

The role of the immune system in governing host-microbe interactions in the intestine

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The mammalian intestinal tract harbors a diverse community of trillions of microorganisms, which have co-evolved with the host immune system for millions of years. Many of these microorganisms perform functions critical for host physiology, but the host must remain vigilant to control the microbial community so that the symbiotic nature of the relationship is maintained. To facilitate homeostasis, the immune system ensures that the diverse microbial load is tolerated and anatomically contained, while remaining responsive to microbial breaches and invasion. Although the microbiota is required for intestinal immune development, immune responses also regulate the structure and composition of the intestinal microbiota. Here we discuss recent advances in our understanding of these complex interactions and their implications for human health and disease.

From birth, the mammalian gastrointestinal tract faces the unique challenge of being constantly exposed to a diverse community of microorganisms, collectively called the microbiota. The human intestine harbors approximately 100 trillion microbes, mainly comprised of over 500 species of bacteria^{1,2}. Bacterial composition varies along the intestinal tract, as each species of bacteria colonizes a specific niche (Fig. 1). This colonization begins at birth, with the first microbial exposure being maternally derived, and from then is shaped by host genetics and by exposure to the surrounding environment³. This inevitable colonization by the microbiota has shaped an intimate relationship where host and microbes have co-evolved for mutually beneficial outcomes. The intestine provides a protected, nutrient-rich environment in which the microbiota establish a diverse, yet remarkably stable and resilient ecosystem⁴. In turn, the microbiota is involved in many aspects of host physiology, as microbial by-products of digestion provide vitamins and nutrients to host cells and contribute to resistance to colonization by potential pathogens⁵.

Despite the mutually beneficial aspects of microbial colonization in the intestine, the sheer abundance and proximity of these microbes to the host epithelium poses a major challenge, as the host must mitigate the potential for opportunistic breaching of the intestinal barrier, as well as invasion of intestinal epithelial cells (IECs). Achieving homeostasis requires the intestinal immune system to act in concert with the host epithelium to find the equilibrium 'set point', which

minimizes the potential for harmful effects from the microbiota or potent inflammatory responses⁶. Minimizing this threat means overcoming considerable challenges. The intestinal immune system must tolerate the microbiota, while simultaneously remaining vigilant against the potential threats posed by these microorganisms. Another challenge comes from the dynamic nature of the intestinal microbial composition, where dramatic shifts in community structure can be exogenously induced by antibiotic treatment⁷, dietary changes⁸ and gastrointestinal pathogens⁹. Large shifts in microbial composition can lead to community imbalance, a state known as dysbiosis. Conversely, endogenous effects such as deficiencies or dysregulation of the intestinal immune system itself can also trigger microbial dysbiosis^{10,11}. In this Review, we highlight recent advances in our understanding of how the intestinal immune system guides dynamic host-microbial interactions toward homeostasis and how breakdowns in this system lead to disease.

Innate epithelial barrier defense

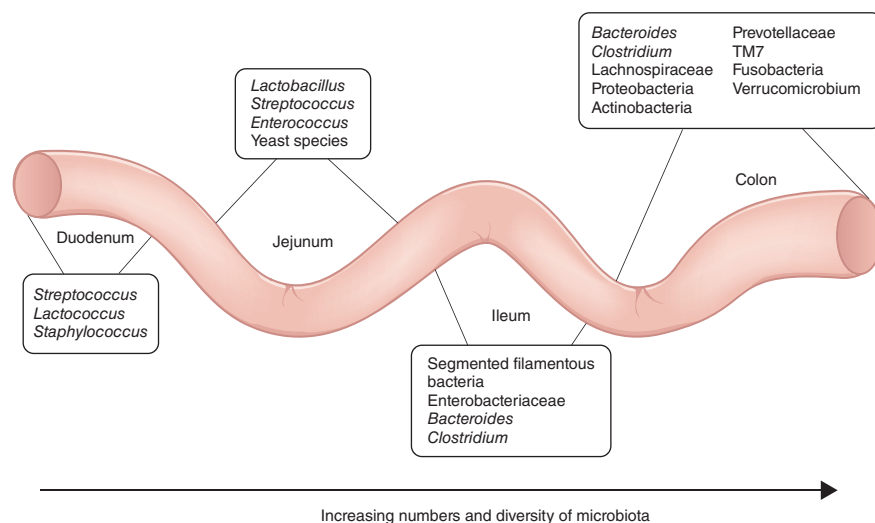
It is of paramount importance to host health that the intestinal microbiota is kept at a distance from IECs, minimizing the likelihood of tissue damage and invasion. Innate immune strategies include the use of a mucus layer, antimicrobial peptides (AMPs) and innate lymphoid cells (ILCs) functioning in concert to confine much of the community to the lumen of the intestinal tract. The strategies used are different in the large intestine and the small intestine but ultimately work to promote mutualistic interactions and anatomical containment of the microbiota (Fig. 2).

In the large intestine, the numbers of intestinal microbiota reach as high as 10^{12} cells per gram of feces. Here the mucus layer is the vital component of the innate immune system, segregating the microbiota from the intestinal epithelium. Specialized epithelial cells called goblet cells secrete mucin glycoproteins (mucus), which assemble into a thick mucus layer extending 150 micrometers away from the epithelium¹². The mucus layer comprises a variety of mucin glycoproteins,

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Figure 1 Spatial distribution and composition of the microbiota along the intestinal tract. The quantity and composition of microbial species differs along the intestinal tract. Phyla, families and genera of the microbiota enriched in each particular niche are listed. Nine main bacterial phyla are represented in the mammalian gut microbiota: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia, Cyanobacteria, Fusobacteria, Spirochaetes and TM7. Most of the bacterial species found in the mammalian intestine are from the phyla Bacteroidetes or Firmicutes. Archaeal and eukaryotic microorganisms also can colonize the intestine in low abundance⁵.



forming a dense inner layer devoid of bacteria and a loose outer layer in which the commensal bacteria reside¹³. The thickness of the inner mucus layer is an important physiological feature and has a role in susceptibility to pathogen-induced and commensal-induced inflammation^{14,15}. The outer mucus layer has a role in shaping the mucosa-associated microbiota by providing glycans as a nutrient source, and the density of the inner layer limits the direct contact of these bacteria with epithelial cells¹⁶. Aside from mucin glycoproteins, goblet cells also produce trefoil factors and resistin-like molecule- β , which aid in maintaining barrier integrity by stabilizing mucin polymers and reducing the susceptibility to inflammation^{17,18}.

In contrast, the mucus layer in the small intestine lacks distinct inner and outer layers, and is discontinuously secreted along the apical surface¹⁶. In this tissue environment, vast arsenals of AMPs take on a larger role in segregating the microbiota from the epithelium. These include defensins and C-type lectins, which are predominately produced by Paneth cells, a lineage unique to the small intestine and strategically located close to epithelial stem cells in the crypt¹⁹. Paneth cells are essential to containing the microbiota, and a loss of Paneth cells results in increased invasion of the epithelial barrier by pathogenic and symbiotic microbes²⁰. Secreted AMPs are retained in the mucus layer, forming a biochemical barrier to protect IECs from microbial contact. For example, the C-type lectin RegIII- γ is effective at preventing epithelial contact by Gram-positive bacteria²¹. Similarly, defensins are cationic membrane-disrupting molecules that can broadly target bacteria, along with fungi and protozoa²². Both gain-of-function and loss-of-function studies suggest an important role for defensins in shaping the mucosal microbial community. A loss of α -defensin function increases the ratio of Firmicutes to Bacteroidetes in the small intestine, and transgenic expression of human α -defensin-5 in mice leads to smaller populations of Firmicutes, most notably those known to directly contact the epithelium²³. Expression of RegIII- γ and of some α -defensins is inducible and requires microbial signals, whereas a subset of α -defensins is constitutively expressed^{24–26}. Many of these proteins kill microbes directly through enzymatic attack of the cell wall, without discrimination between pathogens and commensals. Not all AMPs, however, are microbicidal. Human α -defensin-6 protects against microbial invasion of the epithelium by indiscriminately binding to the microbial cell surface, entangling microbes in a self-assembled ‘nano-net’ of fibrils²⁷. Furthermore, post-translational modifications of defensins may be of importance, based on studies of human β -defensin-1. The redox state of human β -defensin-1 may have a key role in regulating the composition of microbiota at the epithelial surface and perhaps in preventing translocation of the bacteria associated with the mucosa²⁸.

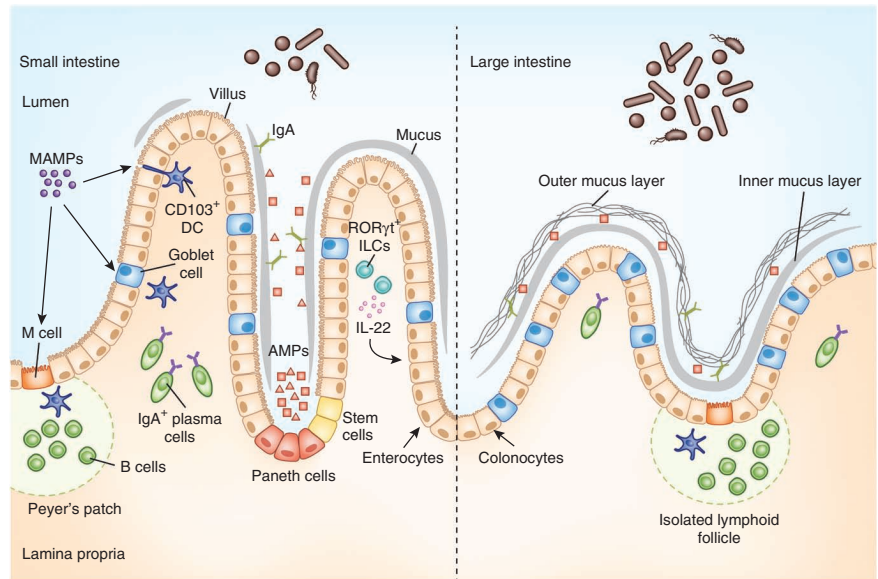
Thus, the local tissue microenvironment tunes the innate immune system to drive protection against epithelial damage.

Innate lymphoid cells

ILCs are a population of lineage marker-negative innate immune cells that rapidly respond to epithelium-derived cytokine signals and are critical to maintaining intestinal homeostasis²⁹. Collectively, they have a cytokine expression pattern resembling that of the T helper cell subsets T_H1 , T_H2 , T_H17 and T_H22 , yet in contrast to T cells, differentiation of ILCs occurs independently of somatic recombination. Development and function of ILCs is dependent on the specific expression of a transcription factor: T-bet (group 1 ILCs), GATA-3 (group 2 ILCs) or ROR γ t (group 3 ILCs)³⁰. Similar to many immune components, ILCs have a bidirectional relationship with the microbiota; responses of ILCs shift depending on microbial composition, and effector function of ILCs impacts microbial anatomical containment and composition³¹. ROR γ t⁺ ILCs secrete interleukin 17 (IL-17) and IL-22, which they produce after detecting microbiota through the aryl hydrocarbon receptor³². IL-22 produced by ROR γ t⁺ ILCs can also be negatively regulated by microbial signals inducing epithelial production of IL-25 or by the presence of an adaptive immune system³³. ROR γ t-deficient mice, which lack ILCs and T_H17 cells and produce less IgA, are defective in their ability to contain the microbial community and exhibit high titers of IgG antibodies to the microbiota, which implies a breach of the intestinal barrier and a systemic immune response to the microbiota³⁴. Studies in *Rag1*^{-/-} mice have shown this defect in anatomical containment of the microbiota may be mediated by a depletion of ILC-specific production of IL-22, leading to increased translocation of the microbiota to the mesenteric lymph nodes³⁵. Production of IL-22 by ROR γ t⁺ ILCs acts directly on the IECs, triggering damage repair and inducing expression of mucin genes and AMPs³⁶.

Studies of the interactions between the other two ILC groups and the microbiota are in their infancy, yet a role for repairing of tissue damage by GATA-3⁺ ILCs through production of amphiregulin and IL-33 has been described³⁷. In *Tbx21*^{-/-} *Rag2*^{-/-} ulcerative colitis mice, the disease was shown to be dependent on ILCs; T-bet⁺ ILCs produce IFN- γ , and deficiency in T-bet results in spontaneous colitis dependent on the microbiota and driven by dysregulated IL-17A production by ILCs expressing the IL-7 receptor³⁸. Additional studies are needed to assess the role of ILCs in regulating the microbiota in human subjects or wild-type mice, as most of these studies have been

Figure 2 Anatomical containment of the microbiota along the intestine. The intestinal epithelium comprises a single layer of enterocytes or colonocytes, and it is the role of the immune system to protect the integrity of this barrier. In the small intestine, absorptive requirements for enterocytes results in a discontinuous mucus layer, with fewer goblet cells. Here Paneth cells are enriched in the crypts, secreting AMPs, which can cross-link with the mucus layer. Through this barrier, sampling of MAMPs can be mediated through antigen uptake by M cells and goblet cells to dendritic cells (DCs), along with direct trans-epithelial luminal sampling from DCs. ROR γ t ILCs can sense microbial signals and produce IL-22 to aid in IEC barrier function. Commensal-specific IgA is produced by plasma cells in the lamina propria, mediated by DCs in a T cell-independent mechanism. The large intestine uses a thick, continuous mucus layer to compartmentalize the microbiota, with IgA and AMPs having a secondary role.



carried out in immunocompetent mice lacking an adaptive immune system. The similarity in functions and cytokine profiles between ILC groups and adaptive T cells indicates that ILCs may have a greater role in microbial colonization events early in life before a fully mature adaptive immune system is present³⁰. It is clear that ILC-mediated regulation of the microbiota provides another layer of protection to IECs from microbial exposure, repairing damaged tissues, promoting barrier functions and preventing systemic inflammation.

Sensing the microbiota

The intestinal immune system in germ-free mammals is underdeveloped, deficient in many immune components including circulating antibodies and mucosal T cells, and does not produce mucus and AMP³⁹. This indicates that the presence of intestinal microbiota induces immune maturation. Simultaneously the immune system must be able to sense what microbes are present and respond appropriately. A variety of proteins called pattern recognition receptors (PRRs) are expressed by IECs and many hematopoietic cells and mediate interactions between the immune system and the microbiota. Expression of these PRRs is crucial for maintaining homeostasis with the intestinal microbiota⁴⁰. PRRs include Toll-like receptors (TLRs) and nuclear oligomerization domain-like receptors (NLRs), which recognize microbe-associated molecular patterns (MAMPs), including lipopolysaccharide, lipid A, peptidoglycan, flagella and microbial RNA/DNA, leading to a variety of downstream signaling pathways⁴¹. PRR-MAMP interactions are crucial in promoting mucosal barrier function, regulating the production of mucin glycoproteins, AMPs, IgA and IL-22 (ref. 42). Accumulating evidence suggests a loss of specific PRRs can lead to an altered microbial composition and intestinal barrier defects leading to microbial invasion of systemic organs. The composition of the ileal microbiota in TLR2-, TLR4-, TLR5- and TLR9-deficient mice was shown to remain stable in the short term, however TLR deficiency lead to longer-term familial shifts in microbial composition⁴³. Specifically, deficiency of TLR5, which recognizes bacterial flagellin, leads to an increased translocation of commensals to the liver and spleen, increased susceptibility to colitis and metabolic abnormalities^{44,45}. These observations suggest that the abnormalities seen in short-term TLR-deficiencies may result from a barrier defect in the containment of commensals, rather than compositional shifts in the microbiota. Mice lacking the adaptor

protein MyD88 have increased colonization of the liver and spleen by commensal microbiota⁴⁶ and reconstitution of MyD88 in Paneth cells limited the microbial penetration into these tissues²⁰. Aside from the well-described role as a TLR adaptor protein, MyD88-deficiency also leads to a loss of signaling events downstream of the IL-1 receptor, including production of IL-1, IL-18 and IL-33 (ref. 47). Recently the inflammasome has garnered increased attention as an important regulator of intestinal homeostasis. In mice, deficiency in the receptor NLRP6 leads to decreased production of IL-18 from the inflammasome, increased susceptibility to dextran sodium sulphate (DSS)-induced colitis and an altered colonic microbiota enriched in the Prevotellaceae and TM7 (ref. 48). NLRP6 also controls the IL-18 dependent downregulation of IL-22 binding protein, a critical regulator of intestinal tissue repair⁴⁹.

Pattern recognition of MAMPs is particularly important in the small intestine, which lacks a thick mucus layer segregating the microbiota⁵⁰. Here recognition of luminal antigens by intestinal dendritic cells is an important mode of sensing and controlling the immune response to the microbiota⁵¹. Recent evidence has shown CD103⁺ dendritic cells, a subset of dendritic cells that are crucial to maintaining intestinal homeostasis, can sample luminal antigens in the small intestine through direct luminal sampling⁵², goblet cell-mediated delivery⁵³ or through M-cell transcytosis in Peyer's patches⁵¹. More experimental data are needed to explore the impact and prevalence of each of these mechanisms. It is hypothesized that different modes of antigen sampling could lead to different outcomes⁵⁴, and future studies in this area will be important to understand how the immune system responds to the intestinal microbial composition. Though innate barriers segregate the microbiota from the IECs, it is clear that controlled sensory mechanisms exist to sample composition of the microbiota, and this is a key regulator of homeostasis.

Generation and function of IgA responses to microbiota

Whereas the innate immune system provides protection via the mucus layer, AMPs and ILCs to indiscriminately control microbial composition and penetration of the epithelium, the adaptive immune system provides an additional layer of protection. This is mediated by the production of IgA, which acts as a link between these two arms of the immune system. Whereas nonspecific IgA binds to microbial surface glycans causing bacterial agglutination⁵⁵, microbe-specific IgA

is the main adaptive immune response controlling the microbiota. Production of IgA results from stimulation of B cells in Peyer's Patches by dendritic cells, which sample the small number of bacteria penetrating through the mucus layer^{56,57}. The cytokine milieu found in this environment, and in particular the presence of TGF- β , results in B cell class switching to produce IgA, which is then transcytosed back into the intestinal lumen⁵⁷. Secreted IgA is preferentially used to recognize the microbiota, and it has a variety of unique properties, tuning IgA to respond effectively to an environment filled with microbial antigens. Host repertoires of IgA coat a majority of the intestinal microbiota without eliciting potent and potentially damaging responses, and seem to be specific for distinct bacterial epitopes of commensals⁵⁸. The repertoire of secreted IgA is constantly shifting to respond to the changing intestinal microbial environment and might be dynamically shaped to mirror the composition of the microbiota⁵⁸. How microbiota-specific IgA mediates epithelial protection is unclear, although several functions have been described. The action of IgA may include trapping of organisms in the mucus, prevention of epithelial-cell invasion and alteration of bound bacteria, including abrogation of bacterial resistance to the oxidative burst response⁵⁹. The importance of IgA in keeping the systemic immune system ignorant of the microbiota is evident by the fact that IgA-deficient mice exhibit priming of IgG responses to organisms which would be expected to form part of the microbiota⁶⁰. In addition, IgA may also have a role in shaping the composition of microbiota. Mice lacking genes for IgA class switching have an expansion of Firmicutes, notably epithelial-associated segmented filamentous bacteria, and *Rag2*^{-/-} mice have an altered microbiota, which can be restored after bone marrow reconstitution⁶¹. Furthermore, mice deficient in somatic hypermutation genes have an expansion of microbiota in the small intestine and impaired mucosal defense⁶². Present literature, based on mouse models, suggests that IgA adds an immunological buffer to host-microbiota interactions in the intestine and the affinity of microbe-IgA interaction required to achieve this buffering appears to be bacterial species-specific⁶³. Yet the mechanisms and signaling pathways of how IgA functions in humans remain poorly understood, and more studies are needed to examine how IgA interacts with human commensals.

T cell-mediated responses

Several T cell subsets are involved in containment of the microbiota, including CD4⁺CD25⁺FoxP3⁺ regulatory T (T_{reg}) cells, CD4⁺FoxP3⁻ T regulatory type 1 (Tr1) cells and T_H17 cells. Their functions range from providing help for B cells in production of IgA to avoiding autoimmunity and chronic inflammation. Production of IgA results from both T cell-dependent and T cell-independent pathways⁵⁷, but the extent of interaction between IgA and T cells in the intestinal submucosa is unclear. Aside from their suppressor function, T_{reg} cells can differentiate to act as helper cells to induce microbiota-specific IgA responses. In one study, depletion of T_{reg} cells resulted in a decrease in IgA⁺ B cells and subsequent adoptive transfer of T_{reg} cells into *Tcrb*^{-/-} $\times$ *Tcrd*^{-/-} mice reversed this process⁶⁴. Whether this interaction occurred in Peyer's patches, isolated lymphoid follicles or in the lamina propria was not examined. *In vitro* studies suggested that this was dependent on the activity of TGF- β ⁶⁴ but additional investigation is required to understand the precise nature and location of this interaction.

In healthy hosts, T_{reg} cells prevent excessive cell-mediated immune responses, which would cause harmful inflammation, allowing tolerance of the microbiota. The importance of T_{reg} cells is highlighted in the *Helicobacter hepaticus* colitis model in *Rag2*^{-/-} mice, where adoptive transfer of T_{reg} cells is sufficient to inhibit inflammation.

This occurs via suppression of T_H1 cell responses and T cell-independent innate immune-induced inflammation, with the latter mechanism being via pathways dependent on IL-10 and TGF- β ^{65,66}. TGF- β also has a key role in maintaining homeostasis of the intestinal microbiota, being required for T cell-dependent regulation of IgA production and suppression of innate immune-induced inflammation. TGF- β also stimulates differentiation of T_H17 cells⁶⁷, and several T_H17 cell-derived cytokines, including IL-17 and IL-22 have key roles in the regulation of normal intestinal microbiota. The balance between differentiation of T_H17 cell and T_{reg} cell subsets is reciprocally regulated in the intestine, mediated by STAT3-dependent cytokines such as IL-6 and IL-23 (refs. 68,69). *Stat3*^{-/-} mice cannot produce an adequate T_{reg} cell compartment, resulting in uncontrolled T_H17 responses and severe colitis⁷⁰. Tr1 cells are complementary to T_{reg} cells in that they are a predominant source of IL-10 in the small intestine, whereas T_{reg} cells largely perform this function in the colon⁷¹. The importance of the differing locations of these two cell types is not completely clear, however, as both can suppress colitis in adoptive transfer studies of mice⁷². We also discuss the role of T cells below, with specific reference to HIV infection.

Immune responses to pathogens alters microbiota

It is clear from the evidence provided so far that the intestinal immune system regulates the spatial containment of the microbiota by recognizing and responding to microbial signals, using multiple layers of immune protection to promote homeostasis. Ingestion of gastrointestinal pathogens represents a threat to intestinal homeostasis. Side effects of the immune response to pathogens can lead to tissue damage and alter the composition of the microbiota, in some cases leading to dysbiosis. Pathogenic insult results in a robust proinflammatory immune response, which can lead to disruption of the intestinal barrier and an altered microbiota, favoring the colonization efficiency and survival of the pathogen^{73,74}. The well-characterized enteric pathogen *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) triggers intestinal immune responses that lead to disruption of the microbiota⁷⁵. In this case, the host immune response to *S. Typhimurium* mediates the disruption of the microbiota rather than the pathogen itself, and the pathogen takes advantage of the host immune response for its own benefit. Intestinal inflammation can promote the production of tetrathionate and nitrates, which are substrates that confer a growth advantage in the gut for *S. Typhimurium* and *Escherichia coli*, respectively^{76,77}. Secreted immune effector molecules such as elastase and AMPs can also be induced by invading Gram-negative pathogens to outcompete the Gram-positive resident microbiota^{78,79}. Similar studies using the intestinal pathogen *Citrobacter rodentium* show how host inflammatory responses can drive a shift in the microbiota favoring the replacement of the resident Firmicutes with Proteobacteria, leading to chronic intestinal inflammation⁷³. Furthermore, during infection with the protozoan parasite *Toxoplasma gondii*, MyD88-dependent production of IFN- γ by CD4⁺ T_H1 cells can cause intestinal dysbiosis leading to death of Paneth cells⁸⁰. Overall, although the immune system has evolved to contain resident microbial challenges, responses can have collateral effects on the microbiota, shifting it to an altered, dysbiotic state. However, in most cases this side effect is favorable for host health in the long term, as it prevents more serious systemic infections such as bacteremia. Host and microbe interactions are constantly evolving; as the host attempts to balance infective responsiveness with tolerance, the microbes relentlessly evolve systems to ensure their survival in the intestinal niche. In a healthy host, many gastrointestinal infections are self-limiting, but the transient and long-term effects of drastically altering the microbiota are just beginning to be resolved.

Table 1 Polymorphisms and mutations associated with IBD

Pathway or site affected	Genes affected in CD	Genes affected in UC	Genes affected in CD and UC
Paneth cells	<i>ITLN1, NOD2, ATG16L1</i>		<i>XBP1</i>
Bacterial sensing	<i>TLR4, TLR9, CD14, MAL</i>		
Innate mucosal defense	<i>NOD2, ITLN1, CARD8, NLRP3, IL18RAP</i>	<i>SLC11A1, FCGR2A/B</i>	<i>CARD9, REL</i>
Autophagy	<i>ATG16L1, IRGM, NOD2, LRRK2</i>	<i>PARK7, DAP</i>	<i>CUL2</i>
Immune cell recruitment	<i>CCL11-CCL2-CCL7-CCL8, CCR6</i>	<i>IL8RA-IL8RB</i>	<i>MST1</i>
Antigen presentation	<i>ERAP2, LNPEP, DENND1B</i>		
IL-23/Th17	<i>STAT3</i>	<i>IL21</i>	<i>IL23R, JAK2, TYK2, ICOSLG, TNFSF15</i>
T cell regulation	<i>NDFIP1, TAGAP, IL2RA</i>	<i>IL2, TNFRSF9, PIM3, IL7R, TNFSF8, IFNG, IL21</i>	<i>TNFSF8, IL12B, IL23R, PRDM1, ICOSLG</i>
B cell regulation	<i>IL5, IKZF1, BACH2</i>	<i>IL7R, IRF5</i>	
Immune tolerance	<i>IL27, SBN02, NOD2</i>	<i>IL1R/IL1R2</i>	<i>IL10, CREM</i>

These changes may promote dysbiosis through loss of immune regulation of the microbiota. CD, Crohn's disease; UC, ulcerative colitis. Adapted from refs. 81 and 105.

Inflammation and immunodeficiency

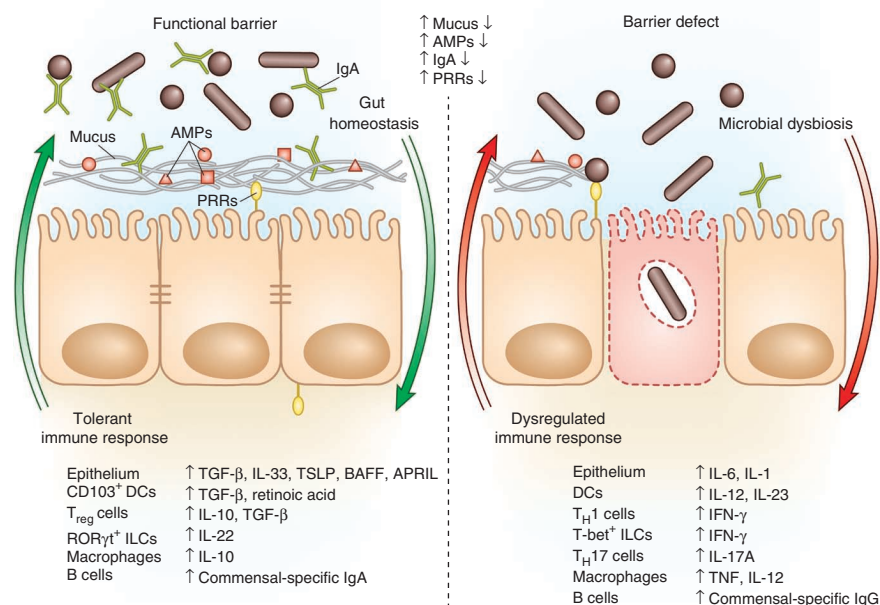
Despite the wide array of immune mechanisms working to promote intestinal homeostasis, chronic inflammation can result from breakdowns in this system. Inflammatory bowel disease (IBD), which encompasses both Crohn's disease and ulcerative colitis, is highly prevalent in developed nations. The currently accepted hypothesis regarding the etiology of IBD is that specific environmental factors trigger intestinal inflammation in a genetically susceptible individual. Although alteration of the intestinal microbiota is one possible 'environmental' trigger, inherited immune defects can also modify the commensal flora in affected individuals, creating a perpetual cycle that ultimately leads to disease. Over 160 genetic loci have been linked with the development of IBD^{81,82} (Table 1), and revealingly many of the identified gene products are involved in mediating interactions between the immune system and intestinal microorganisms. One of the strongest links is between Nod2 mutations and ileal Crohn's disease^{83–85}. A leading

hypothesis is that this is due to an effect on the ileal microbiota, as Nod2-deficient mice have greater bacterial loads in the terminal ileum⁸⁶. Expression of α -defensins is lower in Nod2-deficient mice (compared to wild-type mice) and in humans with Crohn's disease, particularly when a Nod2 mutation is present^{87,88}. Nod2 mutations may interfere with the microbiota, resulting in intestinal inflammation. Alternatively, inhibition of Paneth cell antimicrobial activity may actually be secondary to the inflammation⁸⁹, highlighting the complex nature of the interplay between the host and microbiota in the intestine. Recently, a role for ILCs has been described in the pathogenesis of IBD, as an accumulation of T-bet⁺ IFN- γ -producing ILCs was observed in inflamed tissues of patients with Crohn's disease⁹⁰. It is clear that

IBD encompasses multiple defects in different upstream pathways, resulting in the final common result of chronic intestinal inflammation. It is also important to appreciate the bidirectional relationship that exists as immune dysregulation can drive microbial changes, and shifts in the microbiota can drive immune dysfunction (Fig. 3).

Breakdowns in host-microbial mutualism in the intestine can also occur in a wide array of immunodeficiency-related conditions in humans where the normal functioning of the intestinal immune system is compromised. HIV infection and common variable immunodeficiency (CVID) are examples of acquired or congenital immunodeficiencies that include an enteropathy (chronic intestinal inflammation). Study of affected individuals may therefore provide vital information on immune control of the microbiota. During acute HIV-1 infection, it is estimated that over 50% of the CD4⁺ T cells are preferentially depleted from the lamina propria because of direct infection, immune activation-induced cell death and the cytotoxic

Figure 3 Innate barriers ensure a tolerant response to the microbiota. The presence of a functional barrier, with normal amounts of PRRs, mucus, AMPs and secreted IgA, promotes intestinal homeostasis with the microbiota. The microbiota is segregated away from the IECs, and the intestinal immune system directs a largely tolerant response to the resident commensals. MAMPs stimulate the epithelial secretion of IL-33, TGF- β , TSLP, BAFF and APRIL, all promoting the development of tolerogenic immune cell responses to the microbiota. This cytokine environment enriches for CD103⁺ dendritic cells (DCs), which aid in the development of T_{reg} cells secreting IL-10 and TGF- β . T_{reg} cells and CD103⁺ DCs stimulate the production of commensal-specific IgA. Barrier integrity of the IECs is enhanced by secretion of IL-22 by ROR γ t ILCs in this environment. In immunodeficiency or inflammatory syndromes with an innate barrier defect (for example, IBD, CVID or HIV infection), the intestinal immune system directs a potentially harmful pro-inflammatory response to the microbiota to clear invading bacteria and dysbiosis occurs. In this environment, the epithelium can secrete IL-1 and IL-6 in response to danger signals. Secretion of IL-12 and IL-23 by DCs and macrophages promote a T_H1 and T_H17 response. These T helper cells secrete high levels of IFN- γ and IL-17A, respectively, and T-bet⁺ ILCs also accumulate to produce IFN- γ . A breach in the epithelial barrier by the microbiota in this situation can also lead to higher levels of B cells secreting commensal-specific IgG.



host response⁹¹, contributing to HIV enteropathy. HIV infection also results in relative depletion of T_H17 cells. The exact mechanisms remain unknown and may include direct infection and/or alteration of the T_H17 lineage commitment^{92,93}. Rhesus macaques infected with the homologous retrovirus simian immunodeficiency virus (SIV) as well as HIV-infected humans who have undergone T_H17 depletion exhibit increased translocation of bacteria and viruses^{94–98}. Studies of patients infected with HIV-1, linked with models of *Cd4*^{−/−} mice, provide evidence that CD4⁺ T cells promote anatomical containment of the microbiota and direct tolerogenic responses to microbial signals rather than driving shifts in composition of the microbiota. This has been confirmed in small studies comparing HIV-1–positive and HIV-1–negative humans, where there were no major differences in the overall composition of the microbiome⁹⁹. However, one study did find negative correlations between total bacterial load and duodenal CD4⁺ and CD8⁺ T cell activation (defined by a CD38⁺HLA-DR⁺ phenotype)¹⁰⁰.

CVID is a collection of congenital immunodeficiency syndromes with different underlying causes, which have in common the characteristic of B cell dysfunction. The enteropathy associated with CVID has been poorly described, but it is clear that patients have chronic intestinal inflammation, in some cases similar to celiac sprue¹⁰¹. Whether symptoms are driven by a loss in the regulation of the microbiota has yet to be discovered. However, a recent study described the microbiota-epithelium interactions in B cell-deficient mice as a factor in the normal metabolic functions of IECs and highlighted the similarities seen between B cell-deficient mice and patients with CVID¹⁰².

In many cases, the antibody repertoire in immunodeficient patients is depleted. As IgA is the predominant antibody directed to the intestinal microbiota, gastrointestinal disorders seen in patients with CVID and with HIV infection may be the result of a loss of IgA-mediated regulation of the microbiota. No single pathogen has been associated with CVID gastrointestinal syndromes¹⁰³ and IgG antibodies given to immunodeficient patients to protect against systemic infection do not treat CVID-associated enteropathy^{101,102}. In HIV-infected patients, low numbers of intestinal IgA⁺ plasma cells have also been reported¹⁰⁴. The role of the microbiota in these syndromes is not fully understood and more studies need to be done to determine the etiology of gastrointestinal abnormalities seen in immunodeficiency syndromes. The symptoms of these diseases, however, highlight the importance of a functional intestinal immune system in maintaining host-microbe homeostasis.

Conclusion and perspectives

Over millions of years of host-microbe co-evolution, the intestinal immune system has used various strategies to respond to the microbial environment in a way that benefits host health. These strategies are multilayered, multifunctional and interconnected, and function in a tissue-specific manner to avert immune-mediated epithelial damage. Constant feedback from multiple layers of immunity, including specialized IECs and innate and adaptive immune cells, is required to contain and tolerate the microbial load. As the inherent complexities of the system become clearer, a number of open questions remain. For example, the human gut is colonized with stable communities of eukaryotic microorganisms and a broad diversity of viruses. Being the dominant members of the community, bacteria have been the subject of much of the focus, but how the intestinal immune system interacts with eukaryotic microorganisms is unclear.

As Duerkop and Hooper¹⁰⁶ highlight in this Focus, we are also just beginning to realize how endogenous viruses of our intestinal tract

can interact with our immune system. As techniques for system-wide analysis improve, it will be important to understand the bi-directional relationship of the host-microbial system if new therapeutics are to be developed to combat inflammatory diseases. It is unknown as to what level the microbial composition of an individual is driven by the host immunity and genetics or by inherent dynamics of the microbial system. For example, it is unclear whether microbial dysbiosis seen after deletions of specific immune genes is due to resulting inflammation from proximity of microbiota to IECs or is due to the loss of function of immune effectors themselves. Constant feedback between host and microbe is required to find and maintain a homeostatic balance. In unbalanced situations, such as in immunodeficiency, a working knowledge of how these systems interact will further the potential for targeted system-wide interventions that best improve health and prevent disease.

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COMPETING FINANCIAL INTERESTS

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Erratum: The role of the immune system in governing host-microbe interactions in the intestine

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In the version of this article initially published, a label was missing from Figure 2. The lymphoid structure in the large intestine should be labeled 'Isolated lymphoid follicle'. The error has been corrected in the HTML and PDF versions of the article.