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REVIEW

Microbial Activities and Intestinal Homeostasis: A Delicate Balance Between Health and Disease

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SUMMARY

This review discusses the equilibrium between host and microbial community in the context of health and disease. The focus is on bi-directional pressures between prokaryotes and eukaryotic cells, as well as inter-bacterial interactions resulting in alterations to the microbiota.

The concept that the intestinal microbiota modulates numerous physiologic processes, including immune development and function, nutrition and metabolism, and pathogen exclusion, is relatively well established in the scientific community. The molecular mechanisms driving these various effects and the events leading to the establishment of a "healthy" microbiome are slowly emerging. This review brings into focus important aspects of microbial/host interactions in the intestine and discusses key molecular mechanisms controlling health and disease states. We discuss the evidence of how microbes interact with the host and one another and their impact on intestinal homeostasis. ***(Cell Mol Gastroenterol Hepatol 2015;1:28–40; http://dx.doi.org/10.1016/j.jcmgh.2014.11.004)***

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Appearances can be deceiving. Despite what you see in the mirror, humans are more prokaryotic than eukaryotic, as the bacteria in and on our bodies outnumber our own cells 10 to 1.[1] Microbes are embedded in our biological system and are deeply integrated in our daily life, and an emerging field of research has tackled the inter-kingdom communication network present in the walking mixed cultures we call people.

The gastrointestinal (GI) tract has the highest density and variety of bacteria in the human body (approximately 100 trillion microbes made up of >1000 species) due to the ideal growth conditions provided by this organ. In the colon, there are up to 10^{12} microbes per gram of luminal content which accounts for 60% of fecal weight.[2,3] A healthy adult's intestinal microbiome is diverse, relatively stable over time, and dominated by two phyla, Bacteroidetes and Firmicutes (~95%). Nevertheless, there is considerable interindividual variability at the species level due to genetic, environmental, and nutritional factors.[4] Diet in particular has been shown to rapidly shift this community,[5] resulting in geographic region-specific microbial signatures as seen in rural African children eating a fiber-rich diet compared to their European counterparts.[6] Although understanding the bacterial species present in the gastrointestinal tract is important, recent work has shifted the focus to the existence of a core enteric metabolome, which has the potential to change the way we look at how our gut functions.[7] Because bacterial genes have many overlapping and redundant functions, the end result of their combined metabolism and catabolism can ultimately have a tremendous impact on the intestinal environment and host physiology. As we discuss later, these microbial-derived products are a main component of the host-bacteria and bacteria-bacteria communication network essential to intestinal homeostasis.

External pressures, such as infection or antibiotics, cause a disequilibrium in the microbial community, a phenomenon designated as *dysbiosis* and often associated with pathologies including inflammatory bowel disease (IBD).[8,9] Alternatively, internal pressure resulting from defective host genetics, such as innate and adaptive immune genes, results in mismanagement of the microbiota, again leading to dysbiosis and pathologic conditions seen in the airway,[10] the skin,[11] and the gut.[12,13] IBD is a chronic, relapsing, and remitting inflammatory disease that can be classified as ulcerative colitis (UC) or Crohn's disease (CD). Both forms can be thought of as examples of disrupted communication between the intestinal microbiota and the host. The nature of this communication breakdown is not clear but involves genetic susceptibility related to the epithelial barrier and innate immunity, all of which are important components of host-bacteria interaction.[14]

This review will discuss the complex cross-talk between intestinal cells and the microbiota as well as the antagonistic and mutualistic interactions among enteric bacteria. This multifaceted communication network is what shapes the intestinal environment, drives homeostasis, and is thus essential to understanding how to prevent and treat intestinal disorders such as IBD.

Abbreviations used in this paper: **AMP, antimicrobial peptides; CD, Crohn's disease; CDI, contact-dependent growth inhibition; GI, gastrointestinal; HGT, horizontal gene transfer; IBD, inflammatory bowel disease; MAMP, microbe-associated molecular pattern; QS, quorum sensing; SCFA, short-chain fatty acids; SFB, segmented filamentous bacteria; T6SS, type VI secretion system; UC, ulcerative colitis.**

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Host Effects on the Microbiota

Gastrointestinal Tract Environment

The intestinal microbiota is largely acquired during our first few days of life, although recent studies have shown that infants may not be bacteria free at birth.[15,16] Species diversity and stability increase to an adultlike microbiota by the age of 3 years as children are exposed to a new milieu that includes solid food and individuals with different microbiota.[17–19] The wide topologic and geographic differences that microorganisms encounter along the GI tract dictate their environmental niche for growth and ultimately shape the community as a whole (Figure 1).

The acidity of the stomach limits bacterial growth and only a sparse microbiota ($<10^1$ bacteria/g of contents) is present.[20] This community predominantly consists of Firmicutes and Actinobacteria, but Bacteroidetes, Proteobacteria, and Fusobacteria are also present.[21,22] Although they are similar at the phylum level to the rest of the GI tract, the relative quantities of each organism differ, indicating that the gastric population is distinct.[23] When *Helicobacter pylori* are present, the stomach microbiota is dominated by this Proteobacterium, and diversity is severely reduced.[21,23,24] In the duodenum, stomach acid is neutralized, and bile acids become the driving force of microbiota modulation in the small intestine. For example, cholic acid has been shown to directly increase the cecal Firmicutes/Bacteroidetes ratio and decrease microbial diversity in vivo.[25] Here, diversity and bacterial load (10^3 bacteria/g) are low, but both factors

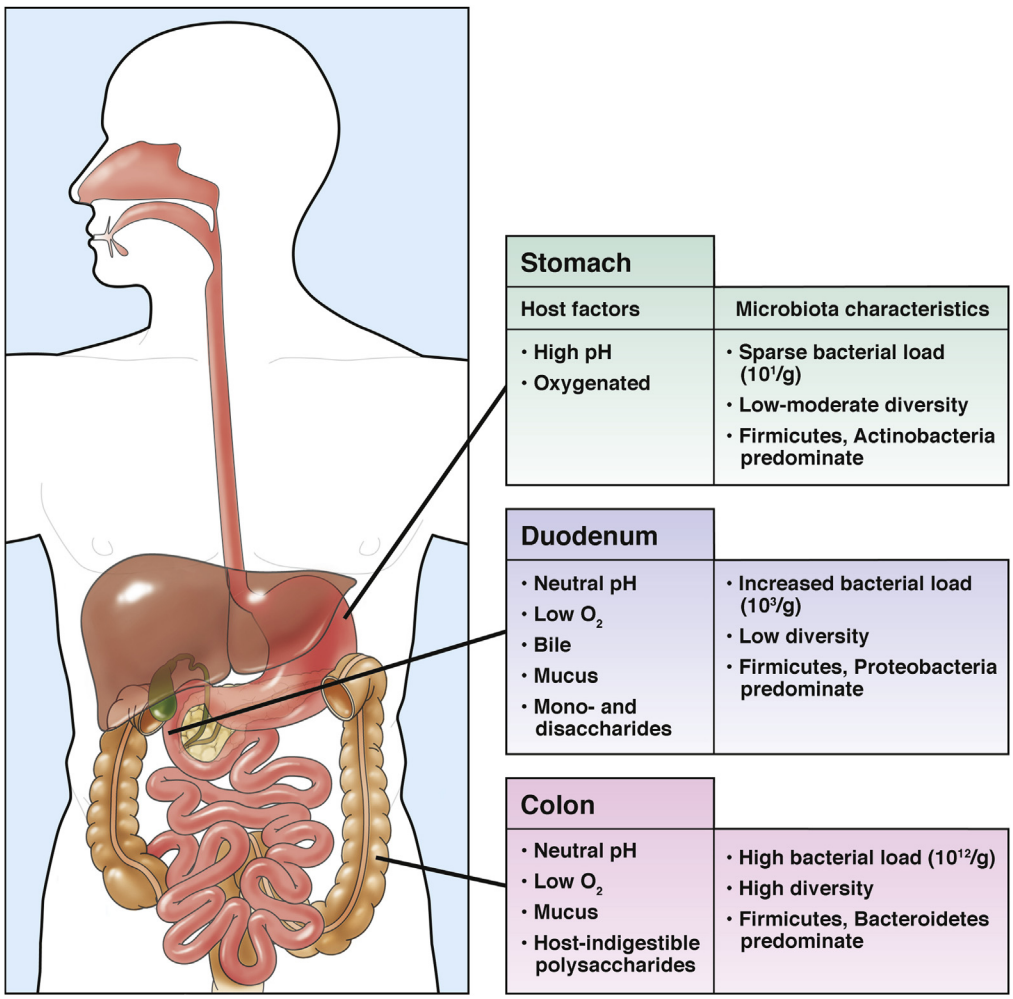

Figure 1. Regional differences in the gastrointestinal tract affect microbial niche. In the stomach, high pH and oxygen content restrict microbial colonization. The major phyla do not significantly differ from those found further along the GI tract, but the predominant bacteria (Firmicutes and Actinobacteria) as well as the species are distinct. Diversity is thought to be moderate, although this may be due to transient organisms. In the duodenum, the stomach acid is neutralized, bile is present, oxygen is reduced by facultative anaerobes, and the host epithelium produces a mucous layer. Most starches have been digested into monosaccharides and disaccharides, which are progressively absorbed throughout the small intestine. These factors result in a microbiota dominated by Proteobacteria and Firmicutes such as Lactobacillales with low bacterial load and diversity. Alternatively, host-indigestible polysaccharides promote the growth of Bacteroidetes and Firmicutes such as Clostridiales in the colon where diversity and bacterial load are high. In addition to these factors, the intestinal barrier and immune system affect which microbes can survive in the gut but do not appear to be region dependent.

increase along the length of the GI tract to the colon, which has 10^{12} bacteria/g.[20]

The radial oxygen gradient with the gastrointestinal tract, whereby oxygen diffuses inward from the epithelium into the mucous layer, creates a microaerophilic zone in which facultative anaerobes such as Proteobacteria thrive in an anaerobic lumen.[26] Although nutrient absorption decreases oxygenation of the mucosal layer by up to 40% due to increased metabolic demands,[27] this is unlikely to substantially affect the microbial community, as 4 days of hyperbaric oxygen treatment are needed to alter the microbiota.[28] However, survival in an atmosphere with fluctuating oxygen does require respiratory flexibility of commensal bacteria. Some strict anaerobes, such as *Bacteroides fragilis, Clostridium acetobutylicum, Faecalibacterium prausnitzii*, have developed molecular switches and alternate respiratory chains, including extracellular thiols and flavins, that allow them to tolerate low levels of oxygen over the short term and thereby achieve a selective growth advantage.[29–31]

In disease states, including these accompanied by intestinal inflammation, diminished vascular perfusion and heightened immune cell metabolic activity result in localized hypoxia or even anoxia within the intestines.[32] It may therefore be surprising that intestinal dysbiosis in IBD patients is characterized by an enrichment of aerobic bacteria including Enterobacteriaceae, which is also seen in experimental models of colitis (eg, *Il10*$^{-/-}$, *Tlr5*$^{-/-}$, TRUC).[33,34] This disparity is puzzling but may be explained by the fitness advantage of Enterobacteriaceae, which can use nitrate generated during inflammation as an alternative terminal electron acceptor.[35] Therefore, respiration has a fundamental influence on the commensal bacteria that are able to thrive in an altered gut milieu.

Dietary Components

Carbohydrates, proteins, and fats all have specific, profound effects on the composition of the intestinal microbiota and have been extensively reviewed elsewhere.[36–39] Notably, Bacteroidetes and Firmicutes such as Clostridiales are able to use host-indigestible polysaccharides and thus dominate in the colon, whereas Proteobacteria and Firmicutes such as Lactobacillales are found in the small intestine along with high levels of monosaccharides and disaccharides (Figure 1).[40] Moreover, dietary vitamins can directly affect bacterial growth, as some microbes such as *Ruminococcus bicirculans* cannot synthesize their own essential factors.[41] Dietary vitamins can also modulate the microbiota by promoting the fitness of *Bacteroides* species (vitamins B_{12}, C, and E) or by decreasing microbial numbers (vitamin A, in particular for segmented filamentous bacteria).[42–44] However, it is not entirely clear whether this is a direct effect on the bacteria or occurs indirectly via the host epithelium or immune system, as with vitamin D.[45] In addition to host nutrient requirements, Suez et al[46] recently demonstrated that indigestible artificial sweeteners directly increase Bacteroidales and decrease Clostridiales in mice and humans. This elegant study demonstrates that the intricate relationship between our gut microbiota and what we consume includes indigestible components and affects whole body health.

Intestinal Immune System and Barrier Function

The gut also plays a very active role in shaping the microbial community to protect the host from the high antigenic potential of bacterial and unwarranted immune stimulation. The dynamic, responsive epithelial cells provide multiple deterrents, including the formation of a mucous layer by goblet cells, secretion of antimicrobial peptides (AMP) by Paneth cells, intercellular tight junction complexes, and microbe-associated molecular pattern (MAMP) recognition systems.[47–49] Similarly, the underlying immune system is very adept at tolerating nonharmful bacteria while recognizing and responding to invading pathogens and opportunistic commensal organisms. Luminal sampling by tolerogenic immune cells in the lamina propria, secretory IgA in the mucous layer, and the complement network operate together to maintain intestinal homeostasis.[48–53] Computer-based mathematical modeling of such a complex interaction supports the theory that these defense systems, along with host-derived nutrients, work together to shape the composition of the microbiota and maintain its stability.[54] Although direct evidence of whether these systems impact microbial species present in the intestine is lacking, host defense is a critical factor in maintaining intestinal homeostasis.

Microbial Influences on the Host

Educating the Immune System

Tolerance of the normal gut microbiota is an absolutely vital element of enteric homeostasis, requiring an extensive network of regulatory immune cells including Tregs and tolerogenic dendritic cells.[55,56] The intestinal architecture of the uncolonized fetal or germ-free gut is immature, with reduced epithelial turnover, thin muscle wall and mucous layers, a decreased number of immune cells, and disorganized gut-associated lymphoid tissue.[57–59] Microbial colonization of mice enhances the intestinal barrier function via MAMP signaling and educates the immune system, resulting in maturation of the gut-associated lymphoid tissue into a tolerant phenotype to prevent unregulated inflammation (Figure 2).[2,60] However, there appears to be a window of opportunity for this codevelopment, as colonization of adult germ-free mice with healthy mouse microbiota does not induce the same protective effects as when germ-free mice are colonized as neonates.[61,62]

Segmented filamentous bacteria (SFB) are able to traverse the mucous layer and are found attached to the small intestinal epithelium of many vertebrates, including humans.[63] Although they are only present in low numbers, SFB are essential to the development of Th17 cells through epithelial cytokine production and dendritic cell processing,[64–66] and they are required for full immune system maturation in mice.[67] This is an important discovery, as aberrant Th17 signaling has been documented in IBD, although evidence that SFB have a similar function in humans is lacking.

Alternatively, *Bacteroides fragilis* express polysaccharide A, which profoundly influences the development of enteric

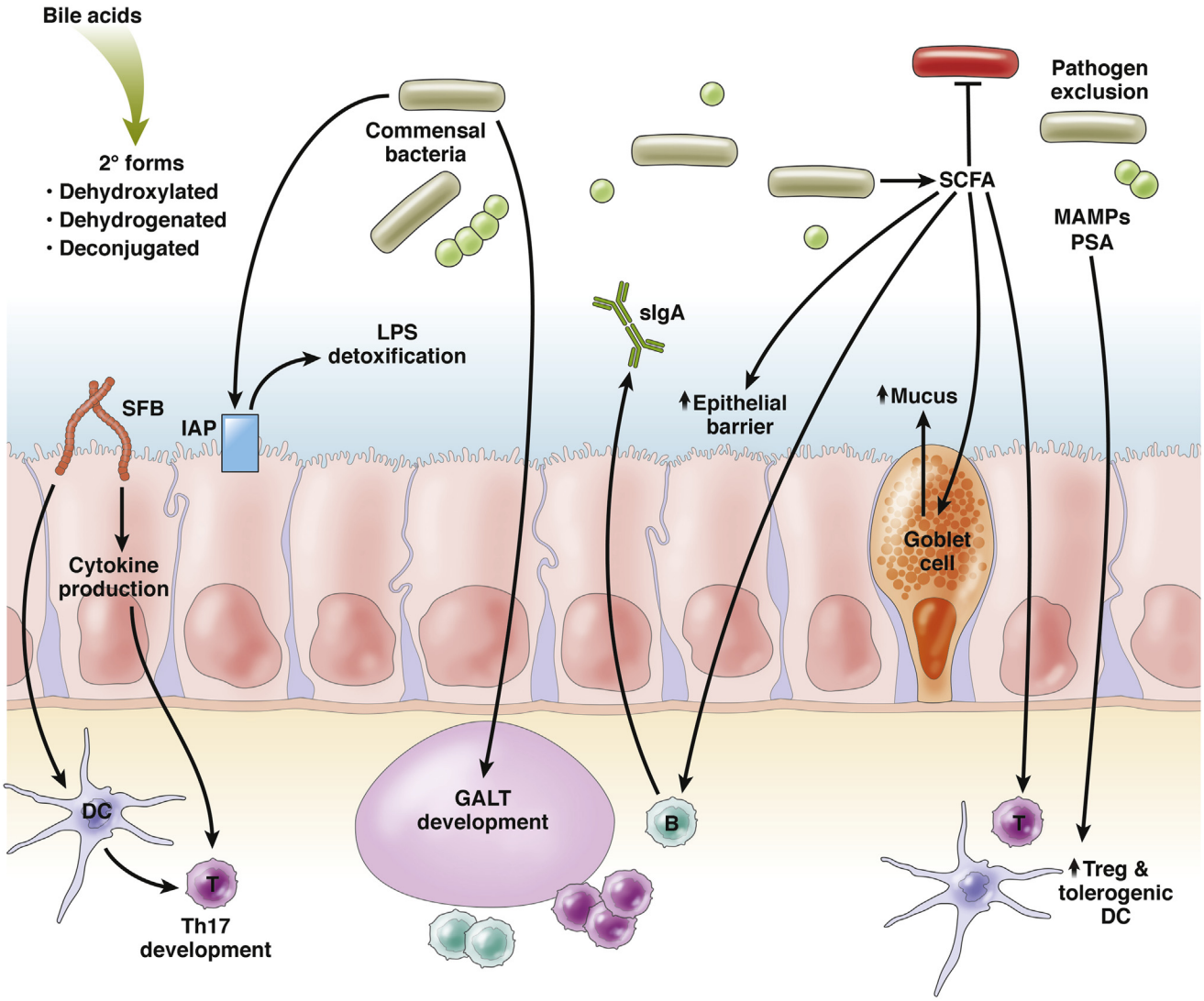

Figure 2. Microbial effects on the host. The microbiota induces host immune tolerance to commensal bacteria directly via a microbe-associated molecular pattern (MAMP) and polysaccharide (PSA) signaling, indirectly through the production of short-chain fatty acids (SCFA) and potentially through expression of epithelial intestinal alkaline phosphatase (IAP), which detoxifies luminal lipopolysaccharides (LPS). Furthermore, segmented filamentous bacteria (SFB) promote immune development of Th17 cells through epithelial cytokine production and antigen presentation by dendritic cells (DC), and the community as a whole is required for proper gut-associated lymphoid tissue (GALT) development. SCFA also induce IgA and mucus secretion into the lumen, promote epithelial barrier integrity, and prevent pathogen colonization. The microbiota also participates in the formation of the active, secondary forms of bile acids.

tolerance by converting proinflammatory $CD4^+$ T cells into Tregs during colonization[68,69] and mediates equilibrium between T_H1 and T_H2 responses.[70] Infants born by cesarean delivery have decreased microbial diversity, including delayed colonization by the *B fragilis* (Bacteroidetes phylum), which is associated with decreased T_H1 activation.[71] Similarly, strains of *Clostridium* clusters IV and XIVa (Firmicutes phylum) can promote differentiation, expansion, and colonic homing of Treg cells through bacterial antigen signaling and short-chain fatty acids (SCFA) stimulation of epithelial transforming growth factor-β1 production.[72] Thus, the far-reaching influences of the microbiota indicate the vital role of bacterial immunomodulation in maintaining whole-body health.

Maintaining Homeostasis

Once an appropriately tolerant milieu is established, maintenance of healthy host-microbial communication is paramount for the host, as dysbiosis jeopardizes protective microbial functions. A recent article by Zelante et al[73] establishes a role for microbial catabolism in balancing T-cell activation of the mucosal immune system. When converted from sugar to tryptophan as the primary energy source, the population of *Lactobacilli* expand and increase the production of indole-3-aldehyde. This metabolite induces interleukin-22 expression through activation of aryl hydrocarbon receptors, conferring both tolerance of the healthy microbiota and resistance to the opportunistic fungal pathogen *Candida albicans*.[73]

Beyond regulation of the host immune system, the microbiota influences many other normal functions of a healthy intestinal tract (Figure 2). For example, the microbiota convert bile acids into secondary forms in the lumen by dehydroxylation, dehydrogenation, and deconjugation.[74,75] Studies in germ-free mice demonstrate that bacterial signaling via G protein-coupled and nuclear receptors (G protein-coupled bile acid receptor [TGR5] and farnesoid X receptor [FXR], respectively) not only controls levels of secondary bile acids but also can modulate synthesis in the liver.[76] Moreover, expression of intestinal alkaline phosphatase, an epithelial-bound enzyme that detoxifies luminal lipopolysaccharide to alleviate inflammation and promote tolerance,[77] is affected by diet and antibiotics. It is tempting to speculate that such modulation could occur via changes in the microbiota.[77,78] Maintaining symbiosis with our bacteria is therefore key to preserving intestinal health and homeostasis.

The Extensive Effects of Fatty Acids

Fermentation, one of the key metabolic pathways employed by the enteric microbiota, produces SCFA, including acetate, propionate, and butyrate.[79] SCFA have been shown to promote homeostatic mechanisms and protect against inflammation in multiple models.[80] This topic has been thoroughly reviewed elsewhere.[60,75,81–83] Briefly, SCFA stimulate protective mucus and IgA production, promote tolerance via Treg induction, inhibit the inflammatory mediator nuclear factor κB, enhance epithelial barrier integrity and repair, and promote competitive exclusion of pathogens (Figure 2). However, butyrate has also been shown to be both protective and deleterious in different models of colorectal cancer, highlighting the complexity of SCFA biology with a disease-specific effect.[80,84] Thus, even predominantly beneficial molecules or microbes can potentially have negative effects in complex environments such as the GI tract.

Microbe-Microbe Interactions

In addition to their host-mediated effect on microbial assembly, composition, and activities, microbial niches are also under intense pressure from other surrounding bacteria. Bacteria use sophisticated intercommunication systems to help maintain their niches; consequently, this microbial network is essential to host homeostasis. These microbial relationships can be antagonistic or mutualistic, depending on the nature of the species (Figure 3). Commensal bacteria combat other microbes using AMP production and targeted attacks by means of specialized secretion systems, and they

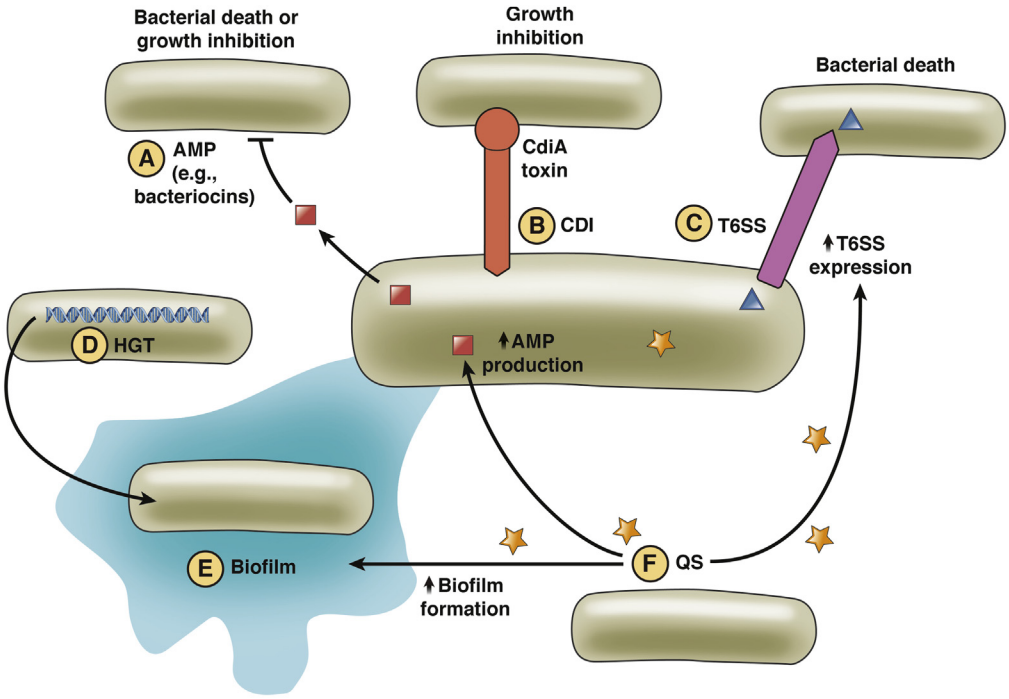

Figure 3. Bacteria use a complex communication network to thrive in an environment. Bacteria interact through combative (*A–C*) and cooperative (*D–F*) methods. (*A*) Antimicrobial peptides (AMP) typically released after cell lysis either kill or inhibit growth of surrounding microbes. (*B*) Contact-dependent growth inhibition (CDI) systems deliver the toxic C-terminal end (CdiA) into the cytoplasm of target cells after contact. (*C*) The type 6 secretion system (T6SS) forcefully injects toxins into attacking bacteria. Bacteria cooperate by increasing survival of similar microbes in their vicinity with (*D*) horizontal gene transfer (HGT) and (*E*) the formation of a protective biofilm. (*F*) Quorum sensing (QS) allows bacteria to talk and coordinate group behavior, and has been implicated in T6SS expression, production of AMP, and biofilm formation. However, several of these mechanisms have not been observed in the healthy human intestinal tract. Red squares represent AMP; blue triangles are T6SS toxin; and yellow stars are QS signaling molecules.

compete for nutrients. Owing to their ruthless requirements for survival, bacteria have also developed cooperative mechanisms such as horizontal gene transfer, biofilm formation, and quorum sensing to ensure the fitness of their own community as a whole.

Combatting

Bacteria express highly potent bacteriocins, microcins, and colicins that fend off other species or pathogens invading their niche without causing collateral damage to eukaryotic cells.[85] Bacteriocins are pore-forming AMP produced by Gram-positive bacteria; they directly relate to the in vivo fitness and competitiveness of *Lactobacillus salivarius* and *Streptococcus pneumoniae* in the gut and nasopharynx, respectively.[86,87] Microcins (<10 kDa) and colicins (30–80 kDa) are produced by Gram-negative bacteria, and they display a wider variety of antimicrobial tactics than bacteriocins, including cell wall pore formation, inhibition of RNA polymerase or tRNA synthetase, nuclease activity, and interference with cell wall synthesis.[88,89]

The degree to which the microbiota is affected by these microbial products depends on the AMP employed. Some have a minimal or subtle influence in various animal models, but others have a profound impact, such as the broad-spectrum lacticin 3147, which drastically reduces Bacteroidetes and Firmicutes and increases Proteobacteria.[90–92] As a similar microbial shift is also seen in IBD,[93,94] one might speculate that bacteriocin overproduction could foster an IBD-like dysbiosis. Interestingly, mice colonized with the bacteriocin-producing probiotic *L salivarius* UCC118 Bac(+) displayed a relative increase in Bacteroidetes and Proteobacteria compared with control mice, although no sign of intestine inflammation was reported in these mice.[95] The importance of bacterial resistance to AMP for survival in the gut is shown in certain pathogens such as *Francisella tularensis* and *H pylori*. These pathogens rely on the phosphatase LpxF to catalyze the removal of the negatively charged 4′-phosphate group from the lipid A backbone, a feature that confers a high resistance level to various AMP.[96,97] Whether a similar system is present in commensal bacteria is currently unknown.

Bacterial-derived AMPs integrate environmental cues and bacterial communication in order to modulate the microbiota composition. Expression of colicin, for example, modulates dynamics within a simple community of three *Escherichia coli* species in vitro and depends on nutrient availability, as the carbon-storage regulator A inhibits lysis-mediated colicin release.[98] Moreover, the colicin produced by the pathogen *Salmonella* Typhimurium is up-regulated under iron-limiting and inflammatory conditions, allowing this bacterium to outcompete other members of the Enterobacteriaceae family during infection.[99]

However, AMP production is costly energywise, and it often requires cell lysis for release. Therefore, bacteria have evolved other methods for toxicity, such as contact-dependent growth inhibition (CDI) systems or type VI secretion systems (T6SS).[88] CDI systems are expressed by a wide variety of Gram-negative and Gram-positive bacteria, both pathogens and commensals.[100–102] Upon contact with another bacterium, the toxic C-terminal end of the cell surface CdiA protein is cleaved and translocated into the target cell where most of these family members exert nuclease activity.[103] Expression of cognate, but not heterologous, CDI proteins can prevent growth inhibition in related species by binding the toxin, thus leading to kin selection.[100] This type of poison-antidote tactic has been shown to be both cooperative and combative in mathematical modeling, and it likely allows for localized regulation of the dynamic microbiota.[104] Interestingly, the CDI system of *Burkholderia thailandensis* E264 is required for formation of cooperative biofilm communities in vitro but acts independently of its antibacterial activity.[105,106]

Conversely, T6SS systems are exclusively found in Gram-negative bacteria and work as contractile nanomachines for injecting toxins into target cells.[107] Remarkably, one group demonstrated that *Pseudomonas aeruginosa* respond defensively after being ambushed by another T6SS or a type IV secretion system in what the investigators termed a tit-for-tat assault, which allows them to distinguish between aggressive and bystander T6SS-negative bacteria in vitro.[108,109] As T6SS only target other Gram-negative bacteria or eukaryotic cells, it follows that these injectors contribute to pathogenesis and interbacterial competition.[110–113] However, only recently have T6SS been shown to aid in colonization of mixed bacterial communities by conferring a competitive advantage to *Vibrio cholera* (an intestinal pathogen) and *Agrobacterium tumefaciens* (Proteobacteria that infect plants).[114,115] Similar to CDI systems, T6SS have also been implicated in the formation and maintenance of biofilms,[116,117] demonstrating their versatility and potential influence on the intestinal microbiota.

Alternatively, pathogens can use T6SS to cause virulence. For example, *Salmonella* Typhimurium requires T6SS expression for full intracellular replication and pathogenesis in mice.[118,119] Similarly, a clinical isolate of the diarrheagenic *Aeromonas hydrophila* requires functional T6SS for virulence.[120] In contrast, the T6SS of enteroaggregative *E coli*, an infectious diarrheal agent associated with IBD, does not appear to contribute to its virulence in a mouse model.[116]

Competing

Carbohydrate utilization is an essential colonization factor and modulates the dynamics of the microbiota.[121] This is the fundamental concept behind nutrient niches in the gut and is best demonstrated by the immediate (albeit often transient) dysbiosis that inevitably follows antibiotic therapy.[9] Antibiotics eliminate a group of bacteria and thus their nutrient source becomes available. This can allow new groups of potentially pathogenic bacteria to thrive on the previously unavailable nutrient source and, potentially, expand to dangerous levels. This was elegantly shown by Ng et al[122] as the postantibiotic expansion of both *Salmonella* Typhimurium and *Clostridium difficile* in vivo depended on their ability to use sialic acid. This monosaccharide is

catabolized by the microbiota but is not normally found in the feces, as it is quickly consumed by other bacteria. After antibiotic treatment, there is a large spike in sialic acid availability, which returns to baseline 3 days after treatment as the microbiota is reestablished. These pathogens exploit the vacant nutrient niche to establish themselves in the intestinal tract and initiate inflammation.[122]

Theoretically, a pathobiont could also take advantage of increased sugar access to expand and cause pathology, similar to how *Bilophila wadsworthia* blooms when supplied with the bile acid taurocholic acid for sulfite reduction.[123] Inflammatory responses can further optimize the milieu for invading species by providing an alternative method of respiration, potentiating horizontal gene transfer of virulence factors such as colicins and allowing the pathogen to outcompete commensal bacteria.[35,99,124,125]

Beyond fighting for sugars as their main carbon source, intestinal microbes also exhibit fierce competition for nitrogen, phosphorus, trace elements, vitamins, and other essential cofactors.[126,127] For instance, bacteria encode a wide variety of transporters for vitamin B_{12}, which indicates the necessity of these factors for survival.[43] Moreover, the availability of iron, zinc, and selenium have each been shown to modulate the microbiota in vivo, theoretically by allowing the expansion of bacteria that are more efficient at competing for those trace elements.[128–131] In fact, *Salmonella* Typhimurium infection causes up-regulation of intestinal epithelial–derived lipocalin-2, an AMP that interferes with bacterial iron uptake. Sensitive bacteria are thus outcompeted by the pathogen because *Salmonella* Typhimurium is resistant to lipocalin-2 action.[132] Interestingly, the probiotic *E coli* Nissle 1917 can reduce the *Salmonella* Typhimurium burden after chronic infection has been established, as it has multiple lipocalin-2–resistant iron uptake systems and can outcompete the pathogen for iron.[133]

The ability of bacteria to metabolize host carbohydrates and bile acids was recently shown to be critical during mouse intestinal colonization, independent of the source of the microbes.[134] Thus, nutrient competition is a key feature of the microbial community dynamic and may play a role in intestinal pathologies that exhibit nutrient deficiencies. For example, IBD is associated with deficiencies in many micronutrient levels, including zinc, vitamin A, and iron, which could potentially influence the underlying dysbiosis and microbial activities.[135,136]

Cooperating and Communicating

Despite the cutthroat enteric battlefield, commensal bacteria also share byproducts rather than competing with their neighbors for the same food source. Intricate resource networks have evolved within the microbiota employing this waste not, want not attitude, and they form the most basic method of bacterial communication via detection of neighboring microbes' substrates. These networks are beyond the scope of this article, but they have been extensively reviewed elsewhere.[40,137]

Another method by which bacterial cooperation occurs is horizontal gene transfer (HGT). Specifically, HGT of the secretome is overrepresented in the intestinal microbiota, as these molecules promote cooperation and social behavior through the production of public goods.[138,139] One well-established example where HGT promotes cooperation is in the production of siderophores; iron chelators secreted to scavenge this essential element.[140] Iron bound by siderophores can be taken up by any bacterium in the vicinity, whether or not it expended the energy to create the chelator. Thus, cheaters arise in these communities, who do not express public goods but benefit from those who do, which hinders cooperation.[141] In limiting conditions, HGT increases the relatedness of cheaters and thus enhances microbiota stability by preventing the fitness advantage of cheating.[142,143]

Secretion of an extracellular matrix, or biofilm, binds bacteria together and protects the bacteria from outside attacks, such as antibiotics or host immunity, while also smothering competing microbes.[144–146] Biofilms aid in the fitness of a bacterial population as a whole by decreasing the diffusion of public goods through the viscous medium and thus preventing others from acquiring them.[147] This function is important as biofilms are especially vulnerable to cheaters, where any reduction in biofilm activity results in a thinning of the biofilm matrix and increased susceptibility to antimicrobials.[148] These structures are not regularly found in the bowels of healthy humans, but are thick, dense, and adherent in untreated IBD patients.[149] This could be related to the dysbiosis that occurs with IBD and may also explain why many patients are refractory to antibiotic treatment despite the apparent microbial involvement in disease.

Tying many of these cooperative pathways together is quorum sensing (QS), the primary method by which bacteria communicate cell to cell and coordinate group behavior. Bacterial signaling molecules are released into the environment and initiate gene regulation once they reach a threshold concentration (typically in the picomolar range), thus providing a means of functionally measuring bacteria density.[150] QS influences many survival mechanisms important to intestinal bacteria, including T6SS expression,[151] antibiotic production,[126] and biofilm formation.[150,152] Cheaters exist within the QS world, and these bacteria benefit from the protective signaling from other members of the same species without expending the energy to produce QS.[153] Similarly, some Gram-negative bacteria eavesdrop by only expressing the QS response transcription factor and not the signaling molecule, which could allow commensal bacteria such as *E coli* to sense the presence of invading pathogens.[154] Alternatively, some bacteria, including multiple Proteobacteria species such as *Klebsiella pneumoniae* and *P aeruginosa*, and even mammalian cells, quench QS by degrading the signaling molecules to prevent competitive colonization.[155,156]

As most studies have examined pathogens, it is not entirely clear whether human commensal bacteria use QS to communicate despite intense speculation.[157] However, QS signaling has been identified in the probiotics *E coli* Nissle 1917 and *Lactobacillus* species and may be involved in their beneficial effects.[158–160] It is likely that bacterial communication is an important component of microbial community assembly, composition, and activities. Further work is

needed to establish the extent to which the various forms of microbial communication are implicated in homeostasis and diseases.

Conclusions and Perspective

The human gut is a dynamic environment where eukaryotic and a multitude of prokaryotic cells establish a complex network of communication that ultimately benefits both parties. It has become clear that disrupting this productive network has dire consequences for the host and may contribute to intestinal pathologies, including IBD and colorectal cancer, as well as extraintestinal disorders such as diabetes and cardiovascular and liver diseases.[2]

Although significant progress has been made in identifying host factors implicated in the disruption of the intestinal microbiota and associated pathologic consequences, a paucity of information is available about the functional consequences of commensal microbial communication. Genetic manipulation of genes implicated in these processes (eg, QS and biofilm formation in IBD) might allow dissection of the roles of bacterial communication during health and disease. Further advances will require the field of microbe-microbe communication to move from the test tube and into the animal, as many of the fascinating interactions in vitro have not been examined in vivo. Thus, a large piece of the health puzzle remains missing.

Although the overwhelming majority of genes (99.1%) examined by metagenomic sequencing of the intestinal tract are bacterial in origin,[1] the viruses, fungi, and archaea present may influence specific host response and intestinal homeostasis.[161,162] For example, susceptibility to enteric viral infection is modulated by bacteria through enhanced viral replication.[163] Bacteriophages in particular are an intriguing area of research as they can enhance bacterial fitness in vivo and alter its colonization.[164,165]

Although there is value in defining which bacterial species are present in the gut, the field is moving toward regarding the enteric microbial community as an organ, rather than as individual parts. For example, does a global "healthy microbiota" exist, and is it associated with a defined metabolomic function? Because microbial metabolism is intrinsically linked to the host, a holistic approach will be necessary to dissect this complex relationship. Such an approach has been successful in defining the relationship between diet, microbes, and cardiovascular diseases.[166] Although tremendous progress has been made in the field of microbiome research, especially regarding intestinal microbiome and IBD, key elements of the dialogues are still missing, and this hinders the generation of novel therapeutic modalities. Replacing an altered microbiota has been validated as a therapeutic alternative to recurrent cases of *C difficile* infection,[167] but it is less clear whether IBD would be a logical candidate for this treatment.[168] In addition, because of the role of host genetics in IBD, it is not clear how stable and functional a transplanted microbe would be. Consequently, a targeted manipulation of microbial activities may represent a better approach to disease treatment and management. Clearly, more research is needed to identify the microbial activities implicated in health and disease and to harness their full potential for therapeutic purposes.

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Conflicts of interest
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