Gastrointestinal Microbiota–Mediated Control of Enteric Pathogens

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Abstract

The gastrointestinal (GI) microbiota is a complex community of microorganisms residing within the mammalian gastrointestinal tract. The GI microbiota is vital to the development of the host immune system and plays a crucial role in human health and disease. The composition of the GI microbiota differs immensely among individuals yet specific shifts in composition and diversity have been linked to inflammatory bowel disease, obesity, atopy, and susceptibility to infection. In this review, we describe the GI microbiota and its role in enteric diseases caused by pathogenic Escherichia coli, Salmonella enterica, and Clostridium difficile. We discuss the central role of the GI microbiota in protective immunity, resistance to enteric pathogens, and resolution of enteric colitis.

Keywords

intestinal microbiota, colonization resistance, Salmonella enterica, Escherichia coli, Clostridium difficile
THE GASTROINTESTINAL MICROBIOTA: DEFINITION AND COMPOSITION

The human gastrointestinal tract (GIT) is home to a complex microbial community with as many as 100 trillion prokaryotic members (119). Although mammals are colonized by many single-cell organisms at various anatomical locations, the GIT harbors the highest number and density of bacteria (67). This complex ecosystem is initially acquired during and for some time after birth. Although the GIT composition varies during the first year of life, at around one year of age the GIT microbiota of an individual becomes stable (87) and remains largely unchanged unless perturbed by antimicrobial treatment or disease (18).

The composition of the gastrointestinal (GI) microbiota is highly variable among individuals. Analysis of stool samples collected from healthy subjects showed that the vast majority of bacteria in the GIT belong to only four phyla: Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria (27, 30, 38, 65). At the genus level, the diversity is so high that no two stool samples (of more than 250 tested samples in the Human Microbiome Project) have the same composition (38). Within the GIT, microbial communities differ in the stomach, small intestine, cecum, and colon, with higher levels of bacterial diversity in the lower segments of the GIT and higher levels of aerobic and facultative anaerobic bacteria in the stomach and upper small intestine (30, 35). Additionally, the bacteria present in stool differ from those that are adherent to the GI mucosa (27).

Despite the high variability in GIT microbiota composition among individuals, metagenomic analysis reveals high levels of similarity between subjects when comparing various metabolic pathways within microbiota genes (38, 57), suggesting a high level of metabolic redundancy among different bacterial taxa.

IMPACT OF THE GASTROINTESTINAL TRACT MICROBIOTA ON THE IMMUNE SYSTEM

The Mammalian Immune System

The immune system is defined as the host’s defense against destructive forces from both outside (e.g., bacteria, viruses, parasites) and within (e.g., malignant and autoreactive cells) the body. Immune responses are generally classified as either innate or acquired. The components and cells that make up these two arms of the immune system are presented in Table 1.

The innate immune system provides immunity to invading organisms without the need for prior exposure to these antigens and includes physical barriers, such as the skin and mucous membranes; cell-mediated barriers, including phagocytic cells, inflammatory cells, dendritic cells, and natural killer cells; and soluble mediators, such as cytokines, complement proteins, and acute-phase proteins (24). This arm of the immune system provides the early phases of host defense that protect the organism during the four to five days it takes for lymphocytes to become activated.

The acquired, or adaptive, immune system develops over an individual’s lifetime. Lymphocytes are an important cellular component of this arm of the immune system that modulate the function of other immune cells or directly destroy cells infected with intracellular pathogens (Table 1). Each developing T or B cell generates a unique receptor, or recognition molecule, such that a set of cells expressing a vast array of diverse receptors is produced, allowing immune cells to selectively eliminate virtually any foreign antigen that enters the body (24). B cells, abundant in lymph nodes, recognize foreign antigen through membrane-bound antibodies, or immunoglobulins, and upon activation become antibody-secreting plasma cells to effectively remove soluble bacteria/antigens (24).
Table 1  The immune system

<table>
<thead>
<tr>
<th>Arm of immune system</th>
<th>Defenses</th>
<th>Components</th>
<th>Functions</th>
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<tbody>
<tr>
<td>Innate immune system</td>
<td>Physical barriers</td>
<td>Skin</td>
<td>Prevent the entry of antigens into systemic circulation</td>
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<td></td>
<td></td>
<td>Mucous membranes</td>
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<tr>
<td>Cell-mediated barriers</td>
<td>Phagocytic cells, e.g., neutrophils, macrophages</td>
<td>Inflammatory cells, e.g., basophils, mast cells Natural killer cells Dendritic cells</td>
<td>Engulf foreign antigens</td>
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<tr>
<td></td>
<td></td>
<td>Inflammatory cells, e.g., histamine, prostaglandins</td>
<td>Release inflammatory mediators, e.g., histamine, prostaglandins</td>
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<td>Soluble factors</td>
<td>Cytokines</td>
<td>Complement proteins</td>
<td>Destroy infected or malignant cells Present antigens to lymphocytes</td>
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<td></td>
<td></td>
<td>Acute-phase proteins</td>
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<tr>
<td>Acquired immune system</td>
<td>B lymphocytes</td>
<td>Plasma cells</td>
<td>Activate/recruit other cells Enhance phagocytosis Promote repair of damaged tissue</td>
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<td></td>
<td>T lymphocytes</td>
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<td></td>
<td>CD4⁺ T cells</td>
<td></td>
<td>Secrete antibodies Induce activation of lymphocytes Promote cell-mediated responses Promote humoral (antibody) responses</td>
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<td>Th1 cells</td>
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<td>Th2 cells</td>
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<td>Th17 cells</td>
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<td>Tregs</td>
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<td>CD8⁺ T cells</td>
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<td>Peripheric tolerance Destroy infected or malignant cells Suppress activity of lymphocytes</td>
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<td></td>
<td>Cytotoxic T cells</td>
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<td>Suppressor T cells</td>
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T cells express a T-cell receptor (TCR) that recognizes a foreign antigen presented in complex with a major histocompatibility complex (MHC) molecule on the surface of an antigen-presenting cell (APC) (24). Subpopulations of T cells include the helper T (Th) cells, which are identified by the presence of the membrane glycoprotein CD4, and cytotoxic T cells that express the CD8 glycoprotein (24). CD4⁺ cells are classified into a number of Th types: Th1, Th2, Th17, and Treg (16, 24, 68). Viral infections or microbes that infect macrophages or natural killer (NK) cells elicit a Th1 response. Th1 lymphocytes secrete interferon gamma (IFN-γ) and tumor necrosis factor beta (TNF-β). A Th2 response elicits Th2 lymphocytes in response to helminths, allergens, and extracellular microbes. Th2 lymphocytes produce cytokines interleukin (IL)-4, 5, and 13, among others. Th17 cells are lymphocytes that produce IL-17 to recruit neutrophils and macrophages and to trigger an inflammatory response in different organs in order to remove extracellular pathogens from the body. Additionally, there is a regulatory arm characterized by regulatory T cells (Tregs) that produce IL-10 to downregulate all other immune arms with the objective of preventing an exacerbated and injury-provoking immune response. Achieving a balance between all these arms is what enables an adequate immune response to fight pathogens and prevents immune-mediated diseases, such as diabetes type 1, rheumatoid arthritis, inflammatory bowel disease (IBD), allergies, asthma, etc.

The Gut-Associated Lymphoid Tissues

The mucosal immune system is strategically located in areas where external pathogens and antigens may gain access to the body. This includes the mucosal-associated lymphoid tissues, which
Figure 1

Diagram of the gut-associated lymphoid tissue (GALT). The GALT consists of aggregated lymphoid follicles (Peyer’s patches and isolated lymphoid follicles) and diffuse or nonaggregated tissue (lamina propria). The epithelium of aggregated follicles contains M cells, which are specialized in antigen sampling. Underneath is the subepithelial dome, which consists of germinal centers (GCs) of B cells in different stages of maturation, as well as dendritic cells (DCs) and T cells. Aggregated follicles are the inductive sites of immunity, where naïve T and B cells are activated to then migrate to the mesenteric lymph node and into the effector sites of the GALT, the lamina propria. Within the lamina propria, mature B cells or plasma cells (PCs) produce IgA, which gets actively transported into the intestinal lumen. DCs in the lamina propria also sample antigens and present them directly to T and B cells. Macrophages are important innate immune cells capable of engulfing and killing microorganisms by phagocytosis. Figure adapted from Reference 2.

Protect sites such as the respiratory, urinary, and reproductive tracts, and the gut-associated lymphoid tissues (GALTs), which protect the intestine. As the intestine is the first line of defense from the environment and must integrate complex interactions among diet, external pathogens, and local immunological processes, it is critical that protective immune responses are mounted against potential pathogens, yet it is equally important that hypersensitivity reactions to dietary and commensal microbial antigens are minimized. The GALT is composed of aggregated tissue in the form of Peyer’s patches and solitary lymphoid follicles, nonaggregated cells in the lamina propria and intraepithelial regions of the intestine, and mesenteric lymph nodes (58) (Figure 1).

Peyer’s patches are aggregates of lymphoid follicles found throughout the mucosa and submucosa of the small intestine. These patches contain both CD4+ and CD8+ T cells, as well as naïve B cells, plasma cells, macrophages, and dendritic cells (58). Overlying the Peyer’s patches are specialized epithelial cells known as M cells, which endocytose, transport, and release antigens from the gut into the Peyer’s patches, where these antigens are presented on APCs to T and B cells (50, 58).

The lamina propria harbors a diffuse population of T and B cells, plasma cells, dendritic cells, mast cells, and macrophages, all covered by a single layer of epithelial cells (58). Intestinal
epithelial cells (IECs) or enterocytes are intimately involved in digestion, absorption, and transport of nutrients but also play a major role in immune regulation of the underlying cells of the lamina propria.

IECs are known to produce different chemokines and cytokines depending on the type of microbial molecules that come in contact with the epithelium (40). IECs have been shown to condition the immune response of dendritic cells by inducing, through an unknown mechanism, the secretion of tolerogenic signals (IL-10) or active immunity signals (IL-12) by the dendritic cells (91). IECs are also involved in antigen transport to underlying antigen-presenting cells, or they can present the antigen themselves to lymphocytes.

The Interactions Between the Microbiota and the Gut-Associated Lymphoid Tissues

Life without symbiotic microorganisms has a profound effect on the host immune system. Experiments in germ-free mice have consistently shown that many components of the immune system remain underdeveloped until bacterial colonization occurs. Structurally, the aggregated lymphoid structures of the GALT, mesenteric lymph nodes (MLNs), and isolated lymphoid follicles and Peyer’s patches are reduced in size and cellularity in germ-free mice (11, 72). Functionally, these mice are depleted in the production of many cytokines, CD4\(^+\) T helper cells, Tregs, B cells, Th17 cells, and antimicrobial peptides, and in expression of the MHC class II, etc. (26, 31, 43, 77, 83, 92, 107). Many of these defects can be reverted upon inoculation with a single species of bacteria (72, 112), indicating that it is the interactions with the microbiota that kick-start the postnatal phase of immune development. Gut bacteria also have critical effects on the development of IECs. IECs express microbial pattern recognition receptors (PRRs) that bind to microbial signals and upregulate the expression of mucus, cytokines, and other immune components (90). In axenic mice, IECs have blunted microvilli, and they display reduced expression of PRRs (1, 122).

Colonization of the host GIT by the microbiota produces inflammatory and tolerogenic signals that stimulate and regulate the different arms of the immune response in order to achieve homeostasis, a state that is favorable to both the microbiota and the host. The Th17 immune response provides a good example of these opposing functions. Despite the fact that Th17 cells are critical in controlling extracellular bacterial and fungal infections (100, 126), overproduction of Th17 cytokines IL-17 and IL-23 is associated with colitis (3, 13) and autoimmunity (64, 118, 126). Thus, the appropriate level of the Th17 response must be achieved to prevent microbial attack and avoid uncontrolled inflammation. An example of a member of the microbiota involved in regulating the Th17 response has been described in segmented filamentous bacteria (SFB), recently named Candidatus Savagella. SFB are a clostridial species that resides in the murine ileum and are a potent stimulator of the Th17 response (31, 126). Colonization with SFB induced the upregulation of cytokines, antimicrobial peptides, and serum amyloid A (SAA), an acute-phase protein secreted during inflammation. SAA is believed to promote the Th17 differentiation of CD4\(^+\) T cells (5, 42). Not surprisingly, microbial signals have also been found to limit the Th17 immune response. Unspecified commensal bacteria induce IECs to produce IL-25, which inhibits IL-23 production by dendritic cells, thus preventing Th17 differentiation (128). In addition, polysaccharide A (PSA) from Bacteroides fragilis downregulated the Th17 response and induced propagation of Tregs in mice that are genetically predisposed to develop autoimmune encephalomyelitis. This treatment was sufficient to prevent disease in this animal model (85). These examples show how microbial signals can act as the tipping forces that increase or decrease Th17 immune cell activation.
Although certain microbes induce a proinflammatory response and others a tolerogenic one, how the immune response differentiates between them is unknown. One way that microbiota may induce a tolerogenic immune response is via their interactions with Tregs. Tregs have a central role in regulating inflammatory responses, and they have been involved in the modulation of disease in animal models of asthma (94) and colitis (4) upon changes in the intestinal microbiome. Although a large proportion of Tregs are developed in the thymus with the purpose of generating tolerance toward self-antigens and preventing autoimmune responses, a subset of Tregs are trained by the GIT microbiota. Analysis of several repertoires of the TCR α chain revealed that the TCRs from Treg cells of the colonic lamina propria are highly heterogeneous compared with Treg cells from other lymphoid organs. Moreover, the study showed that the mouse microbiota was essential for the induction of this particular population of colonic Treg cells from naïve T cells, implying that there may be post-thymic mechanisms of immune cell education that occur via interactions with the commensal microbiota (60). Thus, besides protecting the host from microbial attack, postnatal bacterial colonization may serve the immune system with another important function: to be used as a training ground for a specific population of immune cells.

THE ROLE OF THE MICROBIOTA IN ENTERIC INFECTION

Although the GIT microbiota is often referred to as commensal, with the implication that these organisms are neither harmful nor beneficial to their host, numerous studies clearly demonstrate that the composition and diversity of the GIT microbiota are crucial to host health and disease. Increasing numbers of studies provide evidence for the importance of the GIT microbiota in many human disorders, including immune-mediated and metabolic diseases such as inflammatory bowel disease (30, 54, 70, 120), obesity (65, 110, 129), asthma, atopy (10, 51, 93), etc. In the following sections, we focus on the key role of the GIT microbiota in the susceptibility to and recovery from enteric infection.

Colonization Resistance

The microbiota can determine host susceptibility to bacterial infections. Germ-free and antibiotic-treated mice are significantly more susceptible to infectious disease caused by intestinal pathogens, including *Shigella flexneri* (103), *Citrobacter rodentium* (125), *Listeria monocytogenes* (127), and *Salmonella enterica* (29, 81, 99). The microbiota can prevent or ameliorate infection by direct microbial antagonism or by indirectly promoting appropriate host immune defenses. Direct microbial effects, also defined as colonization resistance (114), include competition for nutrients and host receptors and secretion of antimicrobial substances (97). For example, the probiotic strain *Escherichia coli* Nissle 1917 reduces *Salmonella Typhimurium* colonization of the mouse intestine by competing for iron, a limiting nutrient in vivo (25). As an example of indirect effects via immune modulation, administration of the Toll-like receptor (TLR) 5 ligand flagellin to antibiotic-treated mice inhibited colonization with vancomycin-resistant *Enterococcus* (VRE) by upregulating the secretion of the bactericidal lectin REGIIIγ in IECs and Paneth cells (56). Flagellin stimulation has also been shown to induce differentiation of nonspecific IgA+ plasma cells via activation of TLR5 in intestinal dendritic cells (111). Colonization with *B. fragilis* is sufficient to prevent murine experimental colitis induced by *Helicobacter hepaticus*. *B. fragilis* is coated with PSA, and this capsular component was shown to suppress IL-17, increase IL-10 production, and ameliorate disease in this model (73). Collectively, these findings suggest that the GI microbiota alter the course of bacterial infections both directly and indirectly via the host’s immune response. In the following...
section, we discuss in detail interactions between the GIT microbiota and three major enteric pathogens: *E. coli*, *S. Typhimurium*, and *Clostridium difficile*.

**Attaching/Effacing Pathogens: Enterohemorrhagic *Escherichia coli*–Enteropathogenic *Escherichia coli*–*Citrobacter rodentium***

*E. coli* is a common commensal inhabitant of the mammalian intestinal tract that can become a GI or extraintestinal pathogen by genetically acquiring pathogenicity islands and plasmids (21). Among the several *E. coli* pathotypes that cause enteric infections, two of the best studied are enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC). Both EPEC and EHEC are attaching and effacing (A/E) pathogens, which are characterized by their ability to adhere tightly to the host intestinal epithelium and cause localized destruction (effacement) of microvilli (20, 53). These phenotypes are conferred by the chromosomal pathogenicity island known as the locus of enterocyte effacement (LEE), which encodes a type III secretion system (T3SS) and a variety of effector proteins that are secreted into host cells, leading to rearrangement of the actin cytoskeleton (95). Additionally, EHEC but not EPEC carries the *stx* genes, encoding the Shiga toxin that is responsible for the more severe outcomes of EHEC infection, such as hemolytic uremic syndrome (21). Because EPEC and EHEC are human-specific pathogens that do not cause representative infections in model hosts such as mice (78), *C. rodentium*, which carries the LEE and is a natural pathogen of mice, is frequently used as a surrogate to study these host-pathogen and microbiota-pathogen interactions and mechanisms (79).

*C. rodentium* infections of mice have provided convincing evidence that the microbiota plays a critical role in determining the outcome of infection with A/E pathogens. As one extreme example, germ-free mice have an impaired ability to clear *C. rodentium* infections. Specific pathogen-free C57BL/6 mice, possessing an intact microbiota, clear *C. rodentium* infections within 22 days, whereas germ-free mice of the same strain remain colonized with *C. rodentium* even 42 days post infection (dpi) (52). Less drastic perturbations of the microbiota, such as treatment with antibiotics, can also affect the outcome of infection. Treating mice with metronidazole prior to *C. rodentium* infection leads to more severe cecal inflammation 6 dpi (125). Notably, the overall abundance of gut microbiota was not altered by the metronidazole treatment in this study, suggesting that specific microbes whose abundance was altered by metronidazole play an important role in protecting or predisposing the host to *C. rodentium*–induced colitis (125).

Even differences in microbiota composition between strains of mice can have drastic effects on *C. rodentium* infection. Mouse strains have long been known to vary in their susceptibility to *C. rodentium*: Resistant strains, such as NIH Swiss and C57BL/6, survive and clear the infection, whereas *C. rodentium* infection is lethal to susceptible strains, such as C3H/Hetj (113). In order to assess whether differences in gut microbiota composition in these mouse strains are responsible for the different infection outcomes, microbiota (fecal) transplantation was performed. In these experiments, recipient mice were first treated with antibiotics to deplete their natural microbiota, then repeatedly gavaged with donor fecal extracts from either the same or a different strain of mice to establish a new gut microbial community. Remarkably, transplantation of resistant microbiota into a susceptible recipient significantly increased survival of *C. rodentium* infection compared with mock-treated susceptible mice (33, 121).

There are several mechanisms by which the microbiota can affect the ability of A/E pathogens to infect their hosts (Figure 2a). One of these mechanisms is competition for nutrients. Other enterobacteria, particularly commensal *E. coli*, are especially effective at competing for nutrients with A/E pathogens. Indeed, precolonization of mice with three commensal strains of *E. coli* prevents subsequent colonization by EHEC (63). There are likely several limiting nutrients for which
Figure 2
The gastrointestinal (GI) microbiota during Citrobacter rodentium infection in mice. (a) Early stage of infection: C. rodentium enters the GI tract (GIT), where short-chain fatty acids (SCFAs) (as well as other potential mechanisms) lead to an increase in T3SS (type III secretion system) expression and attachment to the epithelium. Variation in GI microbiota composition between mice strains results in varying susceptibility to the pathogen. A GI microbiota poor in commensal Escherichia coli allows superior pathogen proliferation in the GIT. (b) Late stage of infection: Two populations of C. rodentium are present in the GIT. One is attached to the epithelium and the other replicates in the lumen. Microbiota-mediated increase in mucin secretion by goblet cells helps detach the pathogen and push it out of the GIT. (c) End stage of infection: The regeneration of the GIT microbiota leads to effective pathogen exclusion. Abbreviation: A/E, attaching and effacing.
EHEC and commensal enterobacteria compete, including proline and mono- and disaccharides (47, 76). The importance of competition for sugars is supported by studies of germ-free mice monoclonized with *C. rodentium* (52). When these mice are subsequently colonized with commensal *E. coli*, which like *C. rodentium* preferentially metabolizes monosaccharides, the intestinal load of *C. rodentium* is reduced by several orders of magnitude. However, colonization with *Bacteroides thetaiotaomicron*, which can metabolize polysaccharides that are not used by *C. rodentium*, fails to decrease *C. rodentium* colonization unless mice are fed a simple sugar diet that forces the bacteria to compete for monosaccharides (52). Colonization of the epithelial surface, rather than the lumen of the intestine or the outer mucus layer where the microbiota reside (45), may be one strategy that A/E pathogens employ to reduce competition for nutrients. *C. rodentium* T3S mutants that cannot adhere to the host epithelium can colonize germ-free mice but are unable to establish an infection in mice with an intact microbiota (52). Association with the epithelium may allow A/E pathogens to access nutrients that are not available to microbiota in the lumen of the intestine, such as the products of plasma membrane damage (75).

The GIT microbiota also exerts indirect effects on A/E pathogens via the host immune system (Figure 2b). The barrier posed by the mucus layer is a central part of the defense against A/E pathogens (and most other enteric pathogens). Mucus secretion from goblet cells in the colon is enhanced during the clearance phase of *C. rodentium* infections, and *Muc2*−/− mice that lack the major intestinal mucin protein have a higher rate of mortality during *C. rodentium* infection than wild-type mice (8, 36). The microbiota appear to play an important role in the maintenance of the mucus layer, as mice treated with the antibiotic metronidazole have a thinner inner mucus layer and are more susceptible to *C. rodentium*-induced colitis (125). The microbiota from resistant mouse strains also promotes the production of proinflammatory cytokines, such as TNF-α and MIP-2α (33, 121). These cytokines enhance host defenses against *C. rodentium* by increasing the production of antimicrobial peptides Reg3β and Reg3γ and by promoting infiltration of neutrophils into the colonic mucosa and submucosa (33, 121). IL-22 appears to be particularly important for protection against *C. rodentium* because treatment of susceptible mice with anti-IL-22 antibodies abrogates the protection normally provided by transplantation with resistant microbiota (121).

In addition to changing the ability of A/E pathogens to survive in the GIT by competition and modulation of host immunity, the microbiota also releases metabolites that alter A/E pathogen gene expression. Short-chain fatty acids (SCFAs) are major microbiota-derived metabolites produced from the anaerobic fermentation of dietary fiber in the cecum and the colon (23). When added to culture media at a concentration similar to that found in the human colon, the SCFAs acetate, propionate, and butyrate activate expression of several EHEC virulence genes, including the LEE-encoded T3S proteins EspB and Tir and the chromosomally encoded adhesin Iha (37, 80). SCFAs, particularly butyrate, produced by intestinal microbiota could therefore be a cue for EHEC to trigger the expression of genes needed for adherence and colonization of the colon. Mucus-derived sugars that are liberated by the microbiota could be another signal that EHEC uses to sense its location. Fucose, which is cleaved from intestinal mucin by species such as *B. thetaiotaomicron*, represses expression of the LEE via the two-component system FusKR (86). Because expression of the LEE represents a significant metabolic burden, this fucose-mediated repression could help EHEC to conserve energy while it is present in the mucus layer rather than in close proximity to the epithelium where the T3SS is needed (86). In addition to these examples, there remain additional uncharacterized metabolites produced by the microbiota that affect EHEC virulence gene expression. For example, growth of EHEC either in a human microbiota–conditioned medium or in the presence of probiotic *Lactobacillus* or *Bifidobacterium* species represses the expression of the Stx2 Shiga toxin (14, 23). Further studies are needed to reveal the full scope of how the microbiota affects infections by A/E pathogens.
**Salmonella enterica**

Unlike the A/E pathogens that generally remain in the intestinal lumen, *S. enterica* are a group of facultative intracellular pathogens that can invade the host tissue as well as proliferate in the gut and as such can generate illnesses ranging from mild gastroenteritis to acute systemic colonization, also known as typhoid fever. Once the pathogen reaches the host GIT, it uses a T3SS encoded by *Salmonella* pathogenicity island 1 (SPI-1) to invade M cells in Peyer’s patches along the small intestine (46). Once internalized, a second T3SS encoded by *Salmonella* pathogenicity island 2 (SPI-2) is activated by the pathogen, allowing it to cross the epithelial barrier. In the lamina propria, *S. enterica* is internalized by macrophages that transfer the pathogen to the internal organs (22). Moreover, *S. enterica* can be sampled from the intestine by dendritic cells. These phagocytic cells internalize the pathogen directly from the gut lumen and are hijacked by *S. enterica* for systemic transfer and colonization (115) (*Figure 3a*).

Different serovars of *S. enterica* are linked to different manifestations of disease. In typhoidal salmonellosis, caused in humans by serovars Typhi and Paratyphi, the pathogen invades the internal organs without generating intestinal inflammation or significant colonization of the GIT. Non-typhoidal salmonellosis results in bacterial GI colonization accompanied by inflammation and pathology, but there is little to no systemic infiltration of the pathogen. *S. enterica* serovar Typhimurium (*S. Typhimurium*), an agent of gastroenteritis in humans, can be used to model both typhoidal and non-typhoidal salmonellosis in mice; the shift between the two diseases is moderated by the GIT microbiota. Oral inoculation of C57B/6 mice with *S. Typhimurium* results in high levels of colonization of the liver, spleen, and mesenteric lymph nodes but not intestinal colonization or pathology. However, if mice are treated 24 hours before infection with a high dose of the antibiotic streptomycin (20 mg), the animals develop GI colonization and inflammation (6). Low doses of antibiotic treatment (streptomycin, vancomycin, or metronidazole at doses of 150–600 mg/L of drinking water) also result in increased susceptibility to *S. Typhimurium* gastroenteritis, accompanied by GI pathology and proinflammatory cytokine secretion (TNF-α, MCP-1, KC, IL-6) (29, 99). The low-dose streptomycin treatment alters the GIT microbiota composition and reduces microbial diversity but does not significantly reduce the total bacterial count in the gut (29, 32, 99). Similar to the case with *C. rodentium*, these data suggest that it is not the overall abundance of GIT microbiota but rather their diversity or the presence or absence of certain organisms that determines susceptibility to *S. Typhimurium* gastroenteritis. Antibiotic treatment also affects *S. Typhimurium* colonization of other mouse strains, such as 129 SvJ. Unlike the C57B/6 mice that succumb to *S. Typhimurium* infection within several days, the 129 SvJ mice are more resistant because of host genetic differences and can recover and clear the pathogen. Following *S. Typhimurium*

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**Figure 3**

The gastrointestinal (GI) microbiota during *Salmonella enterica* serovar Typhimurium gastroenteritis in mice. *(a)* Early stage of infection: *S. Typhimurium* invades M cells in Peyer’s patches or is taken up by dendritic cells. The initial composition of the GI microbiota is critical for *S. Typhimurium* proliferation in the GI tract (GIT). Only mice pretreated with antibiotics or colonized by a low-complexity microbiota develop gastroenteritis following *S. Typhimurium* infection. Microbiota-mediated release of fucose provides the pathogen with a unique carbon source in the post-antibiotic-treated GIT. *(b)* Late stage of infection: *S. Typhimurium*-induced inflammation leads to the release of thiosulfate and ethanolamine, which are used selectively by the pathogen for growth. Furthermore, neutrophil recruitment to the gut lumen and RegIIIβ further reduce the GI microbiota and thus aid *S. Typhimurium* colonization of the GIT. *(c)* End stage of infection: The GI microbiota regenerates and pushes the pathogen out of the host. Abbreviation: T3SS, type III secretion system.
a Early

S. Typhimurium
Enterocyte
M cell
Goblet cell
Dendritic cell
Macrophage
Neutrophil
Segmented filamentous bacteria
Microbiota
Short-chain fatty acid
Thiosulfate
Fucose
Ethanolamine
RegIIIβ

b Late

c End
infection, up to 25% of infected 129 SvJ mice develop a supershedder phenotype characterized by long-term GI colonization and inflammation as well as continuous fecal shedding of the pathogen. Treatment of the mice with streptomycin or neomycin (5 mg) before infection resulted in all mice having the supershedder phenotype. Even mice that were allowed to recover for seven days after neomycin treatment, a time frame in which the microbiota generally reverts to the pretreated state, remained susceptible to GI colonization, demonstrating the importance of the microbiota for the ability of 129 SvJ mice to clear S. Typhimurium infection (62). Antibiotic treatment was also shown to promote S. Typhimurium GIT colonization in the FvB murine strain (19). In this model, increased susceptibility was observed even after three weeks of post-antibiotic recovery.

Perhaps the clearest evidence for the importance of a complex GIT microbiota in preventing/reducing S. Typhimurium colonization was shown using low-complexity microbiota (LCM) mice. These animals are bred germ free and are colonized at birth with the Altered Schaedler Flora, a combination of eight bacterial strains that are representative of the major phylogenetic groups found in conventional murine microbiota. The LCM mice have significant reduction in GI microbial diversity (8 versus >500 strains in conventionally bred mice) but similar bacterial counts in the gut. These animals also develop gastroenteritis when infected with S. Typhimurium without the need for antibiotic pretreatment (104). When housed with conventionally bred animals (which facilitates microbiota transfer between animals), the LCM mice gained partial resistance to S. Typhimurium–induced colitis. The transfer of complex GIT microbiota from the conventionally bred mice to the LCM mice was not complete, and lingering population changes, specifically increased levels of Enterobacteriaceae, were linked with increased susceptibility to S. Typhimurium colitis (104).

A simplified model of the post-antibiotic-treated gut was used to understand why it was permissive to S. Typhimurium colonization (82). Germ-free mice were colonized with B. thetaiotaomicron and infected with S. Typhimurium. S. Typhimurium isolated from the gut of these mice was shown to upregulate genes involved in sialic acid and fucose catabolism compared with S. Typhimurium isolated from uncolonized germ-free mice. Furthermore, mutants in these metabolic pathways have reduced virulence in both the B. thetaiotaomicron monocloned mice and in antibiotic-treated animals. Consistently, the GI content of both the B. thetaiotaomicron monocloned mice and the antibiotic-treated mice contained elevated levels of sialic acid. B. thetaiotaomicron is able to free N-acetylneuraminic acid (Neu5Ac) from intestinal mucin by the action of a specific sialidase. Under normal conditions Neu5Ac is used as a carbon source by various members of the GIT microbiota, however in monocloned or antibiotic-treated mice the absence of Neu5A metabolizing strains leaves the sugar available for S. Typhimurium consumption.

Hydrogen is another metabolite that has been shown to promote S. Typhimurium proliferation in the gut of mice with a simplified microbiota. LCM mice were infected with an S. Typhimurium transposon mutant library, and mutants of the hyb hydrogen utilization operon were found to have attenuated virulence in the model (69). In competition with infections with the wild-type strain, the S. Typhimurium hyb operon mutant displayed attenuated virulence in both LCM and conventional mice. However, in animals treated with a high dose of streptomycin, the hyb mutant had similar fitness to the wild-type strain. Hydrogen is produced by the GIT microbiota during carbon fermentation and was shown in this study to be important in the early stages of S. Typhimurium GIT colonization. Other microbiota-induced metabolic changes in the gut may also affect S. Typhimurium colitis. A directed metabolomics survey of low-dose-streptomycin–treated mice found a reduction in SCFA concentration in the ceca (32). The SCFAs propionate and butyrate have been shown to suppress SPI-1 expression in vitro (39, 61), and propionate was also demonstrated to kill S. Typhimurium under gut-like in vitro conditions (17). Collectively,
these studies strongly support the notion that antibiotic treatment promotes metabolic changes in the GIT that can be conducive to S. Typhimurium colonization (Figure 3a).

GI inflammation induced by S. Typhimurium has been generally viewed as a mechanism for pathogen control and expulsion. Recent studies show instead that the pathogen actually manipulates inflammation as a source of nutrients and to out-compete the GIT microbiota in the gut lumen (Figure 2b). In antibiotic-treated animals, wild-type S. Typhimurium induces colitis and inflammation and out-competes the GIT microbiota (98, 105). An S. Typhimurium strain lacking the SPI-1 and SPI-2 secretion systems (the avir strain) can establish initial colonization in the gut of antibiotic-treated mice, but the infection is not sustained and the pathogen is out-competed and expelled by the GIT microbiota. However, in mice that develop spontaneous colitis or if gut inflammation is induced by T-cell transfer, S. Typhimurium avir can colonize the GIT to the same extent as the wild-type strain (105). This indicates that gut inflammation is actually supporting S. Typhimurium colonization. In the gastroenteritis model, S. Typhimurium infection strongly induces the expression of RegIIIβ, a lectin secreted by the GI mucosa that was also shown to have bacteriocin activity against gram-positive and gram-negative bacteria, including E. coli, but is harmless to S. Typhimurium itself (106). The elevation of RegIIIβ in the intestine was shown to be inflammation dependent, as it does not occur in immune-deficient mice and may be one mechanism by which the pathogen uses the immune response to clear the GIT microbiota. An alternative or parallel mechanism involves S. Typhimurium–induced neutrophil infiltration into the gut lumen. Neutrophil recruitment during S. Typhimurium infection is virulence factor and colitis dependent (98), and elimination of neutrophils by antibody treatment results in a diminished ability of S. Typhimurium to expel the GIT microbiota (34). Furthermore, neutrophil elastase, a serine protease produced by neutrophils during inflammation, was demonstrated to shift the GIT microbiota of mice in a manner that supported S. Typhimurium GIT colonization (34). Neutrophil efflux to the GIT lumen is also accompanied by increased production of reactive oxygen species (ROS) and nitric oxide (NO). Previously believed to be important in controlling pathogens, ROS and NO in fact assist S. Typhimurium in its competition with the GIT microbiota. The GIT microbiota produces H2S, which is subsequently metabolized into thiosulfate by the gut lumen. Inflammation-induced release of ROS and NO oxidizes thiosulfate into tetrathionate, an electron acceptor used selectively by S. Typhimurium that also limits the ability of other bacteria to replicate (124). Indeed, the trrA gene responsible for tetrathionate utilization is crucial to the ability of S. Typhimurium to out-compete the GIT microbiota. Tetrathionate respiration in vivo is coupled with the consumption of ethanolamine, a nutrient that is not fermented by the GIT microbiota and as such serves as an abundant carbon source for S. Typhimurium in the GIT (109). The ability of S. Typhimurium to consume ethanolamine was demonstrated to promote colonization of the inflamed GIT but was dependent on the pathogen’s ability to reduce tetrathionate. The picture emerging from all of the above is that the mucosal defense benefits S. Typhimurium by helping the pathogen to out-compete the GIT microbiota.

Mice infected with an SPI-2 S. Typhimurium mutant present a self-limiting infection similar to S. Typhimurium gastroenteritis in humans. In this model of the disease, the microbiota begins to regenerate and out-competes the pathogen (Figure 3c). The starting point for microbiota regrowth remains unknown. It may result from a reduction in the availability of the nutrients required by S. Typhimurium for GIT proliferation, such as thiosulfate or sialic acid, but may also be the result of other, yet undiscovered signals. What is clear is that it is a GIT microbiota–, rather than immune–, dependent process. T- and B-cell-depleted mice can clear S. Typhimurium GIT infection but only if they are colonized with a complex microbiota prior to pathogen inoculation (28). In the model described in this study, all of the mice were treated with high-dose streptomycin
prior to S. Typhimurium infection. How exactly the GIT microbiota exerts immune-independent protection on the host even though it is cleared from the GI before infection is just one of the issues that require further study in the field of host-Salmonella-microbiota interactions.

**Clostridium difficile**

Unlike *S. enterica* or pathogenic *E. coli*, *C. difficile* is often present in the GIT microbiota of healthy individuals, ranging from 1% to 50% of the population (7, 41, 74). As a minor member of the GIT microbiota, *C. difficile* has no adverse effects on human health. High colonization by the bacteria, however, leads to a condition known as *C. difficile*-associated diarrhea (CDAD) and is the main cause of pseudomembranous colitis, a severe and sometimes deadly form of antibiotic-associated diarrhea (55).

*C. difficile* is a spore-forming gram-positive bacteria that induces severe colitis through the action of two unique toxins: Toxin A and Toxin B (117). Both toxins are glucosyltransferases that are delivered into host cells, where they modify Rho and Ras family GTPase and induce epithelial cell necrosis (44, 48, 49). Furthermore, these toxins have been shown to disrupt epithelial barrier function by modifying tight junction protein interaction (84).

It remains unclear whether patients who develop CDAD acquire *C. difficile* from the environment or from overgrowth of indigenous bacteria (123). It is, however, remarkably clear that the GIT microbiota provides the host with a high level of resistance to this disease. CDAD in adults is almost exclusively found in antibiotic-treated or otherwise susceptible hospitalized patients (88). CDAD is more common in infants under one year of age (59), even without additional risk factors, but children of that age have been reported to harbor an unstable GIT microbiota (87). Furthermore, the vast majority of animal models of *C. difficile* colonization involve either germ-free mice or various animals that require aggressive antimicrobial therapy prior to inoculation with the pathogen (9) (Figure 4a).

Similar to *S. Typhimurium*– and *C. rodentium*–induced colitis, increased susceptibility to CDAD can be linked with population shifts of the GIT microbiota rather than complete microbial depletion. Stool samples of hospitalized CDAD patients that were collected prior to the onset of disease harbored a microbial population of reduced diversity with diminished proportions of the phylum Bacteroidetes and families Bacteroidaceae and Clostridiales as well as increased levels of the family Enterococcaceae (116). Additionally, a study that sampled the GIT microbiota of patients suffering from CDAD found that those who experience repeated infection have a higher level of *C. difficile* colonization of the gut and an overall reduction in microbiota diversity when compared with healthy subjects as well as with patients suffering an initial episode of the disease (15). In an animal study, mice treated with a single dose of clindamycin became susceptible to *C. difficile* colitis (12). Similar to the effect of low-dose streptomycin treatment, clindamycin administration did not reduce overall bacterial counts in the gut but did significantly lower population diversity. The post-treatment microbial population was dominated by Enterobacteriaceae species and largely reduced in Lachnospiraceae and *Barnesiella* populations.

A key question in the study of CDAD is how antimicrobial treatment and the subsequent change in the GIT microbiota break the natural resistance to *C. difficile* over-colonization. Ng et al. (82) reported that sialic acid metabolism by *C. difficile* is critical for colonization of antibiotic-treated mice as well as germ-free mice monocolonized with *B. thetaiotaomicron* (82). In both murine populations, sialic acid is more available than in conventionally colonized untreated animals. A more recent study employed metabolomics to survey the *C. difficile* susceptible GIT (108). Significant alterations in many metabolites were observed in mice treated with cefoperazone in comparison to untreated animals or those that were allowed to recover from the treatment to the point at which they were once again resistant to *C. difficile*. The bile acid taurocholate was among
The gastrointestinal (GI) microbiota during *Clostridium difficile* infection. (a) Early stage of infection: Antibiotic treatment increases susceptibility to *C. difficile* colonization, possibly by reducing concentrations of microbiota-produced factors that repress *C. difficile* spore germination. Sialic acid, released by the GI microbiota in the antibiotic-treated GI tract (GIT), is used by the pathogen as an energy source. (b) Late stage of infection: *C. difficile* release of Toxin A and Toxin B leads to epithelial necrosis and tight junction disruption. There is an increase in concentrations of *C. difficile* spore germination–promoting molecules. (c) End stage of infection: Transfer of healthy microbiota (fecal transplant) and restoration of gut homeostasis are currently the best treatments for *C. difficile* infection.
the metabolites significantly increased in the cecum of susceptible mice. In vitro assays confirmed that taurocholate is an inducer of \textit{C. difficile} spore germination. This is consistent with previous in vitro studies that reported primary and secondary bile acids as activators and inhibitors of \textit{C. difficile} spore germination, respectively (101, 102). The metabolomic screen of Theriot et al. (108) also found increases in various sugars in the antibiotic-treated ceca, and the ability of \textit{C. difficile} to grow on these carbon sources was confirmed. As such, a possible mechanism of \textit{C. difficile} expansion in the post-antibiotic-treated gut is that increased availability of various nutrients and changes in spore germination signals together lead to increased pathogen proliferation (Figure 4b). The GIT microbiota may also have other means to control \textit{C. difficile} population levels. \textit{Bacillus thuringiensis} isolated from stool of a healthy human was shown to produce thuricin CD, a bacteriocin that can kill \textit{C. difficile} but not other gram-positive bacteria common to the human GIT (89). Although this study does not link the presence or absence of \textit{B. thuringiensis} to CDAD, it does demonstrate the potential role of direct bacteria-bacteria interactions in controlling pathogens in the gut.

Although several antibiotics can be used to treat CDAD, recurrent infections are frequent because of \textit{C. difficile}'s ability to form resistant spores. Approximately 15–30% of CDAD patients treated with broad-spectrum antibiotics, such as vancomycin and metronidazole, experience recurrence of infection after the first episode and repeated recurrence occurs in as many as 60% of patients (96). New narrow-spectrum antibiotics, such as fidaxomicin, are emerging as improved treatments for CDAD infection (66). Nevertheless, fecal transplant, the transfer of an entire gut population directly to the patient GIT, is currently the most efficient therapy for the disease (71) (Figure 4c).

CONCLUDING REMARKS
The GIT microbiota plays a crucial role during the various stages of bacterial enteric colitis. Although pathogenic \textit{E. coli}, \textit{S. Typhimurium}, and \textit{C. difficile} have very different virulence pathways and, appropriately, unique interactions with the host and its microbiota, commonalities do exist. The susceptibility to all three pathogens can be increased by a single dose of an antibiotic that does not clear the GIT microbiota but rather shifts it to a composition more permissive to pathogen colonization. Furthermore, all three pathogens are able to utilize metabolites made available through alterations to the GIT bacterial population. Finally, recovery from gastric bacterial infection depends on the restoration of a complex microbial flora in the GIT. In recent years, much data have accumulated about the host-mediated interaction between the GIT microbiota and bacterial pathogens. Future efforts will likely turn toward deciphering the direct molecular interaction between the microbes harbored by the human body and those that try to attack it.

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114. van der Waaij D, Berghuis-de Vries JM, Lekkerkerk-van der Wees JEC. 1971. Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. J. Hyg. 69:405–11
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Errata
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