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Regulation of the Immune System by the Resident Intestinal Bacteria

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Abstract

The microbiota is an important factor in the development of the immune response. The interaction between the gastrointestinal tract and resident microbiota is well-balanced in healthy individuals, but its breakdown can lead to intestinal and extra-intestinal disease. We review current knowledge about the mechanisms that regulate the interaction between the immune system and the microbiota, focusing on the role of resident intestinal bacteria in the development of immune responses. We also discuss mechanisms that prevent immune responses against resident bacteria, and how the indigenous bacteria stimulate the immune system to protect against commensal pathobionts and exogenous pathogens. Unraveling the complex interactions between resident intestinal bacteria and the immune system could improve our understanding of disease pathogenesis and lead to new therapeutic approaches.

Keywords

Microbiota; Th17; regulatory T cell; IgA; innate lymphoid cells

The mammalian gastrointestinal (GI) tract is colonized by trillions of microorganisms, including bacteria, fungi, parasites, and viruses. This internal microbial community that is most abundant in the distal intestine is referred to as the microbiota^{1, 2}. In healthy individuals, the intestinal microbiota comprises more than 1000 species of bacteria that contribute to GI homeostasis³. One key role of the resident intestinal bacteria is to promote metabolism of nutrients. Some bacterial members of the microbiota is capable of fermenting diet- and/or host-derived undigested carbohydrates, as well as synthesizing vitamins

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involved in host energy acquisition³. In addition, the resident intestinal bacteria regulate immune homeostasis and pathogen clearance^{2, 4}. The microbiota plays a critical role in the development and function of the GI immune system, but the immune system also regulates the composition and/or function of the microbiota. This bi-directional interaction between the microbiota and the immune system is well-balanced in healthy individuals, but its breakdown can lead to GI diseases, such as inflammatory bowel diseases, as well as extra-intestinal disorders, including metabolic disease². We review the role of the microbiota, and in particular the resident intestinal bacteria, in the regulation of immune responses in the context of GI homeostasis, host defense, and pathogenesis of GI disease.

Resident Intestinal Bacteria in the Function and Development of the Immune System

Structural development of Gut-Associated Lymphoid Tissues (GALTs)

The role of the microbiota in the development of the immune system has been studied for more than a century. A major advance was the establishment of germ-free (GF) and gnotobiotic animal systems. Rearing of GF animals was first attempted in 1890s and succeeded in the early 1910s. Subsequent advances in the methodology eventually led to the establishment of the basic principles of GF technology in the 1940s⁵. Although the microbiota was found to be important for optimal acquisition of nutrients in the intestine, the average survival period of mice housed under GF conditions was longer than that of conventionally raised mice, indicating that the microbiota is not essential for animal survival⁶. However, GF animals exhibit impaired development of GALTs, such as Peyer's patches (PPs) and isolated lymphoid follicles⁶⁻⁸. Notably, a greater number of immunoglobulin (Ig)E⁺ B cells and lower number of IgA⁺ B cells were found in the PPs of GF mice, compared with conventionally raised mice⁹. Colonization of GF mice by commensal microorganisms induces immune reactions in lymphoid cell clusters called germinal centers and IgA development^{10, 11}. Recent studies have begun to elucidate the roles of the microbial molecules, and host signaling pathways that mediate the development of GALTs. For example, NOD1-mediated signaling is required for the development of isolated lymphoid follicles⁸.

Effector T Cells

The microbiota also have an important role in balancing effector T-cell immune responses in the GI tract¹². GF mice have reduced numbers of T-helper (Th)1 and Th17 cells, so the intestinal T-cell immune response in GF animals is primarily controlled by Th2 cells^{13, 14}. The imbalance in Th-cell responses in GF mice can be reversed by reconstitution with conventional microbiota, indicating that microorganisms shape intestinal Th-cell mediated immunity¹⁴.

IL17 responses were first identified in the GI tract in association with *Helicobacter pylori* infection and subsequently an important advance was made when a particular species of commensal *Clostridia*-related bacteria, called segmented filamentous bacteria (SFB), were found to induce Th17-cell development in the small intestine¹⁵⁻¹⁸ (Table 1). Th17 cells are a lineage of CD4⁺ Th cells, which defend against extracellular microorganisms and are also

involved in the development of autoimmune diseases¹⁹. GF mice or mice given antibiotics have greatly reduced numbers of Th17 cells in the small intestine^{16, 20, 21}. Conventionally raised mice from colonies lacking SFB also have low numbers of Th17 cells and the numbers of Th17 cells increase upon SFB colonization^{16, 18}, indicating that SFB induces the development of intestinal Th17 cells.

However, SFB are not the only bacteria that can induce Th17 cells. For example, colonization of GF mice with Altered Schaedler Flora (ASF), a cocktail of 8 defined commensals, also significantly increased the numbers of Th17 cells, although the ASF is less effective than SFB²². Enteric pathogens can also induce a Th17-mediated response, even in the absence of commensal microbes. For example, monocolonization of GF mice with the enteric pathogen *Citrobacter rodentium*, which is used to model enteropathogenic *Escherichia coli* and enterohemorrhagic *E coli* infection in mice, increases the numbers of intestinal Th17 cells²³. Furthermore, colonization of GF mice with the human intestinal microbiota promotes development of Th17 cells, even though SFB is not included^{14, 24}. So, SFB, as well as other members of the human intestinal microbiota and certain bacterial pathogens, are strong inducers of Th17-cell development.

T Regulatory (Treg) cells

Similar to effector T cells, the resident intestinal bacteria is essential for the development and function of Foxp3⁺ Treg cells in the intestine. In the absence of the microbiota, the number of inducible Foxp3⁺ Helios⁻ Treg (iTreg) cells, but not Foxp3⁺Helios⁺ thymic-derived natural Treg cells, is significantly reduced in the colonic lamina propria (LP), but not the LP of the small intestine or mesenteric lymph nodes^{22, 25}. Reduced development of iTreg cells in GF mice can be restored by re-colonization with the intestinal microbiota, indicating that commensals are involved in the generation of colonic Treg cells.

Certain bacterial species within the intestinal microbiota have been shown to possess Treg-inducing activity. For example, colonization of GF mice with *Clostridia* species resulted in robust accumulation of iTreg cells, whereas colonization with mouse intestinal *Bacteroides* spp., *Lactobacillus*, or SFB induced no or small numbers of iTreg cells in the colonic LP²⁵. Atarashi et al., identified 46 strains of the *Clostridium* genus belonging to clusters IV and XIVa (also known as *Clostridium leptum* and *coccoides* groups, respectively) as Treg-inducing *Clostridia* in mice²⁵ (Table 1). Colonization of GF mice with ASF is also sufficient to induce the development of iTreg cells in the intestine²² (Table 1). ASF contains 3 *Clostridia* species belonging to the *Clostridium* cluster XIV; these *Clostridia* species may be responsible for the Treg-inducing capacity of the ASF²⁶. These Treg-inducing *Clostridia* species are present in the mouse and human intestine. In fact, Atarashi et al. further identified 17 Treg-inducing *Clostridia* strains in the microbiota of healthy humans²⁴ (Table 1). These 17 strains belong to the *Clostridium* clusters IV, XIVa, and XVIII, so similar bacteria have Treg-inducing activities in humans and mice²⁴.

Unlike the mouse intestinal microbiota, *Clostridia* are not the only bacterial species in the human microbiota that promotes Treg-cell development. For example, colonization of mice with *Bacteroides fragilis*, a human commensal, robustly facilitates the differentiation of Treg cells and interleukin (IL)10 production by Treg cells, whereas mouse commensal

strains of *Bacteroides* only weakly induce development of Treg cells^{24, 27, 28} (Table 1). However, it is unclear whether *B. fragilis* promotes induction of intestinal Treg cells in humans. Although commensal *Clostridia* are potent inducers of Treg cell development in the intestine of mice and humans, other bacterial species might also induce Treg-cell responses.

Development of B Cells

B cells protect against microbial infections by producing Igs²⁹. Early stages of B-cell development occur not only in fetal liver and the bone marrow, but also in the intestinal mucosa³⁰. Receptors on developing B cells within the intestine are edited differently from those on bone marrow B cells. Intestine-specific editing of B-cell receptors is regulated by extracellular signals induced by resident microorganisms³⁰. The commensal microbiota therefore regulates development of intestine-specific B-cell receptor repertoires.

The microbiota also influences Ig responses within the intestinal mucosa. IgA is secreted primarily on the surface of mucosal tissues, including those of the GI tract, and regulates mucosal homeostasis. Within the GI tract, most IgA-producing B cells mature in PPs upon stimulation by commensal microorganisms³¹, although little is understood about the mechanisms of this process. One possible mechanism involves the effects of the microbiota on the development and organization of GALTs, where IgA-producing cells are generated. In systemic lymphoid organs such as the spleen, intestinal lymphoid follicles contain IgD⁺-IgM⁺-naïve B cells under physiological conditions. In response to antigen stimulation (associated with infection or vaccination), germinal centers form in lymphoid follicles. Here, B cells undergo maturation and proliferation, including class-switching recombination and somatic hypermutation, resulting in appropriate antigen-specific humoral responses³¹. Unlike in systemic lymphoid organs, germinal center formation is observed under steady-state conditions in GALTs—especially PPs, because the GI tract is continuously exposed to commensal bacterial antigens. In GF mice, the lack of microbiota-derived signals results in immature formation of germinal centers in the PPs and reduced generation of IgA-producing B cells³¹.

Innate Lymphoid Cells (ILCs)

ILCs are innate immune cells that have functional characteristics commonly associated with T cells³². ILCs arise from common lymphoid precursors; there are 3 subgroups, based on their different functional properties: T-bet⁺ (group 1), GATA-3⁺ (group 2), and RORγt⁺ (group 3)³². There is increasing evidence of the important roles for group 3 (RORγt⁺) ILCs in intestinal immunity. These ILCs resemble Th17 cells in their cytokine profile (production of IL22 and/or IL17 upon IL23 and IL1β stimulation)³². The extent to which the commensal microbiota influences the development of group 3 ILCs in the intestine is controversial¹². Some studies have shown that the microbiota is required for the differentiation of group 3 ILCs^{33, 34}. Consistently, the proportion of RORγt⁺NKp46⁺CD127⁺NK1.1⁻ or RORγt⁺NKp46⁺CD127⁺NK1.1^{int} ILCs is significantly reduced in GF mice^{33, 34} (Table 1). In the absence of the microbiota, production of IL22 by ILCs is reduced, indicating that the function of ILCs is regulated by commensals³³ (Table 1). In contrast, other studies found that the microbiota does not affect development of RORγt⁺ ILCs^{35–37}.

Lamina Propria Phagocytes

Mononuclear phagocytes such as macrophages and dendritic cells (DCs), along with polymorphonuclear phagocytes (neutrophils), protect many organs against microbial infections, including the GI tract. The resident microbiota appears to regulate the functions of these phagocytes within the intestine. The microbiota is required to upregulate production of pro-IL1 β (the precursor of IL1 β) in resident macrophages³⁸ (Table 1). However, full processing and secretion of pro-IL1 β does not occur in phagocytes until they encounter pathogenic microorganisms. Pathogens such as *Salmonella* and *Pseudomonas aeruginosa* can rapidly induce secretion of the mature form of IL1 β from intestinal macrophages, which contributes to the elimination of infectious pathogens³⁸. Unlike commensals, these pathogens can inject flagellin into the cytosol via type III secretion systems, thereby activating the Nod-like receptor (NLR)C4 inflammasome to induce caspase-1–dependent processing of pro-IL1 β ³⁸. Commensals therefore regulate the protective functions of intestinal macrophages by promoting constitutive expression of pro-IL1 β .

The intestinal microbiota also contributes to the anti-inflammatory functions of intestinal macrophages. Intestinal macrophages spontaneously produce the anti-inflammatory cytokine IL10, which is important for GI homeostasis^{39–41} and controlled by the microbiota⁴¹ (Table 1). In addition, the microbiota modulates the function of neutrophils. During intestinal infection with the pathogen *Clostridium difficile*, commensals translocate to the intestinal tissue, where they induce expression of pro-IL1 β in recruited neutrophils; this protects against *C. difficile* infection-associated mortality, by promoting the removal of translocated commensals⁴² (Table 1). The intestinal microbiota also influences systemic neutrophil responses. For instance, peptidoglycan molecules derived from resident bacteria can prime circulating blood neutrophils to boost their bactericidal ability via NOD1 signaling, which protects against infection with *Streptococcus pneumoniae*⁴³ (Table 1). The microbiota is therefore important for the regulation and function of intestinal phagocytes.

Regulation of the Immune System by Resident Intestinal Bacteria

Intestinal Th17 cells

In vitro studies reported that transforming growth factor (TGF) β and IL6 or IL21 regulate differentiation of Th17 cells, whereas their expansion is mediated by IL23^{44–48}. However, intestinal Th17 cells develop in mice deficient in IL6, IL23, or the IL21 receptor, so other factors appear to contribute to this process in vivo^{16, 49}. Mice deficient in IL1 β or the IL1 receptor have reduced numbers of intestinal Th17 cells⁴⁹. IL1 β production in resident intestinal phagocytes is reduced in GF mice, so commensal microorganisms might induce production of IL1 β in the intestine to promote Th17 development⁴⁹. Administration of IL1 β is sufficient to induce development of intestinal Th17 cells in GF mice⁴⁹. The intestinal microbiota therefore appears to promote development of Th17 cells, at least in part, through the induction of IL1 β (but not IL6)⁴⁹ (Table 1).

How does the microbiota induce IL1 β and Th17 cells in the intestines? Mice deficient in MyD88, an adaptor required for Toll-like receptor (TLR), IL1-like, and IL18 receptor signaling, produce lower levels of IL1 β , and develop fewer Th17 cells in the intestine⁴⁹.

Commensals might therefore induce IL1 β via TLR–MyD88 signaling in the innate immune response pathway⁵⁰.

Atarashi et al. reported that luminal ATP produced by the microbiota promotes development of Th17 cells by activating DCs in the LP²⁰ (Table 1). However, SFB-induced development of Th17 cells was shown to be independent of ATP¹⁸. Ivanov et al. reported that colonization by SFB leads to production of serum amyloid A proteins by intestinal epithelial cells; this might induce Th17-cell development independently of ATP¹⁸ (Table 1). However, little is known about the in vivo roles of serum amyloid A proteins in inducing intestinal Th17 cells. Nonetheless, the microbiota shapes the balance of effector T cell populations—especially of Th17 cells. However, further research is needed to understand the mechanism by which commensals induce Th17 cells in the intestine.

Foxp3⁺-inducible Treg Cells

There is increasing evidence that commensals induce the development of Treg cells via multiple mechanisms. There is controversy over the involvement of host pattern recognition receptors in this process. Induction of colonic Treg cells by mouse *Clostridium* clusters IV and XIVa was found to be independent of TLRs, NODs, and Dectin1²⁵ (Table 1). However, ASF (containing 3 *Clostridium* cluster XIV strains)-induced development of Treg cells is reduced in mice deficient in MyD88 and TRIF²² (Table 1). Moreover, activation of TLR2 signaling by polysaccharide A was required for induction of Treg cells and production of IL10 in mice following colonization with *B fragilis*²⁸ (Table 1). Further studies are needed to determine the precise roles of pattern recognition receptors in the development of Treg cells.

Commensal metabolites were reported to induce the development of Treg cells. Among bacterial metabolites, short-chain fatty acids (SCFAs) have been proposed to regulate development and function of colonic Treg cells^{51–53}. In GF mice, reduced concentrations of luminal SCFAs (such as acetate, propionate, isobutyrate, and butyrate) correlated with impaired development of intestinal Treg cells^{51–53}. Importantly, reconstitution with commensal bacteria or administration of SCFAs restores the numbers of Treg cells in GF mice, supporting a role for bacterial metabolites in Treg cell development^{51–53}. Several G protein-coupled receptors (GPRs) expressed by intestinal epithelial cells and/or hematopoietic cells can be activated by SCFAs. So, SCFAs affect different types of cells in the intestine to regulate homeostasis in the GI tract^{54, 55}. SCFAs affect Treg cell function via epigenetic regulation of the *Foxp3* gene^{51–53}. SCFAs, particularly butyrate, can act directly on T cells, increasing acetylation of the *Foxp3* locus^{51–53} (Table 1). Consistently, mice deficient in *Ffar2*, which encodes Gpr43, have reduced numbers of Treg cells, which cannot be restored by SCFA administration⁵¹. In addition to their direct effects on Treg cells, SCFAs can also promote development of Treg cells via non-immune cells. For example, colonization of GF mice with 17 human *Clostridium* strains induced the luminal levels of acetate, propionate, isobutyrate, and butyrate to promote TGF β production by the intestinal epithelium²⁴. SCFA-induced production of TGF β appears to be necessary for the induction of Treg cells by commensal *Clostridia* strains in humans and mice^{24, 25}.

Although little is known about the mechanism by which SCFAs, via TGF β , promote development of Treg cells, LP DCs appear to be involved. CD103-expressing subsets of DCs in the LP (including CD103⁺CD11b⁺CD11c⁺ and CD103⁺CD11b⁺CD11c⁺) induce differentiation of Treg cell from naïve CD4⁺ T cells via TGF β and retinoic acid (RA)^{56–58}. In addition to DC subsets, F4/80⁺CD11b⁺CD11c⁺ LP macrophages produce RA and induce the differentiation of intestinal Treg cells in the presence of TGF β ⁴⁰. SCFA-induced TGF β might therefore regulate intestinal Treg cells along with LP DCs and macrophages. Depletion of CD103-expressing LP DCs reduces numbers of intestinal Treg cells, even in the presence of the intestinal microbiota⁵⁹. Consistent with these observations, it was recently reported that Gpr109a, a GPR family member, is expressed in intestinal macrophages and DCs⁶⁰. Notably, butyrate and niacin (vitamin B3) produced by commensal bacteria can activate Gpr109a-expressing DCs and macrophages via Gpr109a to promote the differentiation of Foxp3⁺ Treg cells and IL-10-producing Tr1 cells⁶⁰.

In addition to de novo generation of Treg cells, the intestinal microbiota can promote homing of Treg cells to the large intestine. Kim et al. found that the GPR15 receptor is primarily expressed on Treg cells in the large intestine, where it acts as a homing molecule for Treg cells²⁴ (Table 1). Mice deficient in GPR15 are characterized by reduced numbers of Foxp3⁺ Treg cells in the large intestine, but not the small intestine or the spleen⁶¹. GPR15 expression on Treg cells is reduced when the resident microbiota is depleted with broad spectrum antibiotics⁶¹. GPR15 expression is induced by SCFA administration, so homing of Treg cells appears to also be regulated by bacterial metabolites⁵¹. Likewise, dietary folic acids regulate the survival of Foxp3⁺ Treg cells in the colon⁶². Mammals do not have synthetic pathways for de novo production of folic acids, so folic acids derived from commensals are likely to be involved in the induction of intestinal Treg cells⁶³.

Induction of Treg cells by the microbiota protects mice from colitis, infection with enteric pathogens, and allergic diarrhea^{24, 25, 64}. For example, *Clostridium*-induced Treg cells suppress colitis and allergic diarrhea in mice^{24, 25}. Likewise, IL10-producing Treg cells, induced by *B fragilis*, reduce intestinal inflammation caused by *Helicobacter hepaticus*^{28, 64}. These studies indicate that certain commensal species, particularly those belonging to the *Clostridium* genus, contribute to the de novo generation, homing, and functional maturation of Treg cells in the colon to help maintain GI homeostasis.

IgA-producing B Cells

There are several pathways by which the microbiota regulates development of IgA-producing cells in the intestine. One pathway involves MyD88 signaling. The intestines of mice deficient in MyD88 have reduced numbers of CD11b⁺ IgA⁺, but not CD11b⁺ IgA⁺, plasma cells⁶⁵ (Table 1). By unclear mechanisms, commensals activate MyD88 signaling in LP DCs and follicular DCs—cells that promote the generation of IgA⁺ B cells. In response to microbial stimulation, follicular DCs in PPs secrete TGF β , CXCL13, and B-cell activating factor (BAFF, a member of the TNF family), which stimulates class switching and IgA production⁶⁶ (Table 1). Likewise, the microbiota activates MyD88 signaling in subsets of LP DCs, leading to their expression of RA, TGF β , TNF α , inducible nitric oxide

synthetase, BAFF, and proliferation-inducing ligand to promote the generation of IgA⁺ B cells^{67–69} (Table 1).

Although the microbiota is important for the development and maturation of intestinal B cells, the specific bacteria that mediate these processes have not been identified. Monocolonization of GF mice with SFB, in addition to Th17 cells, can induce IgA production^{11, 70} (Table 1). However, the amount of IgA in SFB monocolonized mice is low compared to mice colonized with conventional microbiota, indicating that other bacterial species, or mixtures of commensals, are required for maximal induction of intestinal IgA¹¹. On the other hand, transient monocolonization with a mutant strain of *E coli* that is unable to proliferate in vivo induces amounts of intestinal IgA comparable to those observed following colonization with multiple commensal bacteria⁷¹. Although the IgA induced is specific for this bacterium, exposure of the *E coli*-colonized mice to different bacteria limits the persistence of *E coli*-specific IgA memory response⁷¹. Continuous stimulation by bacteria might therefore be required for sustained production of commensal-specific IgA in the intestine.

Group 3 Innate Lymphoid Cells

The mechanisms by which commensal bacteria regulate the development and function of ILCs are also unclear. One mechanism could involve the direct activation of ILCs by bacterial components. Human CD127⁺ RORγt⁺ ILCs express TLR1, TLR2, and TLR6, and stimulation with TLR2 ligands induces the production of IL22 in these cells⁷². In addition, bacterial metabolites can activate ILCs. Indole-3-aldehyde, a tryptophan catabolite produced by the microbiota, stimulate ILCs through the aryl hydrocarbon receptor (AhR) to induce production of IL22 by ILCs⁷³ (Table 1). Microbial products therefore appear to directly activate ILCs. There is also evidence that the intestinal microbiota regulates the function of ILCs indirectly, by activating other immune cells. For example, commensal bacteria can orchestrate the recruitment of CX₃CR1⁺ intestinal macrophages to the intestine, which subsequently modulate IL-22 production by ILCs^{74, 75} (Table 1). Additionally, in response to bacterial products, CD103⁺CD11b⁺ LP DCs produce IL23, through stimulation of TLR5, leading to secretion of IL22 by RORγt⁺ ILCs⁷⁶ (Table 1). On the other hand, GF mice have increased production of IL22 by RORγt⁺ ILCs, indicating that the microbiota could be a negative regulator of IL22 production³⁷.

IL25, a cytokine generated by intestinal epithelial cells upon stimulation with commensals, activates LP DCs via the IL17BR to inhibit the production of IL22 by ILCs through an unknown mechanism³⁷ (Table 1). The microbiota therefore acts as a positive and negative regulator of intestinal ILCs. The dual role of the microbiota in ILC activation and development might arise through differences in access of microbes to immune cells. Under steady-state conditions, the intestinal epithelium is intact and the microbiota may stimulate epithelial cells to repress ILC function. However, during inflammation, the epithelial barrier is disrupted, allowing the translocation of commensals and luminal products into the intestinal tissue, which activates LP immune cells, including ILCs.

Activation of ILC functions by commensals is important for protection against microbial infection. For example, IL22-mediated production of RegIIIγ, a secreted antimicrobial

peptide, by the intestinal epithelium protects against enteric infection by *C. rodentium*^{33, 77, 78}. Likewise, administration of tryptophan catabolites increases production of IL22 by ILCs, which is associated with protection against fungal infection and dextran sodium sulfate-induced colitis⁷³. In addition, the intestinal microbiota contributes to the stabilization of ROR γ t expression in group 3 ILCs by stimulating epithelial cells to produce IL7—a cytokine that stabilizes expression of ROR γ t³⁶. GF mice express reduced levels of IL7 in the intestinal epithelium, which subsequently downregulates expression of the ROR γ t in ILCs³⁶. Decreased ROR γ t expression in ILCs leads to the conversion of ILCs into IFN γ -producing pathogenic ILCs, which promote intestinal inflammation³⁶ (Table 1). The microbiota can promote GI homeostasis and protect against infection and inflammation by regulating intestinal ILCs—particularly group 3 ROR γ t ILCs.

Lamina Propria Phagocytes

LP mononuclear phagocytes, including macrophages, express pro-IL1 β even in the steady state, which is important for rapid production of mature IL1 β in response to pathogen invasion^{38, 49}. Accumulation of pro-IL1 β in LP macrophages requires resident microbiota and commensal-induced MyD88 signaling^{38, 49} (Table 1). Likewise, the anti-inflammatory function of intestinal macrophages, such as spontaneous production of IL10, is also controlled by the microbiota, in a MyD88-independent manner⁴¹ (Table 1). In addition, the function of neutrophils is regulated by commensal by-products such as SCFAs, which directly activate neutrophils and increase their bactericidal activity through GPR43⁷⁹ (Table 1).

Regulation of Resident Intestinal Bacteria by the Immune System

Compartmentalization of Intestinal Microbes

Although the resident intestinal bacteria are important for the development of intestinal immune responses, excessive immune stimulation by commensals can lead to inappropriate activation of immune cells and harmful intestinal inflammation. To avoid excessive stimulation by resident microbes, the intestinal immune system is separated from luminal microorganisms by a physical barrier. In the colon, the epithelial surface is covered by an inner mucus layer that shields the immune system from microbes and mechanical stress, whereas large numbers of commensal bacteria colonize the outer mucus layer^{80, 81} (Figure 1). Also, RegIII γ limits the penetration of luminal bacteria into the intestinal mucosa and acts as an additional barrier⁸² (Figure 1). Group 3 ILCs contribute to the induction of RegIII γ by epithelial cells by producing IL22. Depletion of IL22-producing ILCs leads to dissemination of the commensal *Alcaligenes xylosoxidans*, which promotes intestinal damage and systemic inflammation⁸³.

Secreted IgA also has an important role in the compartmentalization of the commensal microbiota on the mucosal surface (Figure 1). Polymeric IgA is transported via the polymeric Ig receptor (pIgR), expressed on intestinal epithelial cells, and released into the intestinal lumen as secretory IgA⁸⁴. Secretory IgA binds to luminal commensal bacteria and prevents their penetration across the epithelial barrier^{31, 85}.

Neutrophils also contribute to the compartmentalization of commensal organisms. Although there is no evidence that neutrophils have a role in the compartmentalization of commensals under steady-state conditions, neutrophils migrate to the intestinal lumen after pathogen invasion and build intraluminal cellular casts that encapsulate commensals, thereby limiting their translocation⁸⁶ (Figure 1). Furthermore, neutrophils are recruited to the intestinal tissue and limit the translocation of commensals after epithelial damage from release of toxins by *C difficile*⁴². The mucosal epithelium and the immune system therefore limit access of the commensal microbiota to host tissues, to maintain their symbiotic relationship.

Local Hyporesponsiveness Against Resident Intestinal Bacteria

In addition to compartmentalization of luminal microorganisms, the GI immune system has developed multiple mechanisms to avoid inappropriate immune responses to the commensal microbiota. Different subsets of regulatory Treg cells in the intestine (including Foxp3⁺ iTregs and IL10-producing Foxp3⁻ Tr1 cells)⁸⁷ are thought to be required to suppress proliferation of commensal-reactive effector T cells. In the absence of these Treg cells, commensal-reactive T cells in the intestine proliferate, leading to colitis^{88, 89}. Adoptive transfer of T cells specific for flagellin antigens derived from certain commensals, such as *H hepaticus*, causes colitis in mice, but not in GF mice^{90, 91}. Different subsets of Treg cells (CD25⁺ and CD25⁻ CD45RB^{low}CD4⁺ T cells) suppress proliferation of commensal-reactive T cells⁸⁹, via IL10 but not TGFβ. These findings indicate that CD25⁺Foxp3⁺IL10⁺ Treg cells and CD25⁻Foxp3⁻ IL10⁺ Tr1 cells are both involved in this regulatory mechanism^{87, 89}. So, suppression of commensal reactive effector T cells by Treg cells is important for prevention of immune reactivity against the commensal microbiota (Figure 1).

A recent study identified a unique role for ILCs in regulating effector T-cell responses against commensals (Figure 1). Hepworth et al. found that intestinal group 3 ILCs express MHC-II and are capable of processing and presenting antigens. Notably, MHC-II⁺ ILCs present commensal antigens to bacteria-reactive CD4⁺ T cells to inhibit their activation and to suppress pathologic immune responses⁹² (Figure 1). In addition to Treg cells, intestinal mononuclear phagocytes, such as macrophages, also contribute to intestinal homeostasis, by limiting excessive immune responses against commensal bacteria (Figure 1). Macrophages recognize and eliminate bacteria via phagocytic activity and induction of robust inflammatory responses. Beside their anti-microbial and inflammatory roles, macrophages can reduce inflammation by producing anti-inflammatory cytokines such as IL10 and TGFβ⁹³. In the GI tract, intestinal macrophages are hypo-responsive to microbial ligands, and do not produce significant amounts of inflammatory molecules upon bacterial stimulation³⁸⁻⁴⁰. Yet, intestinal macrophages retain their phagocytic and bacteriocidal functions and spontaneously secrete IL10^{39, 40, 94} (Figure 1). Intestinal macrophages can phagocytose luminal bacteria that penetrate the epithelial barrier without inducing excessive inflammatory responses (Figure 1). Taken together, the intestinal immune system has developed multiple regulatory mechanisms of tolerance against the resident intestinal bacteria.

The Immune System Shapes the Composition and Function of the Intestinal Bacteria

The intestinal immune system actively influences the structure and function of the commensal microbiota. Defective intestinal IgA responses, due to deficiency of or mutations in activation-induced cytidine deaminase, alter the composition of the gut microbiota^{95, 96} (Figure 2). Likewise, loss of inhibitory co-receptor programmed death-1 reduces the bacterial-binding capacity of IgA, which alters the intestinal bacterial community⁹⁷. In addition to altering the composition of the resident intestinal bacteria, binding of IgA to the commensal *Bacteroides thetaiotaomicron* affects expression of bacterial genes⁹⁸ (Figure 2). Importantly, an altered microbial community can facilitate outgrowth of potentially pathogenic microorganisms (pathobionts) or modify gene expression in the commensal bacteria, allowing harmless bacteria to become harmful. Defects in the function of group 3 ILCs can lead to abnormal expansion of SFB⁹⁹ (Figure 2), which can increase responses of intestinal Th17 cells and promote the development of spontaneous colitis under certain conditions⁹⁹ (Figure 2). This abnormal accumulation of SFB appears to require a lack of IL22 production by ILCs⁹⁹. The composition of the microbiota in IL22-deficient mice is altered compared with wild-type mice; this alteration is transmissible and colitogenic¹⁰⁰. Furthermore, T-bet deficiency in Rag-deficient mice leads to accumulation of potential pathobionts *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Helicobacter typhlonius*, which are associated with spontaneous colitis^{101–103} (Figure 2).

Defects in the NLR family have also been associated with dysbiosis. Mice deficient in NLRP6 have impaired production of IL18, which is associated with an abnormal expansion of the commensals *Prevotellaceae* and TM7¹⁰⁴ (Figure 2). However, the observation that immune factors such as IL22 and host innate receptors regulate the composition of the intestinal microbiota should be taken with caution—recent studies evaluating TLR-deficient mice found that the composition of the microbiota largely depends on maternal transmission of the microbiota from isolated mouse colonies, rather than any specific TLR defect¹⁰⁵. Because few of these studies were performed in littermates, the observed changes in mutant mice might result from differences in ancestry, rather than an immune defect.

Perspectives

Mounting evidence indicates that the intestinal microbiota regulates the development and function of the immune system, which in turn, shapes the microbial community and/or regulates immune responses against the microbiota. However, diet is an important determinant of the composition and distribution of the microbiota⁴. Bacterial species in the intestine have distinct nutritional requirements, based on their distinct metabolic pathways and abilities to access luminal nutrient sources. Commensal microorganisms regulate each other through competition as well as by providing nutrients based on their metabolic profiles^{23, 106}. Therefore, competition for nutrients among different commensal species determines, at least in part, the balance and structure of the intestinal microbial community.

However, the immune system could, at least partially, regulate the composition and/or amounts of luminal nutrients. For instance, enteric inflammation may influence the intestinal microbial community by altering luminal metabolites and nutrients in the intestine. There is evidence that intestinal inflammation can affect luminal nutrient availability, leading to a

microbial shift within the intestine^{107, 108}. More research is needed to increase our understanding of how the immune system affects luminal metabolic profiles; this could provide new insights into the mechanisms by which immune cells control the intestinal microbiota. Moreover, novel nutritional therapies, based on better understanding of the metabolic pathways that regulate commensal populations, could be developed to correct disease-associated dysbiosis.

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Figure 1. Multiple Firewalls Limit Immune Responses Against Resident Intestinal Bacteria

The intestine has firewalls that limit inappropriate immune responses against commensal bacteria. One mechanism involves compartmentalization of the resident bacteria by a thick mucus layer, anti-microbial peptides (such as RegIII γ), or secreted IgA, which all prevent access of the resident bacteria to the epithelium. Neutrophils can migrate out to the intestinal lumen and build intraluminal cellular casts that encapsulate commensals, limiting the penetration of luminal microbes. A second mechanism involves microbial hypo-responsiveness of resident intestinal macrophages. In contrast to monocytes and macrophages in systemic tissues, intestinal macrophages are hypo-responsive to microbial stimuli and produce only limited amounts of inflammatory cytokines such as TNF α or IL12. Furthermore, resident phagocytes spontaneously produce anti-inflammatory cytokines, such as IL10. A third mechanism involves active suppression of microbe-reactive effector T cell responses by Foxp3⁺ iTreg cells and Foxp3⁺ Tr1 cells via production of IL10. In addition, MHC-II⁺ ILCs present bacterial antigens, which limits activation of commensal-reactive CD4⁺ T cells.

Figure 2. Regulation of the Resident Intestinal Bacteria by the Immune System

Direct binding of IgA to the commensal *B. thetaiotaomicron* regulates bacterial gene expression. IgA also regulates the composition of the commensal bacterial community. IL-22 produced by ROR γ ⁺ group 3 ILCs induces production of RegIII γ by IECs, which limits the growth of SFB. Overgrowth of SFB increases numbers of Th17 cells and Th17-cell-mediated intestinal inflammation. ROR γ ⁺ ILCs limit expansion of the commensal pathobiont *A. xylosoxidans* in PPs. Expression of T-bet by ILCs limits the accumulation of certain commensal pathobionts, such as *K. pneumoniae*, *P. mirabilis*, or *H. typhlonius*. IL-18 is induced by the intestinal epithelium via a NLRP3-dependent pathway. IL-18 limits the overgrowth of the pathobionts Prevotellaceae and TM7 via unidentified mechanisms.

Table 1

The resident intestinal bacteria regulate the development and function of intestinal immunity

Immune cell	Bacteria (bacterial molecules)	Host cell mediator	Host receptor	Mediator	Note	Ref
Effector T cells						
Th17	Segmented filamentous bacteria	Intestinal epithelium	unknown	Serum amyloid A	MyD88, TRIF, RIP2 independent	16, 17, 18
	Not identified	LP DC and Macrophage	unknown	IL-1 β	MyD88 dependent	48
	Not identified (ATP)	CD70 ^{hi} CD11c ^{low} DC	P2XR, P2YR	IL-6, IL-23	MyD88, TRIF, RIP2 independent	20
Regulatory T cells						
Foxp3 ⁺ Treg	<i>Clostridium</i> clusters IV and XIVa	Intestinal epithelium	unknown	TGF- β	MyD88, TRIF, RIP2, Card9 independent	25
	Altered Schaedler flora	unknown	unknown	unknown	MyD88, TRIF dependent	22
	<i>Bacteroides fragilis</i> (PSA)	CD11c ⁺ DC	TLR2/MyD88	unknown		27, 28
	Not identified (SCFAs)	Treg cells	GPR43	unknown	Epigenetic modification of Foxp3	51, 52, 53
	Human 17 <i>Clostridium</i> strains (SCFAs)	Intestinal epithelium	TGF- β	unknown		24
	Not identified (Butyrate, niacin)	LP DC and Macrophage	GPR109a	RA, IL-10	Induce Foxp3 ⁺ IL-10-producing Tr1 cells	60
IgA-producing B/plasma cells						
IgA ⁺ B cell	Segmented filamentous bacteria	unknown	unknown	unknown		11, 70
	Not identified	Follicular DC	TLR/MyD88	TGF- β , CXCL13, BAFF	Bacterial ligands synergize with RA	66
	Not identified (flagellin)	CD11c ⁺ CD11b ⁺ TLR5 ⁺ DC	TLR5	RA		69
	Not identified	TNF- α /iNOS-producing DC	TLR/MyD88	TGF- β , BAFF, APRIL	Reduced in GF mice	67
	Not identified	LP DC	unknown	RA	Synergize with IL-5, IL-6	68
CD11b ⁺ IgA ⁺ PC	Not identified	unknown	MyD88	unknown	CD11b ⁺ IgA ⁺ PCs are microbe-independent	65
Group 3 Innate lymphoid cells						
ROR γ t ⁺ NKp46 ⁺ CD127 ⁺ NK1.1 ^{int} ILC	Not identified	unknown	unknown	unknown	Reduced in GF mice	33, 34
NKp46 ⁺ ILC	<i>Lactobacilli</i> (indole-3-aldehyde)	NKp46 ⁺ ILC	Ahr	Direct	Enhance IL-22 production	73
CD4 ⁺ ROR γ t ⁺ CD127 ⁺ LTi cell	Not identified	CX ₃ CR1 ⁺ DC	unknown	IL-23	Enhance IL-22 production	74, 75
c-kit ⁺ CD127 ⁺ Scal ⁺ NKp46 ⁺ CD4 ⁺ ILC	Not identified (flagellin)	CD103 ⁺ CD11b ⁺ DC	TLR5	IL-23	Enhance IL-22 production	76

Immune cell	Bacteria (bacterial molecules)	Host cell mediator	Host receptor	Mediator	Note	Ref
ROR γ ⁺ LTi and ROR γ ⁺ NKp46 ⁺ ILC	Not identified	Intestinal epithelium	unknown	IL-25	Activates IL-17BR ⁺ DC to suppress IL-22 by ILC	37 Kamada and Núñez
ROR γ ⁺ NKp46 ⁺ CD127 ⁺ NK1.1 ^{low} LTi	Not identified	Intestinal epithelium	unknown	IL-7	Stabilizes the expression of ROR γ T	36
Mono-, poly-morphonuclear phagocytes						
CD11b ⁺ macrophage	Not identified	unknown	MyD88	unknown	Induce pro-IL-1 β	38
CD11b ⁺ macrophage, CD11c ⁺ DC	Not identified	unknown	MyD88	Direct	Induce pro-IL-1 β	49
CD11b ⁺ CD11c ^{-/-} macrophage	Not identified	unknown	unknown	unknown	Enhance IL-10 production	41
Neutrophil	Not identified	Neutrophils	unknown	Direct	Induce pro-IL-1 β	42
	Not identified (SCFAs)	Neutrophils	GPR43	Direct	Induce ROS, increase phagocytic activity	79
	Not identified (PGN)	Neutrophils	NOD1	Direct	Enhance bactericidal capacity	43