gene for the toxin required to kill the wasp eggs (599).

Although phages make significant contributions to the pathogenicity of many bacteria, there is mounting evidence that the host eukaryotic virome may provide protection from bacterial infection through activation of the host immune system and direct interaction with pathobionts (600). Mouse models have been used to demonstrate the ability of latent murine herpesvirus (MHV68) or cytomegalovirus (CMV) infection to activate macrophages and increase basal immune function in a manner that provides protection from bacterial challenges with *Listeria monocytogenes* and

Yersinia pestis (Fig. 4) (601). The activation of basal immune function to protect against bacterial pathogens was further examined by MacDuff et al. Their work showed that in immunodeficient mice, chronic MHV68 infection increased basal levels of gamma interferon (IFN-γ) and its effector molecules, allowing for rapid clearance of *L. monocytogenes* (602). Additionally, murine norovirus (MNV) infection was found to restore intestinal physiology and immune function in germfree or antibiotic-treated mice in an IFN-α-dependent manner, allowing the MNV-infected mice to cope with challenges from chemically induced intestinal injury and *C. rodentium* infection (603). This work highlights the ability of the mammalian virome to maintain intestinal health through activation of the host immune system (Fig. 4).

It has also been postulated that the virome can protect the host from bacterial infection by serving a barrier function. Phage concentrations are increased in the mucus layer of humans, mice, and marine invertebrates, which results from a weak adherence between Ig-like domains of phage capsids and the glycan residues of mucin. This layer then provides a site for lytic phage infection of bacteria, which protects the underlying epithelial cells. In an *in vitro* model, the presence of phage increased bacterial cell death and decreased epithelial cell mortality in a mucus-dependent manner (604). Further research into this model suggests that the interaction with mucin reduces diffusive motion, increasing the number of encounters with potential bacterial prey on the mucus (605). Together, the results present a model for phage-driven, non-host-derived immunity of mucosal surfaces termed by those authors the "bacteriophage adherence to mucus" (BAM) model (Fig. 4) (604–606).

The growing body of research on the virome during health and disease states is transforming our understanding about the role of viruses in the human body. Studying viral community changes during IBD has led to insights into a potential role for phages during gut dysbiosis and the possibility of identifying specific phages as biomarkers of disease. Furthermore, it is commonly observed that phages can facilitate the virulence of bacterial infections, but the role of eukaryotic viruses and phages as potential players in host immunity is now being appreciated.

Horizontal Gene Transfer and Antibiotic Resistance in the Microbiome

Horizontal gene transfer (HGT) represents a route for the dissemination of genes between bacteria, including genes coding for favorable fitness-related traits, virulence factors, and antibiotic resistance. There are several ways in which phages are involved in HGT in the microbiome: transduction, by which phage mediates the transfer of bacterial DNA from one bacterium to another; specialized transduction, where prophage excision involves packaging of both the prophage and adjacent host genes; and lysogenic conversion, during which a prophage causes a phenotypic change in the host cell (607). Analyses of HGT events across metagenomic data sets have demonstrated that human-associated bacteria exhibit far more gene transfer than those bacteria not associated with humans. After examining gene transfer events in different ecological niches, Smillie et al. suggested that HGT is enriched in bacterial communities inhabiting specific body sites, such as the gut (608). The rich density of bacteria and phage within the human microbiome makes it a potential hot spot for phage-mediated HGT of virulence and antibiotic resistance genes (ARG).

Antibiotic resistance genes are present in bacterial populations within the human intestinal microbiome as well as the human gut virome (609, 610). *In vitro* studies of commensal and pathogenic bacteria have found that many species are capable of phage-mediated gene transfer (595, 611–614). While HGT is normally limited to transfer within a species, some phages are capable of transferring virulence and antibiotic resistance genes between bacterial species and even genera (Fig. 4) (612, 615). For example, strains of *Lactobacillus gasseri* contain multiple integrated prophages, which can transduce both other strains of *L. gasseri* and strains of *L. acidophilus* (611). This expands the possibilities for the transfer of genes to opportunistic pathogens residing within the human microbiome, resulting in more-challenging bacterial infections. The transfer of virulence-, resistance-, and fitness-enhancing genes via phage has also been

shown to occur in hosts infected with *S. aureus*, *E. coli*, *P. aeruginosa*, and *S. enterica* serovar Typhimurium (568, 579, 616). In one example in a moth larva model, *S. aureus* phage φ11 was released from a subpopulation of lysogenized cells, and these phages then infected neighboring susceptible bacteria that contained a resistance gene. These newly infected bacteria underwent cell lysis, and some of the resulting φ11 phage particles packaged the resistance gene of the lysed neighbors and transduced members of the lysogenic population, thus providing them with novel resistance (616). Those authors termed this process "autotransduction" and postulated that it may increase the fitness of a bacterial population through the acquisition of beneficial genes of neighboring populations (616).

Many phages carrying virulence and resistance genes reside in bacteria as prophages. Some species of bacteria carry prophages that can undergo spontaneous induction into lytic growth; for example, *L. gasseri* contains phage that can undergo spontaneous induction at fairly high levels, which could contribute to HGT in human gastrointestinal and vaginal microbiota (611). However, in many species, induction of prophages to excise from their host and infect other bacteria occurs through environmental or host triggers, including UV radiation, antibiotics, hydrogen peroxide, ROS, and, more broadly, SOS response activation (Fig. 4) (568, 617–619). Recent work has shown that inflammation-induced production of ROS triggers the SOS response, leading to subsequent prophage induction and HGT of a phage-encoded virulence factor (568). Within the mouse gut, *S. enterica* serovar Typhimurium triggers inflammation, which leads to the production of ROS and RNS; these trigger the *S. enterica* SOS response, in turn activating the *tum* antirepressor of prophage induction, thus allowing for the lytic induction of the SopEφ prophage. The new phage progeny then infect naive *S. enterica* and through lysogenic conversion transfer the phage-encoded virulence factor SpoE (568). Therefore, the host inflammatory response to bacterial infection may contribute to horizontal gene transfer by phages in the microbiome (Fig. 4).

Antibiotics are powerful therapeutic tools that have profound effects on the human microbiome, including the induction of prophages leading to HGT and the potential spread of antibiotic resistance (Fig. 4) (620, 621). For example, the Stx-encoding prophages in pathogenic Shiga toxin-producing *E. coli* (STEC) were shown to be induced by treatment with the fluoroquinolone antibiotic ciprofloxacin (622). In mice, ciprofloxacin triggered not only prophage induction but also transmission of Stx phage to naive bacteria. Ciprofloxacin treatment increased Stx transductants and Stx production, resulting in more-severe STEC infection and ultimately a significant increase in mortality of the mice (622). This represents one of the challenges of treating enteric infections, as antibiotics can exacerbate ongoing infections by inducing prophages and spreading virulence. Later experiments in the murine intestine showed that antibiotic treatment can increase potential phage-bacterium interactions within the microbiome as well as enrich the enteric population of phages for carriage of traits, including resistance to the antibiotic used as well as to other antibiotics (621). Naive bacteria that were infected *ex vivo* with phages from antibiotic-treated mice obtained higher levels of resistance than phages from non-antibiotic-treated mice, suggesting that antibiotic resistance in the intestinal phageome can be enriched by drug treatment and has the potential to provide functional benefits to members of the microbiome through HGT (621). However, it is possible that the contribution of phages to ARG transfer is somewhat overestimated due to use of lax metagenomic data annotation for ARG databases, bacterial DNA contamination, and unverified resistance annotations (623).

There is no doubt that phages play a role in the mobilization of virulence and resistance genes through HGT. Through transduction and lysogenic conversion, phages are able to disseminate genes and modulate the functional capacity of the microbiome. Intriguing research suggests a role for phages in liberating DNA for the process of natural transformation through bacterial lysis, further implicating the role of phages in HGT (624). Factors including antibiotics and host inflammation have also been shown to impact phage-bacterium interactions within the context of the microbiome (Fig. 4). Together, these findings reveal the importance of phage-mediated HGT within the

microbiome and its impact on host health. Analyzing metagenomic data sets for HGT events has been shown to be a powerful approach for understanding the dynamics of gene flow in the context of the human microbiome, and future studies should aim to utilize these analytical techniques to better understand the role of bacteriophage in HGT within the human microbiome (608, 625).

Phage Therapy: a Weapon against Antibiotic Resistance?

Since Félix Hubert d'Hérelle coined the term "bacteriophage" in 1917, researchers have been experimenting with utilizing phages for the treatment of bacterial infections in humans (626). In a summary of this early work, d'Hérelle articulated enthusiasm for the possibility of treating deadly bacterial infections such as *V. cholerae*, septicemic *Staphylococcus*, and *Y. pestis*. Concluding his report, d'Hérelle recognized that extensive research will be required to fully demonstrate the efficacy of phage therapy (626). Almost one century later, the field of phage therapy finds itself in a similar position: excited over the possibility of a novel treatment for bacterial pathogens but in need of more clinical trials in order to understand the efficacy and safety of bacteriophages as treatments. The discovery of antibiotics almost completely diverted attention away from phage therapy research in the United States, although bacteriophages have still been used and developed as therapies in Eastern Europe (627). However, the current antibiotic resistance crisis is weakening our antibiotic arsenal, and focusing more attention on the potential utilization of bacteriophages may be beneficial in clinical practice to combat multidrug-resistant (MDR) bacterial infections (628, 629). Recent studies have begun to evaluate the safety, efficacy, and possible role of phage therapy in treating bacterial infections.

While there are currently no FDA-approved bacteriophage treatments for bacterial infections in humans, there has been research into efficacy and safety in animal models as well as a limited number of cases involving humans. Animal models have shown efficacy when utilizing lytic bacteriophage treatments as prophylaxis or following symptoms for infections involving *K. pneumoniae*, *Enterococcus faecium*, *V. cholerae*, *E. coli*, *C. difficile*, *S. aureus*, and *P. aeruginosa* (Fig. 4) (630–636). In these studies, treatment with lytic phage showed positive health outcomes that could match or exceed those of traditional antibiotic therapies or enhance treatment efficacy when combined with antibiotics. Furthermore, research has shown that phage therapy can succeed in treating antibiotic-resistant *Enterococcus* infections in a mouse model, supporting its potential benefit in a world of increasing resistance to current drugs (631, 637).

While results from animal models have suggested that bacteriophages are able to successfully treat bacterial infections, there is far less evidence for phage-based treatments in humans. When used to treat acute *V. cholerae* infection in humans, bacteriophages displayed no clinical efficacy, compared to the traditional antibiotic therapy (tetracycline), which was successful in treating the infection (638). Additionally, more recent clinical applications of phage therapy were unsuccessful in treating *E. coli* gastrointestinal infections in humans while also highlighting the need for more highly targeted phage cocktails to be used in treatment (639). However, despite these failures, phage therapies are generally considered to be safe, and studies have reported no adverse effects related to phage treatment (640–643). Another benefit observed was that unlike antibiotics, which have a broad effect range and can cause intestinal dysbiosis, phage therapies are much more targeted and can preserve the integrity of the host microbiome (632, 642). Despite the lack of widespread use, in the United States there have been recent case reports in which experimental phage therapy was administered to patients as a last resort when infections were not responsive to antibiotic therapy. In these limited reports, administration of phage cocktails was associated with resolution of serious bacterial infections (644, 645). In the United Kingdom, a controlled study of patients with drug-resistant *P. aeruginosa* ear infections showed significant improvement in patients receiving bacteriophage therapy compared to placebo (641). While these cases showcase the potential power of phage therapies, controlled clinical studies are still required before such treatments become mainstream.

The use of bacteriophage therapy is now evolving beyond the idea of using lytic phages to kill bacteria. Instead, researchers are devising ways of using phages to sensitize bacteria to antibiotics in order to enhance their efficacy and circumvent certain mechanisms of resistance. Pioneering work by Lu and Collins utilized a genetically modified lysogenic phage of *E. coli* that was able to modulate antibiotic efficacy *in vivo* through the regulation of gene networks involved in antibiotic sensitivity (646). Researchers increased the lethality of several antibiotics by suppressing the bacterial SOS response through a phage-borne *lexA3* gene, thus opening the possibility of utilizing engineered lysogenic phages as adjuvants to increase antibiotic efficacy in treating bacterial infections (646). Although not intended for use *in vivo*, Edgar et al. proposed a method to resensitize bacteria to antibiotics using engineered phages: to combat drug resistance developed through mutations in bacterial genes, phages modified to contain the sensitive version of the resistance genes were used to lysogenize bacteria (647). In this case, a sensitive *gyrA* gene was introduced via phage to a *gyrA* mutant *E. coli* strain that was resistant to the quinolone nalidixic acid. Upon lysogeny with the phage carrying a sensitive *gyrA* gene, the *E. coli* strain became more susceptible to nalidixic acid (647). In a similar vein, a separate group used phages to deliver a CRISPR-Cas system to target and destroy antibiotic resistance plasmids within bacteria to increase the effectiveness of antibiotic therapies (648). Additionally, Chan et al. found that exposing MDR *P. aeruginosa* to both bacteriophage and antibiotics can help to overcome resistance mechanisms (649). Their MDR *P. aeruginosa* strain attained resistance through an efflux pump, which is the same system that the lytic phage OMK01 uses to bind to and infect the bacteria. Losing a component of the efflux pump in the bacteria made the bacteria susceptible to tetracycline but resistant to OMK01; when the efflux system was functional, the bacteria were tetracycline resistant but also sensitive to phages (649). This trade-off displays the potential use of combined antibiotic and phage therapy to target MDR bacteria in novel ways. Together, these findings represent a novel method for utilizing phage to enhance the efficacy of antibiotics for treating bacterial infections.

Although phage therapy provides a novel method of killing compared to that of antibiotics that may circumvent problems with MDR bacterial infections, bacteria also possess defenses against phages that present potential roadblocks to developing phage therapies. Bacteria can develop a number of resistance mechanisms against phages, including prevention of phage attachment and subsequent DNA injection, restriction-modification and CRISPR-Cas systems to destroy phage genomic DNA, and abortive infection by which an infected bacterium sacrifices itself to prevent phage replication and further infections. These systems, along with strategies employed by phages to circumvent bacterial defenses, are described in detail elsewhere (650–652). The results of research done in animal models as well as some intriguing case reports show promise for the future of phage therapy, although more controlled and targeted human trials are needed. Furthermore, utilizing phages as an engineerable tool to treat MDR infections could play an important role in combating the antibiotic resistance crisis.

Summary

Study of the virome is limited and mainly focused on the intestine and phages. Unlike bacterial and fungal studies, there is no universal marker or phage equivalent for the 16S rRNA gene or ITS markers. This makes characterization of viral communities difficult and heavily dependent on metagenomic sequencing, which is limited by the low number of viral sequences in databases; for example, there are 2,040 complete *Caudovirales* genomes in the NCBI database. Thus, a majority of sequences from these metagenomic studies cannot be classified and are from unknown viruses, the "viral dark matter." The recent identification of crAssphages as the most prevalent component of the human virome is an example of how much of this field remains to be explored and presents an opportunity to uncover the role of these bacteriophages in the context of the human microbiome. In addition, challenges remain in collecting viral DNA without

introducing contamination and in obtaining sufficient quantities for metagenomic analysis, and culture-based work is extremely limited due the unknown host range of most phages. In addition to taxonomic characterization, there is a substantial knowledge gap in understanding the interactions between the virome and the microbiome and how these interactions may impact human health and disease. Research has shown that gut dysbiosis can lead to a shift away from a core phageome and that phages are capable of increasing pathogen virulence and drug resistance through horizontal gene transfer. However, it also appears that the human virome may be able to help prevent bacterial infection and maintain health. The role of phages in the clinical setting has recently generated interest to help combat the growing threat of antibiotic resistance, leading to the development of new and creative strategies to potentially utilize phages in the treatment and prevention of MDR bacterial infections.

CONCLUSION

While dominated by bacteria, the microbiome is increasingly recognized as a rich community that includes archaea, fungi, viruses, and sometimes eukaryotic parasites. All of these players can impact the eukaryotic host as well as interact with each other in a variety of ways, ranging from symbiotic cooperation to antagonistic competition. As we recognize the importance of these interactions, we must utilize multi-omics approaches inclusive of the entire host-associated community, as looking at only certain communities in isolation limits the conclusions that can be drawn. For example, from one perspective, *Lactobacillus* species can be associated with host health, promoting immunotolerance toward the microbiota and colonization resistance against potential pathogens such as *Candida*; however, studies of helminths and viruses have revealed that *Lactobacillus* species may actually promote helminth infection or contribute to phage-mediated horizontal gene transfer, which can have negative impacts on the host. Moving forward, we encourage the increased use of metagenomics, metatranscriptomics, and metaproteomics to survey the full diversity of the microbiota, the interactions of its members, and its function in promoting health or contributing to disease states.

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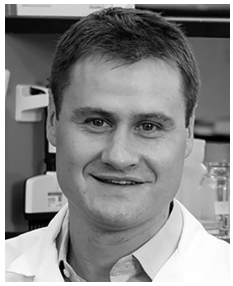

Peter Belenky received his B.Sc. in Biochemistry from Brandeis University and completed his Ph.D. in biochemistry at Dartmouth Medical School. Dr. Belenky conducted his postdoctoral training as an HHMI associate in the laboratory of Dr. James Collins at Boston University. Currently, he is an assistant professor in the Department of Molecular Microbiology and Immunology at Brown University. His research is focused on defining microbial responses to antimicrobials in the microbiome and on identifying how host factors can impact these responses. He is also interested in determining how host health and colonization with nonbacterial microorganisms influence the bacterial composition of the microbiome. His work is supported by the NIH as well as the DOD.