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Intestinal Bacteria and the Regulation of Immune Cell Homeostasis

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Abstract

The human intestine is colonized by an estimated 100 trillion bacteria. Some of these bacteria are essential for normal physiology, whereas others have been implicated in the pathogenesis of multiple inflammatory diseases including IBD and asthma. This review examines the influence of signals from intestinal bacteria on the homeostasis of the mammalian immune system in the context of health and disease. We review the bacterial composition of the mammalian intestine, known bacterial-derived immunoregulatory molecules, and the mammalian innate immune receptors that recognize them. We discuss the influence of bacterial-derived signals on immune cell function and the mechanisms by which these signals modulate the development and progression of inflammatory disease. We conclude with an examination of successes and future challenges in using bacterial communities or their products in the prevention or treatment of human disease.

Keywords

commensal; bacteria; IBD; allergy; antibiotic; germ-free; microbiota; colitis

INTRODUCTION

Microorganisms are the most abundant life form on earth. Although many are free living, some have evolved to participate in close and often long-lasting interactions with multicellular species. Some of these relationships are pathogenic, whereas others are beneficial to the multicellular host. Such beneficial relationships have evolved to represent a conserved feature of multicellular life, important for normal development and physiology in plants (1), insects (2), nematodes (3), fish (4), birds (5), and mammals (6).

After birth, the epithelial surfaces of mammals are colonized with viruses, fungi, bacteria, protozoa, and helminths, creating complex microbial communities in multiple environmental niches. The mammalian intestine is the best studied of these microbial environments. By adulthood, the mammalian intestine is colonized by members of all three domains of life; bacteria are the most abundant, with more than 100 trillion individual organisms (7). Several

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terms have been used to describe the relationship between microorganisms and multicellular organisms (see sidebar), but for the purposes of this review we use “intestinal bacteria” to refer to all bacteria that inhabit the intestine and the term “beneficial” to specify those that participate in a mutualistic or commensal relationship. In some cases, millennia of evolution have resulted in mutualistic relationships between beneficial bacteria and the multicellular host (8). For example, beneficial bacteria supply essential nutrients, aid in the digestion of otherwise indigestible compounds, promote angiogenesis and enteric nerve function, defend against opportunistic pathogens, and contribute to the development and regulation of the mammalian immune system (9, 10).

Although signals derived from intestinal bacteria are important for normal mammalian development and physiology, alteration of these communities (dysbiosis) in patients or animal models is associated with multiple disease states including inflammatory bowel disease (IBD) (11), obesity (12), cancer (13), diabetes (14), and allergy (15). In many cases, altered immune responses to intestinal bacteria contribute to inflammation (16, 17), implicating dysbiosis as a biomarker and a potential trigger for disease. As such, understanding how signals derived from intestinal bacteria influence the mammalian immune system has important implications for defining the etiology of human inflammatory diseases as well as for the development of preventative or therapeutic intervention strategies.

This review focuses on the influence of intestinal bacteria on the mammalian immune system. We first give an overview of the role of intestinal bacteria in mammalian health and disease. We then review the acquisition and composition of bacterial communities in the mammalian intestine, with particular attention given to genetic and environmental factors that influence bacterial community structure. Next, we discuss immunomodulatory signals derived from intestinal bacteria, the receptors that recognize them, and their influence on innate and adaptive immune cell homeostasis. Finally, we discuss the prospects of exploiting our knowledge of signals from intestinal bacteria to prevent or treat human disease.

INTESTINAL BACTERIA IN MAMMALIAN HEALTH AND DISEASE

Although beneficial bacteria colonize all mammalian epithelial surfaces, the gastrointestinal tract has the largest bacterial burden, with more than 100 trillion individual organisms at a density of 10^{11} to 10^{14} cells per gram of luminal contents (7, 18). The bacterial communities of the mammalian intestine are also some of the best characterized; studies carried out as early as the 1960s using culture-based and microbiological identification methods began to identify the major bacterial groups present in the mammalian intestine (19). Currently, molecular advances in DNA bar coding and 454 pyrosequencing of 16S ribosomal RNA gene segments are allowing previously unattainable insights into nonculturable bacterial communities (20–23) and are placing species estimates from conservative numbers of 1000–2000 to numbers as high as 15,000–40,000 individual members (24).

Over the past century, studies in animals and humans have identified important roles for bacterial signals in promoting the optimal digestion of food (25), maintaining epithelial homeostasis (26), modulating fat metabolism (27), promoting angiogenesis (28) and enteric nerve function (29), supporting resistance to infection (30), and promoting normal

development and regulation of immune cell homeostasis (6) (Figure 1). Despite these beneficial sequelae, dysbiosis may be both a biomarker and a potential contributing factor to human inflammatory diseases.

For example, IBD is thought to result from inappropriate and ongoing mucosal immune responses to normal intestinal bacteria (31). Tolerance to intestinal bacteria is broken in IBD (32), leading to inappropriate local (33) and systemic (34, 35) immune responses to intestinal communities that may contribute to pathogenesis (16). Additionally, bacterial communities from the intestine of IBD patients have a reduced diversity compared with those from healthy individuals (11), and IBD patients display aberrant cytokine production, T cell activation, and IgG antibody responses to intestinal bacteria (32, 33). Genetic susceptibility loci have been identified for the inflammatory bowel diseases Crohn's and ulcerative colitis, including mutations in the pattern-recognition receptor NOD2 (nucleotide-binding oligomerization domain-containing protein 2) (36, 37), a component of the innate immune system that is important for immune recognition and responses to intracellular bacteria (38) (see below). These findings implicate altered immune responses to intestinal bacteria in the pathogenesis of IBD.

Animal models of IBD have provided additional insights into the influence of intestinal bacteria on the pathogenesis of this disease. Reducing microbial stimulation in murine models of IBD, achieved by rearing animals under germ-free conditions, ameliorates intestinal disease. For example, IL-2-deficient mice spontaneously develop intestinal inflammation when raised under conventional conditions but have a delayed and milder disease course when raised under germ-free conditions (39). Similarly, both IL-10-deficient or TCR $\alpha\beta$ -deficient mice develop spontaneous colitis associated with inappropriate inflammatory immune cell responses when maintained under conventional conditions but were protected against disease when maintained under germ-free conditions (40, 41). These findings support an essential role for microbial-derived signals in driving pathogenic inflammatory responses in these models.

Antibiotic treatment can also ameliorate disease in murine models of IBD. For example, mice deficient in the multiple drug resistance gene (*mdr1a*) developed spontaneous colitis when housed under specific pathogen-free conditions but were protected from disease by oral antibiotic treatment (42). In addition, mice deficient in keratin-8, a major intermediate filament protein present in the intestinal epithelia, were protected from spontaneous colitis by oral antibiotic treatment (43), as were IL-10-deficient mice (44, 45). These findings suggest that the role of bacterial-derived signals in disease pathogenesis is not purely developmental; rather, they identify bacterial signals as important in the maintenance of intestinal inflammation.

In addition to contributing to inflammatory states, dysbiosis alone can cause disease in otherwise healthy animals. For example, mice deficient in the inflammatory transcription factor T-bet and the recombinase-activating gene RAG2 (TRUC mice) developed spontaneous colitis that was ameliorated by antibiotic treatment (46). When wild-type mice were cohoused with TRUC mice, they also developed colitis, implicating vertical and

horizontal transmission of colitogenic bacterial communities as a cause of disease in immunocompetent animals (46).

Altered signals from intestinal bacteria may also influence risk of developing asthma and other systemic atopic disorders in humans (17). Atopy describes inappropriate, type 2 inflammatory responses to environmental allergens (reviewed in 47), and some patients with atopic diseases have altered intestinal bacterial communities (15). In addition, antibiotic treatment of children increases the risk of developing asthma later in life (48) (presumably as a result of altering intestinal communities), as does early colonization with the intestinal bacterium *Bacteroides fragilis* (49), a bacterial group that increases in frequency upon antibiotic treatment of mice (50). Similarly, colonization with *Bifidobacterium*, *Clostridium difficile*, or *Escherichia coli* is associated with the development of eczema in humans (15, 51, 52), an association that may be related to formula feeding (53), although this hypothesis remains to be tested directly.

Animal models have provided important insights into the influence of intestinal bacteria on systemic immune responses that may contribute to disease states. For example, outgrowths of *Candida albicans* after antibiotic treatment of conventional mice were associated with the development of a CD4⁺ T cell-mediated allergic airway disease (54). In addition, inflammatory responses following subcutaneous injections of carrageenan, lipopolysaccharide (LPS), TNF- α , IL-1 β , or the chemokine CXCL1 were reduced in germ-free mice (55). These immune defects were reversed through conventionalization, or the systemic administration of LPS, implicating bacterial signals in the regulation of systemic inflammatory responses (55). Finally, intestinal bacteria may also influence the development of type 1 diabetes, as nonobese diabetic mice deficient in the Toll-like receptor (TLR) adaptor molecule MyD88 are protected against diabetes development (14). Taken together, these findings implicate signals from intestinal bacteria in the regulation of local and systemic inflammatory responses that contribute to disease pathogenesis.

BACTERIAL COMPOSITION AND COLONIZATION DYNAMICS IN THE MAMMALIAN INTESTINE

Humans and other mammals are born from a sterile environment and subsequently acquire intestinal bacteria during their first months of life (56). Early studies using culture-based and microbiological identification methods identified lactobacilli, anaerobic streptococci, and members of the *Bacteroides* genus as residents of the normal adult human intestine (19). However, a large percentage of intestinal bacteria are anaerobes that lack the enzymes necessary for the detoxification of oxygen. As such, even under ideal conditions, it is estimated that only half of bacteria in stool are culturable (57).

More recently, DNA bar coding and 454 pyrosequencing of 16S ribosomal RNA gene segments have provided more accurate characterization of intestinal communities. These studies have identified the Firmicutes and Bacteroidetes phyla as the major bacterial groups present in the mammalian intestine (20–23) (Figure 2). Of the Firmicutes, 95% belong to the Clostridia class, whereas large variations exist in the Bacteroidetes phylotypes among individuals (20–22, 58). Other phyla present in relatively low abundance include the

Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia (20, 21, 23, 58, 59). New sequencing methods also allow for metagenomic analysis of intestinal communities and are providing novel insights into the influence of microbial-derived genes and gene products on normal mammalian physiology (24).

Bacterial communities exhibit differences along the length of the colon and between the luminal and mucosal-associated microenvironments (20, 60, 61), suggesting that defined microbial communities at different anatomical locations may be important for normal mammalian physiology (Figure 2*b* and 2*c*). For example, although lactobacilli have been cited for potential probiotic effects (62) and can be isolated from approximately 80% of adults, they represent a relatively low proportion of luminal bacteria (58). However, these potentially beneficial microorganisms represent a much higher proportion of mucosal-associated bacteria (up to 13%) in the mammalian intestine (Figure 2) (50).

Although intestinal bacteria are likely to be continuously acquired, mammals undergo two dominant phases of intestinal colonization, the first during breast milk or formula feeding and the second upon weaning to solid foods. Mammalian breast milk is both a continuous source of defined microorganisms and an important source of passive immunity that shapes developing intestinal communities (63). As such, breast-feeding is considered important for the development and maintenance of normal bacterial communities in the intestine (64). The temporal and spatial patterns of intestinal colonization in infants are variable between individuals (65) and depend on multiple factors, including country of birth (66), prematurity (67–69), mode of delivery (67, 70), history of hospitalization (67), antibiotic use (67), feeding practices (53, 56, 67, 71–74), and other factors (Table 1). For example, vaginally born and breast-fed infants have a predominance of *Bifidobacteria* in their intestine, with smaller contributions of *E. coli*, *Bacteroides*, and Clostridia species (75). In comparison, infants delivered by Cesarean section have delayed colonization kinetics compared with vaginally born infants, as well as persistent changes to community compositions (70), including lower burdens of *Bifidobacteria* and *Bacteroides* and higher burdens of *C. difficile* (67). Although these individuals likely acquire mature adult bacterial communities upon transition to solid foods, these early alterations may not be benign, given that some associations exist between early alterations to intestinal bacteria and increased risk of atopic disease (76, 77).

The initial immune response to colonization of the mammalian intestine is best described in mice and is characterized by a general inflammatory response that peaks within a week of birth and subsequently stabilizes over the first year of life (78). A hallmark of colonization is the production and secretion of IgA into the intestinal lumen by the host (79) in response to small numbers of intestinal bacteria that penetrate the intestinal epithelium (80, 81). Upon penetration, Peyer's patch dendritic cells (DCs) phagocytose penetrating bacteria and initiate IgA responses through T cell-dependent and -independent mechanisms (82–85). These DCs induce IgA class switching in the mesenteric lymph nodes (mLNs), but not in systemic secondary lymphoid structures (80), indicating that induction of this initial IgA response is compartmentalized to mucosal tissues (86). The resulting secretion of IgA across the intestinal epithelium feeds back in a homeostatic mechanism to reduce epithelial penetration by intestinal bacteria (87).

In addition to IgA responses, the mammalian host mounts an innate immune response upon colonization that contributes to maintenance of the mucosal barrier (reviewed in 88). For example, intestinal Paneth cells directly sense intestinal bacteria through cell-autonomous MyD88 activation, resulting in upregulated expression of the antimicrobial peptide REGIII γ (89). In addition, intestinal goblet cells upregulate their expression of RELM β , but not of RELM α or RELM γ , in response to intestinal colonization (90). These early innate responses by intestinal epithelial cells (IECs) have important immunoprotective roles. For example, MyD88-deficient mice fail to upregulate REGIII γ and are susceptible to *Listeria monocytogenes* infection; reconstitution of MyD88-deficient mice with recombinant REGIII γ enhances clearance of this pathogen (91).

The systemic response to colonization, and the subsequent development of systemic tolerance, is less well described. Serum antibodies to components of intestinal bacteria are found in humans and other mammals (92), and these antibodies help contain bacteria to the intestine in the absence of innate mechanisms (93, 94). In addition, patients with IBD display systemic immune responses to intestinal bacteria (34, 35), suggesting that tolerance to intestinal bacteria is important for systemic immune cell homeostasis. Although the mechanisms by which the naive host tolerates intestinal bacteria are an ongoing field of study (see below), it will be interesting to examine whether colonization of the intestine with microorganisms early in life influences immunological thresholds from which subsequent proinflammatory or immunoregulatory responses are determined (48, 49).

INNATE RECOGNITION OF BACTERIAL-DERIVED SIGNALS IN THE INTESTINE

As discussed above, the colonization of the mammalian intestine results in rapid and dramatic responses by the mucosal immune system that are important for maintaining mucosal homeostasis and protecting against intestinal pathogens. These responses are likely mediated in part through the recognition of bacterial signals (cell wall components, DNA segments, metabolites, etc.) by innate TLRs, NOD-like receptors (NLRs), and G protein-coupled receptors (GPCRs) expressed in hematopoietic and nonhematopoietic cells of the intestine. In this section, we review the signals derived from intestinal bacteria, their innate receptors, and the role that IECs and DCs play in recognizing these signals and modulating subsequent adaptive immune responses (summarized in Table 2).

TLR Ligands: Flagellin, Lipopolysaccharide, Polysaccharide A, and CPG Motifs

TLRs are innate pattern-recognition receptors that recognize evolutionarily conserved motifs found in bacteria and other microorganisms (95). TLRs have evolved to recognize multiple microbial-derived products including double-stranded viral RNA (TLR3), gram-negative LPS (TLR4), and gram-negative and gram-positive flagellin (TLR5) (reviewed in 96). Most TLRs are expressed on the surface of cells, with the exception of TLR3, 7, 8, and 9, which are localized to endosomal compartments (97).

Several disease models have provided insights into the role of TLR ligands in modulation of the mammalian immune system and indicate both proinflammatory and immunoregulatory

functions for TLR signals in various disease settings. As discussed above, nonobese diabetic mice deficient in the TLR signaling molecule MyD88 are protected against the development of type 1 diabetes (14), suggesting that microbial-derived signals are central to disease development in this model. In contrast, treatment with flagellin protects against chemical-, bacterial-, viral-, and radiation-induced mortality in animal models (98), indicating that this molecule has important immunoregulatory roles. In humans, dominant-negative TLR5 polymorphisms reduce adaptive responses to flagellin and are negatively associated with Crohn's disease (99), whereas TLR2 expression is higher in antigen-presenting cells (APCs) from patients with psoriatic arthritis (100). These findings have spurred interest in TLR ligands as therapeutic agents for human disease, and TLR ligands are currently being investigated as treatments for human allergy (101) and as adjuvant therapies for cancer (102).

One mechanism by which TLR ligands may influence disease states is through modulation of mucosal immune cell function. For example, LPS-induced TLR-dependent signaling protects against experimental colitis (103). Cellular studies showed that LPS elicits TLR-dependent NF- κ B activation in IECs, providing a possible mechanism by which IECs monitor and initiate immune responses to intestinal bacteria (104). In addition, LPS causes differential DC migration (105) and differential DC activation, depending on anatomical location; TLR4 signaling on DCs promotes antigen-specific CD4⁺ T cell-mediated pulmonary inflammation (106), whereas intestinal DCs are reported to become hyporesponsive upon TLR4 ligation (107).

Other TLR ligands have proinflammatory and immunoregulatory roles depending on the anatomical location examined. Administration of CpG, a microbial DNA motif that is recognized by TLR9, reduced the susceptibility of TLR4-deficient mice to systemic allergy (108), implicating this molecule in immunoregulatory roles. However, CpG rescued defective IFN- γ and IL-17 production in the intestine of germ-free mice and protected against infection with intestinal parasites (109). In contrast, the *Bacteroides fragilis* cell wall component polysaccharide A (PSA) may have a predominantly immunoregulatory role, given that it promotes normal immune homeostasis (110, 111) and protects against experimental colitis through the suppression of IL-17 production (112, 113).

NLR Ligands: Peptidoglycan

NLRs, such as NOD1 and NOD2, detect intracellular ligands and are recognized as key mediators of proinflammatory and immunoregulatory responses (114). NLRs recognize several bacterial components, including peptidoglycan-containing meso-diaminopimelic acid (NOD1) and muramyl dipeptide (NOD2) (reviewed in 115). In a pathogenic setting, NOD2 ligation initiates NF- κ B activation and upregulation of inflammatory cytokines, including IL-12 (114). In addition, recognition of bacterial signals through NLRs is important for the development of intestinal lymphoid tissues (116), the maintenance of normal intestinal bacterial communities (116), and the mounting of antigen-specific immunity (117).

Approximately 15% of patients with Crohn's disease have homozygous or compound heterozygous mutations in the gene that encodes NOD2 (CARD15) (36, 37). Disease-

associated alleles were shown to impair NOD2 receptor activity (118), leading to the hypothesis that impaired control of intestinal bacteria was central to disease development (119, 120). However, subsequent studies in NOD2-deficient mice, in particular the seemingly unaltered susceptibility of these mice to some experimental colitis models, suggested a more complex immunomodulatory role for NOD2 signaling (121, 122). Indeed, contrary to the prevailing dogma, it was shown that Crohn's disease-associated NOD2 alleles potentiate rather than attenuate NF- κ B signaling (123, 124).

Subsequent studies explored the complex proinflammatory and immunoregulatory roles for NOD2. NOD2 activation was shown to inhibit TLR2-dependent activation of NF- κ B, suggesting one possible mechanism by which Crohn's disease-associated NOD2 alleles could result in an inflammatory state (125). Consistent with this, NOD2-deficient mice displayed TLR2-dependent susceptibility to colitis that was characterized by antigen-specific IFN- γ -producing CD4⁺ T cells (126). Mu-ramyl dipeptide activation of NOD2 also protected mice from experimental colitis (127), and NOD2 transgenic mice exhibited enhanced muramyl dipeptide-mediated resistance to colitis (128). Cellular studies have indicated that administration of muramyl dipeptide decreases the production of IL-12p40, IL-6, and TNF- α by intestinal DCs (127–129). Furthermore, APCs from NOD2-deficient mice produced greater IL-12p70 when stimulated with peptidoglycan, whereas addition of muramyl dipeptide to cultures of APCs from NOD2-sufficient mice lead to decreased IL-12p70 responses (125). While these findings suggest that NOD2 plays an important immunoregulatory role in attenuating TLR2-mediated proinflammatory responses to intestinal bacteria (130), other studies have highlighted synergistic inflammatory and immunoregulatory roles for NOD- and TLR-dependent signaling (124, 131, 132).

NOD signaling also modulates production of the immunoregulatory cytokine IL-10. Animal models have shown that TLR2 and NOD2 can act synergistically to induce IL-10 production by macrophages (133), and Crohn's disease-associated NOD2 alleles suppress transcription of human *IL10* by inhibiting the activity of the nuclear ribonucleoprotein hnRNP-A1 (134). As such, both positive and negative interactions between TLRs and NLRs potentially exist to modulate inflammatory and immunoregulatory cytokine responses. While complex, the intricacies of these interactions likely hold important potential for future preventative and therapeutic interventions for IBD and other inflammatory diseases.

G Protein-Coupled and Other Receptors: Adenosine, Short-Chain Fatty Acids, and Surface Layer A Protein

In addition to the TLR and NOD ligands, immunoregulatory GPCR ligands such as the purine adenosine (Ado) are of growing interest in the fields of IBD and other inflammatory disease research (135). Ado may function as an endogenously generated regulator of inflammation, depending on the receptor it binds. For example, an Ado A2A receptor agonist did not alter the course of dextran sodium sulfate (DSS)-induced colitis (136), whereas Ado A2B-deficient mice had increased susceptibility to DSS colitis (137). Additionally, intestinal bacteria can be a source of ATP that can drive Th17 cell differentiation in the lamina propria by inducing IL-6 and IL-23p19 production by a population of CD70^{high} CC11c^{low} cells (138). Consistent with this, germ-free mice exhibited

lower concentrations of luminal ATP, as well as fewer numbers of Th17 cells in the lamina propria, a defect that could be reversed through the systemic or rectal administration of ATP.

The normal bacteria of the mammalian intestine also produce significant amounts of butyrate, as well as other short-chain fatty acids (SCFAs) (reviewed in 139). The receptor for butyrate, GPR109A, is expressed on IECs and is downregulated in human colon cancer, in a mouse model of intestinal/colon cancer, and in colon cancer cell lines (140, 141). Consistent with the view that GPR109A is a tumor suppressor, expressing GPR109A in colon cancer cells induces apoptosis in the presence of butyrate (142). Butyrate signals through GPR109A in IECs to suppress NF- κ B signaling (142, 143) and reduces production of TNF- α , TNF- β , IL-6, and IL-1 β by lamina propria lymphocytes (LPLs) in Crohn's and ulcerative colitis patients (144, 145), implicating this microbial metabolite in the regulation of multiple cell populations. Additionally, the SCFA receptor GPR43 has recently been identified as a key mediator of microbial-derived immunomodulatory signals, as mice deficient in GPR43 show exacerbated or unresolving inflammation in models of colitis, arthritis, and asthma (146).

Succinate, a component of the citric acid cycle, modulates DC function by signaling through the extracellular GPCR GPR91. In one study, succinate signaling triggered intracellular calcium release, induced migratory responses, and acted in synergy with TLR ligands to induce the production of proinflammatory cytokines by DCs (147). In this study, GPR91^{-/-} mice exhibited reduced Langerhans cell migration to draining lymph nodes and succinate enhanced antigen-specific activation of human and mouse CD4⁺ T cells (147). GPR91^{-/-} mice displayed impaired tetanus toxoid-specific recall T cell responses, further implicating GPR91-dependent succinate signaling as a signal of immunologic danger.

Finally, bacterial adhesion via surface proteins can directly modulate immune cell function. For example, *Lactobacillus acidophilus* NCFM attaches to DCs and induces concentration-dependent production of IL-10 and IL-12p70 in a DC-specific, ICAM-3-grabbing nonintegrin (DC-SIGN)-specific manner (148). This immunomodulatory function may depend on the bacterial surface component surface layer protein A (SlpA) because purified SlpA protein binds directly to DC-SIGN, and T cells primed with DCs that are stimulated with *L. acidophilus* NCFM lacking SlpA produce less IL-4 than do those stimulated with wild-type *L. acidophilus* NCFM. In summary, microbial signals can have proinflammatory and immunoregulatory effects on multiple immune cell lineages. The next section examines how recognition of microbial signals by IECs and DCs can result in modulation of both local and systemic immune responses.

RECOGNITION OF INTESTINAL BACTERIA BY EPITHELIAL CELLS

The intestinal epithelium has a diverse set of physiologic functions, including digestion and absorption of nutrients, creating a physical barrier between the external and internal environments, and immunological surveillance of intestinal bacteria and potential pathogens. Epithelial cells are continually replaced from a pool of Lgr5⁺ multipotent stem cells that reside in crypts of the intestine (149, 150). These epithelial cells provide an effective physical barrier to the outside environment as intercellular tight junctions prevent paracellular traffic and actin-rich microvillar extensions create an apical brush border that

impedes microbial attachment and invasion (151). Uptake of macromolecules, particulate antigens, and microorganisms across the intestinal epithelia occurs only by active vesicular transport across epithelial cells, and as such is regulated by multiple mechanisms (reviewed in 152).

In addition to physical adaptations that control transport of solutes across the epithelium, biochemical adaptations have evolved, including production of heavily glycosylated, mucin-rich secretions from goblet cells that create a relatively impermeable, apically adhered glycocalyx (153). The epithelium also produces antimicrobial peptides, including defensins, cathelicidins, and calprotectins that confer broad-spectrum antimicrobial properties through the formation of pores in bacterial cell walls (154). These adaptations are consistent with the view that IECs, in addition to promoting digestion and absorption of nutrients, perform essential barrier functions that obstruct the entry of beneficial and pathogenic bacteria into the underlying lamina propria.

IECs are in continuous contact with beneficial and pathogenic bacteria and, as a result, are ideally located for immunological surveillance of the intestinal lumen. As discussed above, IECs express TLRs (155, 156), NLRs (38), and GPCRs that recognize microbial components and modulate cellular responses (Figure 3). IEC expression of innate pattern-recognition receptors is important for mounting immune responses to pathogenic microorganisms (115, 157) by promoting the expression of proinflammatory cytokines (95, 114), chemokines, and antimicrobial peptides (97) as well as the direct induction of IgA class switching by B cells (82, 158, 159). Responses by IECs to intestinal bacteria are not uniform, however: IECs selectively initiate proinflammatory responses to pathogenic bacteria while promoting tolerance to beneficial bacteria (151, 157, 160). One example of this is the gram-negative bacteria *Bacteroides thetaiotaomicron*, which induces IEC expression of the antimicrobial peptide REGIII γ , whereas the gram-positive *Bifidobacterium longum*, a common component of intestinal communities, does not (161, 162).

Two mechanisms by which IECs may discriminate between beneficial and pathogenic bacteria are (a) through subcellular sequestering of pattern-recognition receptors away from luminal signals and (b) differential receptor expression. For example, TLR5, which recognizes bacterial flagellin, is expressed exclusively on the basolateral surfaces of IECs (reported in Reference 156). Additionally, TLR3, 7, 8, and 9 are reported to be expressed exclusively in intracellular endosomal organelles (97), and NLRs are localized to the cytoplasm, reducing exposure of these receptors to luminal bacteria (163, 164). In addition, under steady-state conditions, IECs express little or no TLR2, TLR4, or CD14, further minimizing stimulation by luminal bacteria (165, 166). However, subcellular localization and differential expression alone cannot account for discrimination between beneficial and pathogenic bacteria, as GPCRs that recognize and initiate responses to bacterial products are continuously expressed on the apical surface of IECs. As such, further investigations are necessary to fully understand how IECs discriminate against beneficial and pathogenic bacteria.

The recognition of bacterial signals by IECs is essential to mucosal homeostasis, implicating IECs as central modulators of inflammatory responses (103, 116, 167). One mechanism by which IECs may regulate mucosal homeostasis is by influencing DCs, macrophages, and lymphocytes through the local expression of immunoregulatory cytokines, including thymic stromal lymphopoietin (TSLP), IL-10, transforming growth factor- β (TGF- β), prostaglandin E₂, retinoic acid, and IL-25 (168–174). For example, a population of intestinal DCs induced regulatory T cells (Tregs) that expressed the forkhead box P3 transcription factor (Foxp3⁺) in a TGF- β - and retinoic acid-dependent manner in vitro, implicating epithelial-derived signals in the conditioning of DCs and subsequent adaptive responses (175, 176). In addition, deletion of NF- κ B signaling specifically in IECs resulted in the dysregulated expression of DC-derived proinflammatory cytokines and the development of spontaneous or infection-induced intestinal inflammation (177, 178). These findings provided the first in vivo evidence of a crucial role for IECs in the conditioning of intestinal DC responses. In vitro studies recapitulated the in vivo results as monocyte-derived or circulating DCs conditioned with supernatants from Caco-2 cells or IECs isolated from healthy patients induced Foxp3⁺ Tregs, whereas IEC supernatants from Crohn's disease patients did not (179). This effect was dependent on the production of TGF- β and retinoic acid by IECs, but not on TSLP production, as DCs deficient in the TSLP receptor (TSLPR^{-/-}) and wild-type DCs exhibited a similar capacity to convert naive T cells into Tregs (180).

Epithelial-derived TSLP in particular has important immunomodulatory roles. High levels of *Tslp* mRNA are expressed by epithelial cells at the barrier surfaces of the skin, airways, and intestine, and expression can be upregulated by infection, inflammation, and tissue injury (172, 178, 181–184) in an NF- κ B-dependent manner (185). In vitro studies have shown that TSLP-conditioned human DCs can promote Th2 cell responses (172, 186–188) through the inhibition of IL-12 production and the induction of OX40L expression (172, 188). In addition, in vivo studies in the skin and lung (186, 187, 189, 190) have shown that transgenic overexpression of TSLP in cutaneous or pulmonary epithelial cells results in the onset of Th2 cytokine-mediated inflammation resembling atopic dermatitis or asthma, respectively (189, 190). This finding suggests that TSLP is necessary and sufficient for the initiation of Th2 cytokine-driven inflammation (reviewed in 191). Indeed, TSLP expression and TSLP-TSLPR interactions are important for immunity to the intestinal nematode *Trichuris* and for protection against experimental colitis through the in vivo inhibition of IL-12/23p40 production by DCs (173, 178, 192).

Finally, IECs are in direct contact with in-traepithelial lymphocytes (IELs) and express all the molecular machinery required for antigen processing and presentation, including proteolytically active cathepsins, the invariant chain, and MHC class II (MHCII) molecules (193). In addition, IECs isolated from patients with IBD were shown to express MHCII molecules and localize exogenous antigens to the late endosome on their basolateral surfaces (194). In vitro studies have shown that rodent IECs, although less potent than professional APCs, could process and present antigen through the MHCII pathway (195). Despite the ability of IECs to process and present antigen, they are reported to lack expression of costimulatory molecules (196), indicating that IECs cannot prime naive T cells and may instead provide tolerogenic signals. However, the intestine contains a large population of memory/activated T cells that exhibit less stringent requirements for costimulation and

therefore may be influenced by IEC-intrinsic antigen presentation. Additionally, IECs may deliver inhibitory or tolerogenic signals directly to T cells, consistent with their known role in controlling B cell responses (159). Taken together, these studies highlight that IEC-mediated recognition of intestinal bacteria results in IEC-intrinsic gene expression and the production of immunoregulatory signals that can control innate and adaptive immune cell function.

RECOGNITION OF INTESTINAL BACTERIA BY DENDRITIC CELLS

DCs are perhaps the most efficient modulators of adaptive immune responses (reviewed in 197), and they represent an important link between the innate and adaptive immune systems. In the intestine, DCs take on specific phenotypic characteristics and perform distinct functions depending on their anatomical location (198). In the small intestine, DCs reside in the intestinal lamina propria (LP DCs) and in organized lymphoid structures such as Peyer's patches (PP DCs), solitary isolated lymphoid tissue, and mLNs where they function to sample and present luminal and self antigen to T cells (199) (Figure 4). PP DCs can be divided into three groups based on chemokine receptor expression, location within the Peyer's patch, and functional characteristics: CX3CR1⁺ PP DCs are found in close contact with the follicle-associated epithelium, where they participate in a close functional relationship with the epithelial M (microfold) cell (200) to sample luminal antigens in a pattern-recognition receptor-dependent manner (201); CCR6⁺ PP DCs are found in the subepithelial dome but can quickly migrate to the follicle-associated epithelium in response to microbial stimulation (202); and CCR7⁺ PP DCs are found in T cell areas (203), where they orchestrate helper T cell responses (204), T cell migration (205, 206), and IgA production (207, 208) in response to microbial signals (reviewed in 209).

Although DCs resident in the Peyer's patches are important mediators of immune function, Peyer's patches are relatively rare along the length of the intestinal tract. DCs also reside in the lamina propria of the small intestine, where they may be seeded from circulating precursors (210). LP DCs express tight-junction proteins that allow for direct luminal sampling through the extension of dendrites between IECs (211), a process that is reported to be dependent on the CX3C chemokine receptor 1 (CX3CR1) (212) and TLR ligation (213). Accordingly, CX3CR1^{-/-} mice exhibit defective luminal sampling by DCs and impaired resistance to *Salmonella typhimurium* infection. As such, luminal sampling by LP DCs may play a role in the development of protective immune responses in the intestine (212). However, impaired protective immunity in this system may also be due to changes in other myeloid cells such as macrophages.

Intestinal DCs recognize bacteria through the expression of innate pattern-recognition receptors (97) that can modify luminal sampling (213), migration (105, 214), and the induction of T cell differentiation (215–217). Intestinal DCs are tolerogenic compared with systemically circulating DCs (175, 176), a phenotype that may contribute to the generation of oral tolerance (218, 219). For example, stimulation of intestinal DCs with the TLR4 ligand LPS resulted in elevated IL-10 production, whereas stimulation of systemic DCs resulted in proinflammatory activation (220, 221). The mechanisms by which intestinal DCs may be skewed toward a tolerogenic phenotype are still under investigation and include

reduced TLR expression (220, 221), hyporesponsiveness to TLR stimulation (107), and/or negative regulation of the NF- κ B pathway via NOD2 signaling (as discussed above) (125–129). Additionally, IECs may play an active role in regulating the functional capacity of intestinal DCs (see above).

Intestinal resident DCs regulate local T cell responses in part through the production of IL-12 and IL-23. IL-12 is a key regulatory cytokine that induces Th1 cell differentiation (222) and plays an important role in Th1-mediated experimental models of autoimmune diseases, including IBD (223–226). IL-23 is a heterodimer cytokine composed of a p40 subunit (that is shared with IL-12) and a unique p19 subunit (227). Although one study has implicated IL-23 as having a protective role in a murine model of colitis (228), others have shown that IL-23 drives inflammatory Th17 responses (229, 230; reviewed in 231) and is the causative agent in such inflammatory disorders as joint inflammation (232), intestinal inflammation (230, 233–236), and psoriasis (237, 238). The recent association of IBD with the gene that encodes the IL-23R has further increased clinical interest in an IL-23 inflammatory axis (239).

Intestinal DCs (240) transport self (241) and bacterial (80, 197) antigens to the mLNs where they can influence local immune responses. For example, LP and mLN DCs can promote conversion of naive CD4⁺ T cells into Tregs (175, 176, 199) in a retinoic acid- and TGF- β -dependent manner. Intestinal DCs in the mLNs can also target B and T lymphocytes back to the intestine by promoting the upregulation of CCR9 and α 4 β 7 (205, 206, 242–244). Taken together, these findings suggest that the tolerogenic phenotype of intestinal DCs may be important for maintaining mucosal homeostasis. However, the role that commensal bacteria play in maintaining or promoting the tolerogenic phenotype of mucosal DCs remains to be examined.

SIGNALS DERIVED FROM INTESTINAL BACTERIA REGULATE IMMUNE CELL DEVELOPMENT AND FUNCTION

As discussed above, signals from intestinal bacteria appear to influence human and murine models of disease by modulating innate and adaptive immune responses. One model used to study the role of microbial signals in immune cell development and regulation is the germ-free animal (245). Germ-free animals are born and live in a sterile environment and are therefore free of exposure to live microbial signals (10). Additionally, animals with altered intestinal communities, primarily achieved through the administration of antibiotics (30, 50, 103, 109, 168) or selective colonization studies, have provided a complimentary approach to germ-free studies and have identified key roles for bacterial signals in the regulation of immune cell homeostasis. In this section, we review the evidence for developmental and regulatory roles for bacterial signals in the regulation of mammalian physiology as well as innate (summarized in Table 3) and adaptive (summarized in Table 4) immune cell function.

Intestinal Morphology and Function

Germ-free animals display morphologic defects compared with conventionally reared animals. Perhaps most striking is the dramatic enlargement of the cecum (an intestinal

segment located between the distal small intestine and proximal colon) observed in germ-free animals. This enlargement is due in part to the accumulation of undegraded mucus glycoproteins (246) that are produced by the intestinal epithelium and are normally degraded by glycoside hydrolases from intestinal bacteria (namely *Peptostreptococcus micros* and members of the genera *Ruminococcus* and *Bifidobacterium*) (247–249). Accordingly, cecal enlargement can be rapidly reversed through selective monoassociation with *Peptostreptococcus micros* (247). Germ-free animals also accumulate bile acids in their cecum and large intestine that may contribute to cecal distention by causing osmotic imbalances across the epithelial wall (250).

There are also histologic alterations in the architecture of the germ-free intestine. The villi of the cecum are longer and wider in germ-free compared with conventionally reared mice (50), and morphologic studies in rats suggest that colonic crypts are shorter and contain fewer cells in germ-free compared with conventionally reared animals (251). These changes in crypt architecture could be due in part to the decreased turnover of IECs in germ-free compared with conventionally reared animals (252) or could be due to anatomical changes as a result of bacterial reduction (described above).

The intestine of conventionally reared animals undergoes waves of peristalsis that help move luminal contents. Intestinal bacteria have been shown to influence enteric nerve function, as transient manipulations of intestinal communities can lead to persistent neuromuscular dysfunction and enteritis (253). Consistent with this, germ-free animals show defects in small intestinal peristalsis characterized by slower and less frequent migrating motor complexes as a result of reduced responsiveness to enteroendocrine cell products (254). Peristaltic defects can be reversed through conventionalization of germ-free animals, suggesting that bacterial signals dynamically influence the intestinal neuromuscular function (29). Conversely, intestinal motility is important for the maintenance of normal intestinal bacterial communities as the ablation of enteric neurons specifically in the jejunum and ileum results in bacterial overgrowth and jejunoileitis (255).

Gut-Associated Lymphoid Tissues

It is estimated that the intestinal mucosa of humans contains more lymphocytes, and produces more antibodies, than any other organ in the body (256). In mice, most of these cells reside in gut-associated lymphoid tissues (GALT) (257), which include the mLN, Peyer's patches (258), cecal patch, and isolated lymphoid follicles (ILF) that exist along the length of the gastrointestinal tract, with increasing frequency in the colon and rectum (258).

Germ-free mice display reduced intestinal lymphatic tissue and an underdeveloped lymphatic system compared with conventionally reared mice (259, 260). Specifically, Peyer's patch numbers and cellularity are reduced (261), as are the number of ILFs (116). Defects in lymphoid tissue genesis are not limited to the immediate intestinal compartment, as mLNs are smaller, are less cellular, and have fewer germinal centers in germ-free compared with conventionally reared animals (262). These findings suggest that microbial signals are required for lymphoid tissue development and/or maintenance in the mammalian intestine.

Several studies have begun to examine how bacterial signals influence the maintenance of intestinal lymphoid tissues. Defects in intestinal lymphoid tissues observed in germ-free animals are not developmental, as selective colonization of germ-free animals with intestinal bacteria results in the recovery of intestinal lymphoid structures (259) and the recovery of mLN size and cellularity (262). Rather, bacterial signals continuously support mucosal lymphoid tissue maintenance. For example, antibiotic treatment decreases the cellularity of intestinal Peyer's patches (263). In addition, one study showed that peptidoglycan from gram-negative bacteria is both necessary and sufficient to induce ILF formation in the mammalian intestine in a NOD1-dependent manner (116). These findings support an important role for innate recognition of bacterial-derived signals in the maintenance of adaptive lymphoid tissues.

DCs/Macrophages

Signals from intestinal bacteria have developmental and regulatory influences on intestinal APCs. Intestinal DCs are present in reduced numbers in the intestine of germ-free animals (264, 265). These defects appear to be isolated to the intestinal compartment, as DCs from the spleen and mLNs of germ-free animals had normal surface marker expression of CD86 and MHCII and induced similar levels of T cell proliferation in vitro compared with those isolated from conventionally reared animals (266). Intestinal DCs were recruited to the intestinal lamina propria upon monoassociation of germ-free animals with *E. coli*, further suggesting that intestinal bacteria may play an active role in the regulation of intestinal DC populations (264). Monocyte/macrophage development may also be influenced by signals from intestinal bacteria. While monocyte/macrophage numbers in the intestine were either normal (265) or reduced (267), systemic monocyte/macrophage numbers were reduced in germ-free compared with conventionally reared animals (267). Again, systemic and intestinal defects in monocyte/macrophage populations could be recovered upon monoassociation of germ-free animals with *L. acidophilus* and *L. reuteri*, implicating signals from intestinal bacteria in the regulation of these cell types (267).

As discussed previously, some intestinal APCs have a tolerogenic phenotype that promotes tolerance to oral antigens and commensal bacteria (reviewed in 97, 199, 221). However, it is important that intestinal DCs remain responsive to potential pathogens, a characteristic that may be mediated in part through differential TLR expression. For example, TLR5 recognizes bacterial flagellin, a structural protein of flagella that promotes bacterial chemotaxis, adhesion, and invasion of host tissues (268), whereas TLR4 recognizes LPS, a component of the outer membrane of most gram-negative bacteria present in the intestinal lumen. Intestinal CD11c⁺ lamina propria cells selectively express TLR5, but not TLR4, and produce proinflammatory cytokines in response to bacterial flagellin (216). Appropriately, these CD11c⁺ lamina propria cells produced proinflammatory IL-6 in response to pathogenic flagellated *S. typhimurium* in a TLR5-dependent manner, but produced little IL-6 in response to the nonflagellated commensal *Enterobacter cloacae* (216). Thus, intestinal DCs may remain unresponsive to normal intestinal bacteria while mounting proinflammatory responses to potential pathogens.

The tolerogenic nature of intestinal APCs may be imparted directly or indirectly by select intestinal bacteria. For example, *Lactobacillus rhamnosus* GG decreases TNF- α production in LPS-activated macrophages in a contact-independent manner (269), a phenomenon that may be important for controlling pathogenic, proinflammatory immune responses (198). The ability of intestinal bacteria to impart a tolerogenic influence may be lost in IBD, as Crohn's disease patients have higher numbers of proinflammatory intestinal macrophages compared with healthy individuals (270). These cells express both macrophage (CD14, CD33, CD68) and DC (CD205, CD209) markers and evoke Th1 and Th17 cell differentiation (271), suggesting that intestinal APCs that lack tolerogenic properties could contribute to pathogenic states. Finally, while some intestinal DCs are thought to be specialized for induction of tolerance, evidence exists for tolerogenic DCs outside of the intestinal compartment. For example, a CD11c^{lo}CD45RB^{hi} DC subset present in the spleen and lymph nodes of mice produces IL-10 and promotes suppressive functions of Tregs (272). However, whether or not signals from intestinal bacteria influence these systemic DCs remains to be tested directly.

Lymphoid-Tissue Inducer and Natural Killer Cells

Lymphoid-tissue inducer (LTi) cells are ROR γ t⁺ IL-7R α ⁺ innate leukocytes that induce lymph node development in the embryo through the production of lymphotoxin- β and TNF and the recruitment of circulating LTi cells, their precursors, and more mature lymphocytes (reviewed in 273). Clustering of LTi cells in the intestine is mediated by the chemokines CXCL13 and CCL21, and initial clustering seems to be dependent exclusively on CXCL13 expression by stromal organizer cells by retinoic acid and neuronal stimulation (274). In adult mice, clusters of LTi cells are found in the cryptopatches of the small intestine and in secondary lymphoid organs such as the spleen, where they participate in maintaining local lymphoid tissue anatomy (273). LTi cells are an innate source of IL-17 and IL-22 (an IL-10 family member that contributes to epithelial cell resistance and repair by inducing the production of antimicrobial proteins such as β -defensins, RegIII γ , and S100 calcium-binding proteins) (275), although experimental evidence supporting a role for these cells in initiating or regulating immune responses is lacking at present.

The observation that lymphoid follicles are underdeveloped in germ-free mice has led some to speculate that LTi cells might be regulated by bacterial signals; however, one study to date indicated that LTi cell numbers and function were similar in the mucosa of germ-free compared with conventionally reared mice (276). Nevertheless, an intriguing connection exists between LTi cells and another cell population that is regulated by intestinal bacteria: IL-22-producing intestinal natural killer (NK)-like (NK-22) cells. Intestinal NK-22 cells are found in the cryptopatches and lamina propria of adult mice, where they are also an important innate source of IL-22 (276–279). Production of IL-22 by mucosal NK-22 cells may contribute to defense against the extracellular enteric pathogen *Citrobacter rodentium*, as the partial depletion of mucosal NK-22 cells increases the mortality of infected mice (277, 278). In addition, NK-22 cells mediate protection from experimental colitis (280), supporting the hypothesis that these cells are involved in defense against assaults to the enteric mucosa.

LTi cells differentiate into NK-like cells in vitro (281) that express both LTi and NK cell receptors (reviewed in 282). For example, human fetal LTi cells give rise to NKp44⁺ cells that produce IL-17 and IL-22 in vitro (283). The dependency of NK-22 cells on ROR γ t expression further suggests that these cells may derive from LTi cells and has led to speculation that LTi cells and NK-22 cells may be related functionally and developmentally (273). Additionally, intestinal LTi cells and NK-22 cells upregulate IL-22 production shortly after birth, suggesting that colonization of the intestine drives IL-22 production by these cell types in humans and mice (276, 278). Finally, absolute and relative numbers of NK-22 cells are reduced in germ-free compared with conventionally reared mice (276), further implicating signals from intestinal bacteria in NK-22 cell development. These findings suggest that signals from intestinal bacteria promote mucosal homeostasis by inducing IL-22-producing innate leukocytes. However, the relationship between LTi cells and NK-22 cells in vivo remains to be investigated. For example, LTi cells are required for lymphoid tissue development, whereas lymphoid tissue development and the development of IL-22-producing intestinal NK cells are both dependent on bacterial signals. However, LTi development seems to be independent of signals from intestinal bacteria (276). As such, more investigation into the relationship between LTi cells and IL-22-producing intestinal NK cells, and the role that signals from intestinal bacteria may play in the respective development of these cell populations, is needed.

B Cells

There is an intimate relationship between intestinal communities and B lymphocytes that reside in the intestine. As discussed previously, the first immunological response to bacterial signals during colonization is IgA production and secretion into the intestinal lumen (79, 284). In fact, most B cells in the intestine are IgA-producing plasma cells that produce and secrete IgA into the intestinal lumen at an estimated rate of 0.8 g per meter of intestine per day (257, 285) (Figure 4). This secretory immune response, which is characterized by class switching of B cells from IgM to IgA production, is orchestrated through an intimate functional relationship between secretory epithelia and local plasma cells and is mediated by TLRs through both T cell–dependent and –independent mechanisms (82, 158). For example, intestinal bacteria trigger T cell–independent IgA class switching in B cells through IEC secretion of cytokines such as a proliferation-inducing ligand (APRIL) (158, 159). In addition, IEC-derived TSLP and IL-10, produced in response to bacterial signals, may orchestrate local B cell responses (158, 159, 286). Secretory IgA creates a first-line defense against mucosal compromise that is lost during IBD (35, 287), implicating early recognition of bacterial signals and the subsequent modulation of B cell responses as important processes that promote normal mucosal homeostasis.

Several findings in germ-free mice support a role for bacterial signals in B cell development. There are reduced numbers of plasma cells in the germ-free small intestine (262), which correlates with reduced IgA production (288). Further, there are reduced systemic numbers of germinal centers and plasma cells in germ-free mice (289), which correlates with reduced systemic immunoglobulin levels (260, 290–293). Germ-free mice also show reduced antigen-specific immunoglobulins to some antigens (DNP-BSA, *E. coli*) (294, 295) but not to others (RBC, phosphorylcholine, DNP-lys-Ficoll) (294, 296). In general, these intestinal

and systemic immunoglobulin defects are corrected upon conventionalization of germ-free animals, suggesting that these defects are not purely developmental (288, 290, 293, 297).

One exception to the trend of reduced immunoglobulin levels is IgE. There are increased numbers of IgE-bearing B cells in the Peyer's patches of germ-free mice (298) and elevated serum IgE levels in germ-free and antibiotic-treated mice (108), a finding that may be linked to impaired oral tolerance to Th2 antigens in mice with reduced microbial stimulation (108, 299, 300). IgE in germ-free mice is likely composed of natural specificities and induced by a mechanism independent of MHCII cognate help (301). These findings suggest that, in general, microbial signals act as an adjuvant to immunoglobulin responses, and, with the exception of allergic IgE responses, bacterial signals may play an immunoregulatory role.

In addition, accumulating evidence suggests that B cells can play a regulatory role in many models of colitis through the production of IL-10 (reviewed in 302). For example, autoantibodies from B cells suppress colitis in mice deficient in TCR α chain (303) and intestinal inflammation induces a population of CD1d-expressing B cells in (GALT) that produces IL-10 and suppresses progression of colitis (304). Roles for B cell-derived IL-10 in controlling colitis in other animal models have also been reported (305). Although the influence of bacterial signals on regulatory B cell development and function remains to be examined, these findings suggest that B cells play an integral role in controlling inflammation in animal models of colitis.

Th17/Treg Cells

The differentiation of Th17 cells is characterized by ROR γ t expression, requires TGF- β and IL-6 or IL-21, and relies on IL-23 for Th17 cell maturation and survival (reviewed in 306). In the steady state, IL-17-producing cells are present in high numbers in the lamina propria of the small intestine (307), where the Th17-related cytokines IL-22 and IL-17 play a role in host protection against extracellular pathogens (reviewed in 308, 309).

Conventionally reared animals have TLR-independent spontaneous IL-17 production in the lamina propria of the small intestine (307). Spontaneous IL-17 production was absent in the small intestine of germ-free animals, while MyD88-deficient mice had normal numbers of Th17 cells, suggesting that intestinal bacteria signal through a TLR-independent mechanism to promote Th17 cell development (307). Specific bacteria and bacterial-derived stimuli have been identified as key regulators of Th cell responses in the mammalian intestine. For example, colonization of germ-free mice with segmented filamentous bacteria induced strong Th1, Th2, Th17, and Treg responses in the lamina propria (310, 311). Another mechanism by which intestinal bacteria may regulate Th17 cell development independent of TLR signaling is through the production of other bioactive molecules (see above). Indeed, systemic or rectal administration of ATP into germ-free mice stimulates lamina propria APCs to produce IL-6, IL-23, and TGF- β , resulting in Th17 cell differentiation (138).

Although Th17 cells are reduced in the small intestine of germ-free mice, more Th17 cells have been observed in the large intestine of germ-free compared with conventionally reared mice (168). In the large intestine, bacterial signals can regulate Th17 cell development through bacterial-dependent production of the IL-17 family cytokine IL-25 (IL-17E), which

downregulates IL-17 production through the inhibition of IL-23 production by lamina propria macrophages (168). These findings suggest that regulation of Th17 cell differentiation depends on anatomical location. Indeed, the small and large intestines are micro-biologically and immunologically distinct sites. As discussed above, there are site-specific differences in intestinal bacterial communities along the length of the intestine (Figure 2). In addition, there are more IELs and LPLs per epithelial cell in the small intestine, and the lymphocyte composition and migration to the small versus large intestine is differentially regulated (307, 312). Other environmental factors, such as diet and non-live microbial signals, could also influence Th17 populations. Although more studies are required, these fundamental differences may underlie differential regulation of Th17 cells in distinct anatomical locations of the intestine.

Studies from antibiotic-treated mice mirror some of the observations made in germ-free mice and support a role for bacterial signals in influencing homeostasis of Th17 cells. For example, mice treated with vancomycin have fewer Th17 CD4⁺ T cells in the lamina propria of the small intestine (138, 307, 313), whereas mice treated with a complex antibiotic mixture displayed reduced Th17 CD4⁺ T cell frequencies in the mLNs (50). Additionally, Th17 cell differentiation in the lamina propria of the small intestine requires specific intestinal bacteria: cytophaga-flavobacter-bacteroidetes bacteria (307). This induction of Th17 cells is independent of TLR signaling, IL-21, or IL-23, but requires TGF- β activation, suggesting that specific intestinal bacteria may regulate the Th17/Treg balance in the mammalian intestine.

An intimate relationship exists in the intestinal mucosa between proinflammatory Th17 cells and CD4⁺ Tregs, which play an important role in controlling Th17 cell responses (reviewed in 314, 315). Tregs are characterized as CD4⁺ CD25⁺ cells that express Foxp3⁺ and suppress the proliferation of effector T cells in vitro and protect against autoimmune and other inflammatory diseases in several animal models (reviewed in 316). For example, mice carrying a loss-of-function mutation of Foxp3 completely lack Tregs and develop lethal autoimmune disease (317). Additionally, mice engineered to lack the expression of specific regulatory cytokines in T cells, including IL-10 or TGF- β , develop colitis when pathogenic bacteria are present in the intestine (318–320). These findings identify Tregs as an important regulatory cell type that contributes to intestinal and systemic immune homeostasis.

There are conflicting reports regarding the influence of bacterial signals on Treg development and function. Consistent with studies that showed reduced Foxp3 mRNA in CD4⁺ T cells from mLNs of germ-free mice (321, 322), early studies in germ-free animals showed a selective reduction in the percentage of Foxp3⁺CD4⁺CD25⁺ T cells in the mLN of germ-free mice (321). In addition to reduced frequencies, Tregs from the mLN do not suppress CD4⁺ T cell proliferation in vitro in either germ-free animals or in conventionally reared animals (323). Furthermore, Tregs from the mLN of germ-free animals produce less IL-10 and do not protect as well against disease in a transfer model of experimental colitis, compared with Tregs from conventionally reared animals (322). Consistently, some intestinal bacteria may help promote Treg development; for example, *Lactobacillus* and *Bifidobacterium* strains caused expansion of Tregs in the intestinal intraepithelial compartment, which correlated with protection against experimental colitis (324). These

results suggest that signals from intestinal bacteria are important for normal development of Treg numbers and function in the mLNs.

However, bacterial signals seem to have different effects on other Treg populations. One study reported no change in the frequency of lamina propria CD4⁺Foxp3⁺ T cells from the colon of germ-free animals (168), whereas another reported increased percentages of CD4⁺Foxp3⁺ in the germ-free small intestine (307). These differing results could be due to sampling Treg cell subsets from different anatomical locations (mLN versus intestine). Additionally, differences in experimental methods, animal housing methods, diet, nonbacterial microbial stimulation, employed assays, or Treg identification methods could explain these conflicting results.

Th1/Th2 Cells

In humans, as in mice, several distinct patterns of cytokine secretion have been defined among CD4⁺ helper T cell clones. Th1 cells produce IL-2, IFN- γ , and TNF- β , whereas Th2 cells produce IL-4, IL-5, IL-9, and IL-13 (reviewed in 325). These distinct immune responses are important for fighting distinct types of infection; Th1 cell responses are protective against bacterial, viral, and protozoan infections, whereas Th2 cell responses are important in mediating immunity to helminths and ectoparasites.

Inappropriate Th1 and Th2 cytokine responses result in distinct forms of human disease. For example, Crohn's disease is characterized by exaggerated IFN- γ responses, as well as IL-23 and IL-17 responses (reviewed in 231), whereas ulcerative colitis and atopic diseases are primarily associated with elevated Th2 cytokine responses (16). As discussed above, IBD patients display altered bacterial communities in their intestine (326), and tolerance to intestinal bacteria is broken in these diseases (32, 33, 35), suggesting that a dysregulated mucosal immune response to intestinal bacteria could be linked to pathogenesis (16). Consistently, animal models of IBD exhibit CD4⁺ T cells specific for bacterial antigens (327) that induce colitis when adoptively transferred into naive SCID mice (35). The loss of regulatory mechanisms, such as mucosal T cells with regulatory properties that suppress inappropriate Th1 responses (328), may contribute to disease pathogenesis in these models (329).

The influence of intestinal bacteria on Th1 and Th2 cell development and regulation is not limited to the intestinal mucosa. For example, recent epidemiological studies indicate that antibiotic use in infancy may be associated with an increased risk of developing atopy (48). As discussed above, patients with atopic diseases such as asthma have altered intestinal bacterial communities (15), suggesting that bacterial signals influence systemic type 2 inflammatory responses. Consistent with this notion, treating animals with antibiotics during infancy promoted a shift in the Th1/Th2 balance toward a Th2-dominant immunity (330) that could be corrected by oral inoculation with *Enterococcus faecalis* or *Lactobacillus acidophilus* (300).

The balance of proinflammatory or regulatory immune responses to intestinal bacteria may be regulated by mucosal DC populations. For example, subsets of mucosal DCs have tolerogenic capacities that contribute to the induction of tolerance to intestinal bacteria and

food antigens (97, 221). However, other nonhematopoietic cells may influence the balance of inflammatory or tolerogenic responses; IECs may deliver inhibitory or tolerogenic signals indirectly or directly to T cells (discussed above). In addition, T cells express pattern-recognition receptors under certain conditions and could be directly responsive to bacterial-derived signals (331), although this possibility has not been examined in detail.

Finally, granulocytes such as mast cells, basophils, and eosinophils are important effectors of protective and pathogenic type 2 inflammatory responses (332). In addition, granulocyte populations are important sources of type 2 cytokines (333), and basophils have recently been identified as an important cellular source of IL-4 that express surface MHCII and can prime antigen-specific Th2 cell differentiation (334–336), suggesting that basophils may also play a role in the initiation of type 2 responses. Several lines of evidence suggest that granulocytes are dysregulated in human inflammatory disease. For example, tissue from patients with ulcerative colitis show mast cell aggregation along the line of demarcation, dividing inflamed from healthy tissue (337), and the number of NOD2⁺ intestinal mast cells are increased in Crohn's disease patients compared with healthy controls (338), identifying a possible role for these cells in responding to innate, bacterial-derived signals. Mast cells are of particular interest in IBD because they are thought to modify disease progress through release of histamine (339), TNF- α (340), and the T cell chemoattractants XCL-1 (341) and IL-16 (342). Indeed, in a model of experimental allergen-induced asthma, TLR4-defective mice subjected to sensitization and pulmonary challenge with a protein allergen had reductions in airway inflammation, allergen-specific IgE levels, and Th2 cytokine production (106), although rigorous investigation of potential influences that intestinal bacteria have on granulocyte development or function is lacking at present.

CD8⁺ T Cells

The first observation that microbial signals could influence tumor immunity came in the eighteenth century when Deidier reported a correlation between patient infection and the remission of malignant disease, a finding that eventually led to the use of LPS to treat primarily inoperable sarcoma, with a cure rate of better than 10% (343). These early findings indicated that microbial signaling through TLRs is important for initiating or sustaining antitumor immune responses (reviewed in 344). This hypothesis has now been supported by several studies in animal models (345–347) and patients (102), leading to a well-accepted role for bacterial products as adjuvants that promote the recruitment and/or stimulation of CD8⁺ cytotoxic T lymphocytes (348).

The intestinal mucosa of conventionally reared animals normally contains CD8⁺ cytotoxic T lymphocytes. In the conventionally reared mouse intestine, CD4⁺ T cells are found primarily in the lamina propria, whereas CD8⁺ T cells dominate in the intraepithelial compartment. The maintenance of CD8⁺ IELs depends on bacterial signals, as MyD88-deficient (349) and germ-free mice (350, 351) display reduced CD8⁺ T cell numbers in the IEL compartment of the small intestine. The systemic distribution of CD8⁺ T cells also depends on bacterial signals. The liver is a site of activated CD8⁺ T cell sequestering and subsequent apoptosis during systemic viral immune responses, a phenomenon that is dependent on TLR4 ligands such as endotoxin from intestinal bacteria (352).

Cytotoxic activity of IELs is impaired in both germ-free (351) and antibiotic-treated mice, a phenotype that can be reversed through the administration of the TLR4 ligand LPS (345). In germ-free mice, this cytotoxic defect was isolated to $\alpha\beta$ TCR-bearing IELs, whereas the number and cytotoxicity of $\gamma\delta$ TCR-bearing IELs was comparable between germ-free and conventionally reared mice (351). These defects may be in part due to impaired clonal expansions of $CD8\alpha\beta^+$ and $CD8\alpha\alpha^+$ IELs in germ-free mice (353). This functional defect is not limited to intestinal compartments, as conventionally reared mice have a selective reduction in systemic plasmacytoid DCs as a result of enhanced cytotoxic T lymphocyte activity that is not observed in germ-free mice (354). Finally, animals colonized with nonpathogenic *Clostridium* sp. have reduced numbers of naive, splenic $CD8^+$ lymphocytes compared with conventionally reared controls (355), further suggesting that signals from intestinal bacteria play an active role in $CD8^+$ lymphocyte regulation.

Although the adjuvant effect of microbial signals on cytotoxic cells is well established, microbial stimulation can also result in tumorigenesis under some conditions. For example, MyD88-deficient mice are protected against the development of spontaneous intestinal tumors in a model of familial-associated polyposis (356), and germ-free animals have reduced intestinal tumorigenesis compared with conventionally reared animals following the same protocol of colorectal cancer induction (357). Similarly, hepatocellular carcinoma, the most common liver cancer, occurs mainly in males. This gender disparity is also seen in mice given the chemical carcinogen diethylnitrosamine and is dependent on MyD88-mediated increases in serum IL-6 (358). In humans, associations between intestinal bacteria and cancer risk have been explored (reviewed in 359), and studies were performed to identify fecal bacterial communities associated with high colorectal cancer risk (13, 66, 360), although they failed to provide conclusive results. In summary, clarifying the influence of bacterial signals in establishing normal cytotoxic immune capacities has led to exciting therapeutic possibilities, but more research is needed to fully understand the role that signals from intestinal bacteria play in tumorigenesis and control.

MANIPULATION OF INTESTINAL BACTERIAL COMMUNITIES: PROSPECTS FOR PREVENTION AND TREATMENT OF INFLAMMATORY DISEASES

The identification of the important roles that intestinal bacteria play in normal development and regulation of the mammalian immune system provides a rationale for using therapeutic agents based on bacterial-derived signals in preventative or therapeutic endeavors. Live biologics given as supplements to confer some benefit to the host are referred to as probiotics. Several studies in animal models have investigated the beneficial effects of probiotic bacteria, including *Lactobacillus* spp. and others. In one study, administration of *Lactobacillus* spp. protected IL-10-deficient mice from developing spontaneous colitis (361). In another, probiotic mixtures including *Lactobacillus* spp. protected mice against chemically induced colitis (324). Probiotics are thought to mediate their beneficial effects in part through modulation of immune cell function. For example, probiotic bacteria including lactobacilli induce IL-10 production by DCs and inhibit subsequent generation of Th1 cells in vitro (148, 362), and *Lactobacillus* and *Bifidobacterium* strains prevent experimental colitis while expanding IEL $\gamma\delta$ T cells and Tregs (324).

These and other studies have led to clinical trials in human patients examining the potential role for probiotic bacteria in preventing and treating human disease. These treatments have been associated with modulation in DC function (362, 363), as well as the function of other immune cells (364), but have reported mixed successes in the treatment of intestinal inflammatory and atopic diseases (62, 365, 366). One positive study showed that *E. coli* provided an effective probiotic therapy to ulcerative colitis patients (367). In addition, delivery of probiotics seems to be effective for the prevention and treatment of pouchitis, a complication following surgical treatment of ulcerative colitis (368–371). Despite these encouraging results, how probiotics modulate intestinal communities to modify subsequent immunoregulatory signals is unknown. A better understanding of these mechanisms is necessary to realize the full potential of probiotic therapies.

In addition to interventions with unmodified bacteria, more recent work using genetically modified bacteria that act as platforms for the delivery of drugs, antimicrobial agents, vaccines, and other biologically active molecules to physiologically relevant sites has expanded the definition of probiotic therapy. For example, *Lactobacillus* strains have been engineered to produce IL-10 as a therapeutic intervention in animal models of IBD (372), while *Streptococcus gordonii*, engineered to express an antimicrobial antibody fragment, confers a therapeutic benefit to rats with vaginal *Candida albicans* infections (373). In addition, genetically modified lactobacilli that express the tetanus toxin fragment C efficiently induce local and systemic antigen protection when given orally to mice (374), while those that express CD4 molecules and secrete HIV-1 fusion inhibitors have been developed as a step toward the use of topical, genetically engineered bacteria to prevent HIV transmission (375, 376).

In the development of new therapeutics, the use of specific bacterial products with immunoregulatory characteristics, as well as their molecular derivatives, could offer new therapeutic modalities in the prevention and treatment of human disease. For example, identifying specific bacterial compounds that could be administered early in life to promote tolerance could play a role in preventing the development of atopy later in life. Similarly, bacterial products that bolster mucosal barrier function or that regulate inflammatory immune responses in the intestinal mucosa may be important in the treatment of human IBD. Finally, bacterial-derived proinflammatory molecules that modulate immune responses could play a role in vaccine development, cancer treatment, or the treatment of infection. To move such therapies into the clinics, the specific microbial products and their immunoregulatory properties must be precisely defined.

CONCLUSION

Technological and conceptual advances have ushered in an exciting time in mucosal immunology and in our understanding of how bacterial-derived signals influence the immune system. New sequencing techniques are allowing previously unattainable insights into the complex bacterial communities integral to mammalian health. Additionally, established tools such as germ-free animals are being complemented with antibiotic treatment, selective colonization, and treatment with specific microbial products to probe the molecular mechanisms that facilitate cross-talk between intestinal bacteria and their

mammalian hosts. It is hoped that these insights will offer new therapeutic strategies in the prevention and treatment of human diseases.

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Glossary

IBD	inflammatory bowel disease
LPS	lipopolysaccharide
TLR	Toll-like receptor
DC	dendritic cell
IEC	intestinal epithelial cell
NLR	Nod-like receptor
APC	antigen-presenting cell
LPL	lamina propria lymphocyte
IEL	intraepithelial lymphocyte
NK cell	natural killer cell

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COMMON TERMS DESCRIBING INTESTINAL COMMUNITIES

Symbiosis: close and often long-term relationship between different biological species.

Symbiotic relationships can be described as

- Mutualistic: both species derive a benefit from the interaction,
- Parasitic: one member benefits and the other is harmed, or
- Commensal: one member benefits and the other is unaffected.

Dysbiosis: the condition of having microbial imbalances on or within the body.

Probiotic: an organism given as a live supplement to confer a biological benefit to the host.

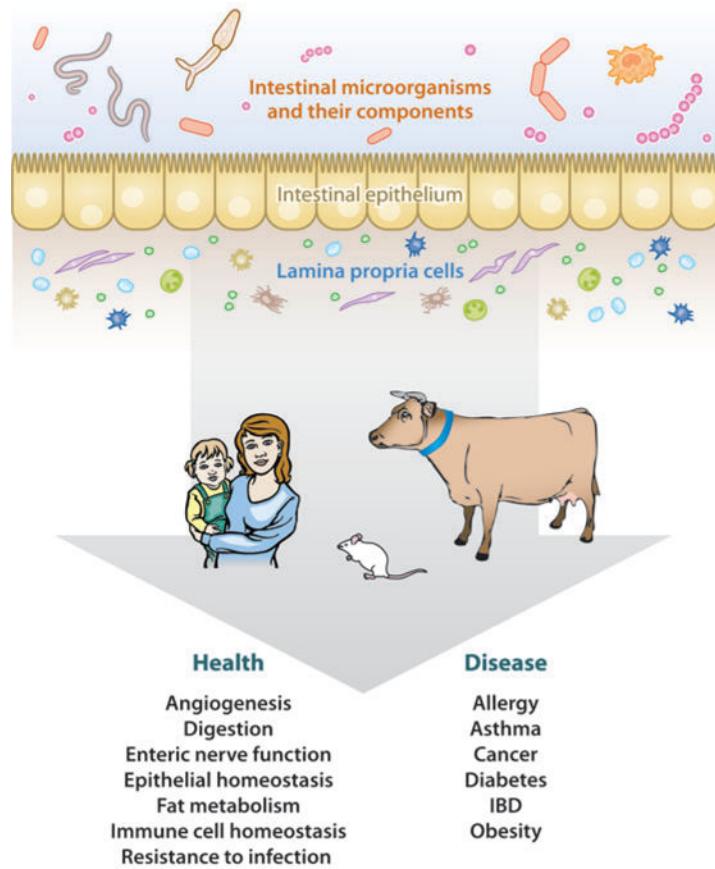


Figure 1. Intestinal bacteria in mammalian health and disease. Schematic of the known influences of intestinal bacteria on normal mammalian physiology and inflammatory disease states.

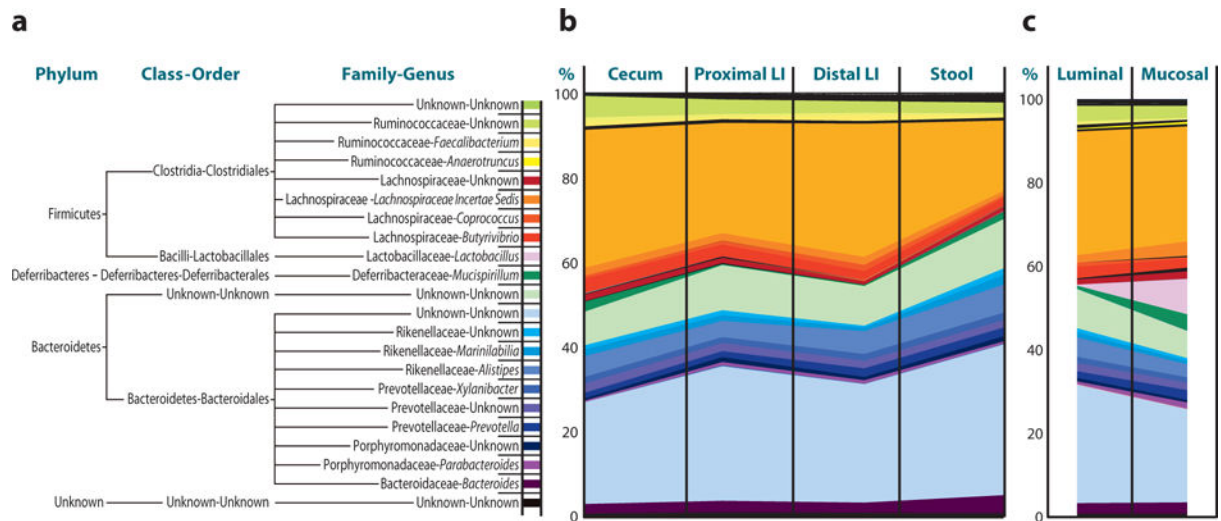


Figure 2.

The composition of bacterial communities along the length and between luminal and mucosal compartments of the mammalian intestine. Stool pellet, luminal content, or mucosal-associated communities were sterilely collected. Total sample DNA was extracted and bacterial 16S rRNA gene fragments were PCR amplified with bar code-tagged primers and subjected to pyrosequencing, and taxonomic assignments for each sequence were obtained using RDP Classifier. (a) Commonly found bacteria in the murine colon. (b) Relative frequencies and distribution of bacteria along the length of the murine colon and in murine stool samples. (c) Relative frequencies and distribution of bacteria between luminal and mucosal-associated compartments of the murine colon. Adapted from Reference 50.

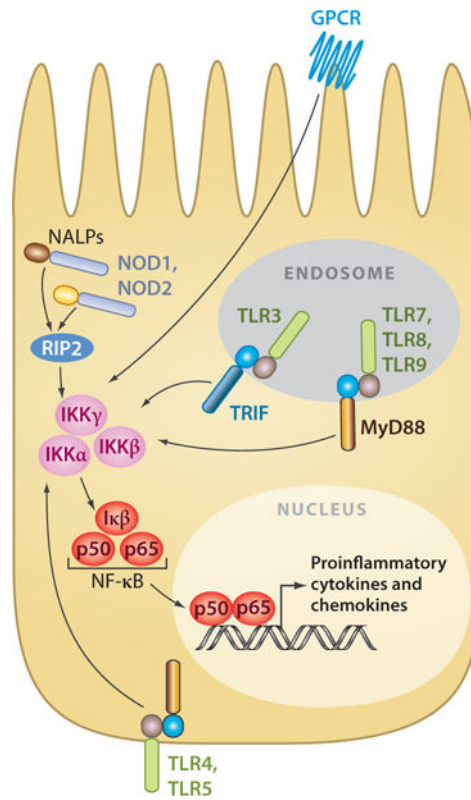


Figure 3. Innate receptors and signaling cascades of mammalian intestinal epithelial cells (IECs). Schematic shows the location of innate pattern-recognition receptors and their signaling cascades in mammalian IEC. Innate pattern-recognition receptors converge on a common NF- κ B signaling cascade to regulate transcription of proinflammatory cytokines and chemokines.

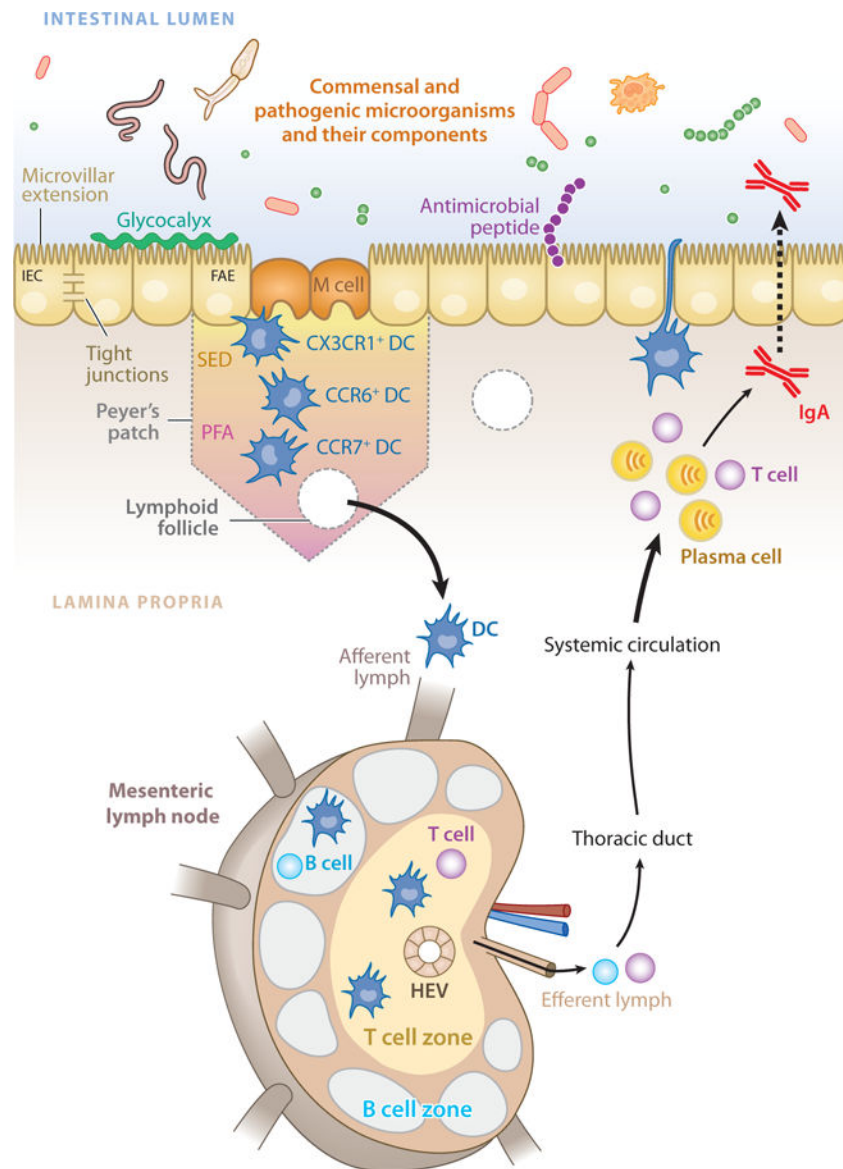


Figure 4.

The mucosal immune system of the mammalian intestine. Innate recognition of signals from intestinal bacteria takes place at the intestinal epithelium, in the lamina propria, and in gut-associated lymphoid tissues such as Peyer's patches and isolated lymphoid follicles.

Specialized intestinal epithelial cells known as M (microfold) cells overlie Peyer's patches and lymphoid follicles to facilitate luminal sampling and to transport microbial components to professional antigen-presenting cells present in the subepithelial dome (SED). Dendritic cells (DCs) in the SED and perifollicular area (PFA) acquire antigens and influence adaptive responses. Additionally, specialized DC subsets directly sample luminal antigens. Intestinal DCs transport antigens to mesenteric lymph nodes through the afferent lymphatic system. DCs in the mesenteric lymph node promote differentiation of regulatory and effector T lymphocytes, as well as class switching of B lymphocytes, which then exit through the

efferent lymph into the systemic circulation. Some of these cells home back to the intestine, where they exert their effector functions.

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Table 1

Factors influencing the acquisition and/or composition of intestinal bacterial communities

Factor	Reported alteration	References
Birth in North America	Increased <i>Bacteroides</i> and <i>Bifidobacterium</i> species, decreased <i>Lactobacillus</i> and <i>Eubacterium aerofaciens</i>	66
Hospitalization/prematurity	Delayed colonization, reduced community diversity, reduced <i>Bifidobacterium</i> species, higher <i>C. difficile</i>	67–69
Delivery by Cesarean section	Delayed colonization, lower <i>Bifidobacterium</i> species and <i>Bacteroides fragilis</i> , higher <i>C. difficile</i>	67, 70
Antibiotic use	Decreased <i>Bifidobacterium</i> and <i>Bacteroides</i> species, increased <i>Campylobacter</i>	61, 67
Formula feeding	Delayed colonization with <i>Bifidobacterium</i> species, more often colonized with staphylococci, <i>E. coli</i> , <i>C. difficile</i> , <i>Bacteroides</i> , and lactobacilli	53, 56, 67, 72, 74
Vegetarian diet	Higher <i>Clostridium</i> species	71
Old age	Lower <i>Clostridium</i> species, higher <i>Ruminococcus obeum</i> and gammaproteobacteria species	377
Older siblings	Higher <i>Bifidobacterium</i> species	67
Infectious colitis	Increased <i>Campylobacter</i> , decreased <i>Lactobacillus</i>	61, 378
Species	Bacterial communities differ between mammalian species	61
Gender	Bacterial communities differ between genders	61
Genetics	Innate immune function regulates intestinal communities in flies	379

Table 2

Bacterial signal receptors, ligands, and immunologic effects

Ligand	Proposed receptor	Immunologic outcome	References
PSA	TLR2	Promotes normal Th1/Th2 balance Enhances response to abscess-forming bacteria Suppresses intestinal IL-17 production Protects against colitis	110, 113 110, 111 112 112
LPS	TLR4	Activates NF- κ B Induces DC migration Activates systemic DCs Inhibits mucosal DCs Protects against colitis	104 105 106 107 103
Flagellin	TLR5	Positively associated with Crohn's disease Protects against chemical-, bacterial-, viral-, and radiation-induced mortality	99 98
CpG	TLR9	Enhances intestinal IFN- γ and IL-17 production Protects against intestinal parasites Protects against systemic allergy	109 109 108
Muramyl dipeptide	NOD2	Activates NF- κ B Promotes lymphoid tissue development Regulates intestinal bacterial communities Promotes antigen-specific immune responses Protects against colitis Promotes tolerance to bacterial products Inhibits IL-12p70 production Protects against IL-12-driven experimental colitis Protects against Crohn's disease	114 116 116 117 127–129 130 125 126, 127 36, 37
Ado	A2A	Protects against colitis Drives intestinal Th17 cell differentiation	137 138
Butyrate	GPR109A	May protect against colon cancer Induces ROS and suppresses NF- κ B signaling in IECs Reduces TNF- α , TNF- β , IL-6, and IL-1 β production by LPLs in IBD patients	140–142, 380 142, 143 144, 145, 381
Succinate	GPR91	Acts on intestinal DCs to trigger intracellular calcium release, induce migration, induce proinflammatory cytokine production, and enhance antigen-specific T cell activation	147
SlpA	DC-SIGN	Induces IL-10 and IL-12p70 production by DCs	148

Table 3

The role of microbial signals in the development and regulation of innate immune cell function

Parameter	Evidence from germ-free compared with conventionally reared animals (reference)	Evidence from antibiotic-treated animals (reference)	Evidence from ex-germ-free animals (reference)
Secondary lymphoid tissue development	Lymphatic tissue and lymphatic system underdeveloped (259, 260, 382) Peyer's patch number and cellularity reduced (261, 382) Reduced ILFs (116) mLNs smaller and less cellular, with reduced germinal centers (262)	Subcutaneous cefmetazole decreases cellularity of Peyer's patches (263)	Recovery of lymphatic tissue and the lymphatic system upon conventionalization of germ-free animals (259) Recovery of mLN size and cellularity upon conventionalization of germ-free animals (262) Peptidoglycan from gram-negative bacteria necessary and sufficient to induce ILF genesis via NOD1 in epithelial cells (116)
Macrophage development	Decreased surface expression of macrophage activation markers in the intestine (383) Intestinal macrophages present at high levels from birth (265) Reduced monocyte/macrophage numbers in the ileum and spleen (267)		Recovery of ileum and spleen monocytes/macrophages upon monoassociation of germ-free mice with <i>L. acidophilus</i> and <i>L. reuteri</i> (267)
DC development and function	Normal expression of surface markers and ability to stimulate T cell proliferation by DCs from the spleen and mLNs (266) Reduced DCs in the intestine (264, 265)		Recruitment of DCs to the lamina propria upon monoassociation of germ-free animals with <i>E. coli</i> (264) Lamina propria APCs stimulated by ATP to produce IL-6, IL-23, and TGF- β , resulting in Th17 cell differentiation (138)
LTi/NK cell development	Reduced NK-22 cells (276)		LTi cells and NK-22 cells upregulate IL-22 production shortly after birth (276, 278)

Table 4

The role of microbial signals in the development and regulation of adaptive immune cell function

Parameter	Evidence from germ-free compared with conventionally reared animals (reference)	Evidence from antibiotic-treated animals (reference)	Evidence from ex-germ-free animals (reference)
B cell development and function	<p>Reduced numbers of plasma cells in small intestine (262)</p> <p>Decreased IgA production in small intestine (288)</p> <p>Reduced systemic germinal centers and plasma cells (289)</p> <p>Low systemic immunoglobulin levels (260, 290–293)</p> <p>Decreased IgM and IgG response to DNP-BSA (294)</p> <p>Normal IgM response to sheep red blood cells or phosphorylcholine (294, 296)</p> <p>Normal IgM and IgG response to DNP-lys-Ficoll (294)</p> <p>Delayed and reduced primary antibody titers against heat-killed <i>E. coli</i> (295)</p> <p>Increased antibody response to ferritin and DNP-Ficoll (384, 385)</p> <p>Increased IgE-bearing B cells in Peyer's patches (298)</p>	<p>Increased IgE responses caused by antibiotic treatment (108)</p>	<p>Recovery of intestinal IgA upon conventionalization of germ-free animals (288, 297)</p> <p>Recovery of systemic germinal centers and plasma cells upon conventionalization of germ-free animals (289)</p> <p>Recovery of systemic immunoglobulin levels upon conventionalization of germ-free animals (290, 293)</p>
Th17/Treg cell development and function	<p>Reduced Th17 cells in the small intestine (307)</p> <p>Increased Th17 cells in the large intestine (168)</p> <p>Reduced Foxp3 mRNA in CD4⁺ T cells from the mLNs (321, 322)</p> <p>Selective reduction in the percentage of Foxp3⁺CD4⁺CD25⁺ T cells in the mLN (321)</p> <p>CD4⁺ T cell proliferation not suppressed as well in vitro by Tregs from the mLN (323)</p> <p>Less IL-10 produced by Tregs from the mLN (322); disease not protected as well by Tregs (322)</p> <p>Increased frequency of CD4⁺Foxp3⁺ in the small intestine (307)</p> <p>Similar frequency of lamina propria CD4⁺Foxp3⁺ T cells from the colon (168)</p>	<p>Fewer Th17 cells in the lamina propria of the small intestine of mice treated with vancomycin (138, 307, 313)</p> <p>Reduced frequency of Th17 cells in the mLN of antibiotic-treated mice (50)</p>	<p>Monoassociation of germ-free mice with cytophaga-flavobacter-bacteroidetes rescues Th17 cell defect (307)</p>
Th1/Th2 balance cell development and function	<p>Decreased delayed-type hypersensitivity in response to sheep red blood cells (386)</p> <p>Reduced αβ TCR-bearing IELs (387)</p> <p>Decreased response to T cell mitogens (294, 388)</p> <p>Normal response to Ova but reduced oral tolerance (389–392)</p> <p>Reduced tolerance to Th2 antigens (393)</p> <p>Normal graft-versus-host reaction (394)</p> <p>Reduced T cell numbers in the jejunum (264)</p>	<p>Long-term Th2-skewed immunological memory caused by Kanamycin treatment (330)</p> <p>Enhanced IgE responses caused by antibiotic treatment (108)</p>	<p>Recovery of response to T cell mitogens upon conventionalization of germ-free animals (388)</p> <p>Recovery of αβ TCR-bearing IELs upon conventionalization of germ-free animals (387)</p> <p>Recovery of jejunal T cells upon mono-association with <i>E. coli</i> (264)</p> <p>Recovery of oral tolerance upon conventionalization of germ-free or antibiotic-treated animals (300, 393, 395, 396)</p> <p>Recovery of long-term Th2-skewed immunological memory upon Kanamycin treatment through oral inoculation with <i>Enterococcus faecalis</i> and <i>Lactobacillus acidophilus</i> (300)</p>
CD8 ⁺ T cell development and function	<p>More diverse repertoire of CD8⁺ IELs in germ-free rats (353), no difference in mice (397)</p> <p>Reduced number and cytotoxicity of CD8⁺ IELs (262, 350, 387, 397)</p> <p>Delayed development of IELs (265)</p>		<p>Recovery of CD8⁺ IEL diversity upon conventionalization of germ-free animals (353)</p> <p>Recovery of number and cytotoxicity of CD8⁺ IELs upon conventionalization of germ-free animals (262, 350, 387, 397)</p> <p>Reduced numbers of naive, splenic CD8⁺ lymphocytes in animals colonized with nonpathogenic <i>Clostridium</i> sp. compared with conventionally reared controls (355)</p>