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The intestinal microbiota in health and disease: the influence of microbial products on immune cell homeostasis

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Abstract

Purpose of review—A vast and diverse array of microbes colonizes the mammalian gastrointestinal tract. These microorganisms are integral in shaping the development and function of the immune system. Metagenomic sequencing analysis has revealed alterations in intestinal microbiota in patients suffering from chronic inflammatory diseases, including inflammatory bowel disease and asthma. This review will discuss the mechanisms through which the innate immune system recognizes and responds to the intestinal microbiota as well as the effect of specific microbiota-derived signals on immune cell homeostasis.

Recent findings—Recent studies in murine model systems have demonstrated that manipulation of the intestinal microbiota can alter mammalian immune cell homeostasis. Specific microbial signals have been identified that can impact immune cell function both within the intestinal tract and in peripheral tissues. These microbiota-derived signals can either have an immunoregulatory effect, creating an immune state that is refractory to inflammation, or conversely, act as an adjuvant, aiding in the propagation of an immune response.

Summary—Associations between alterations in the microbiota and human disease implicate intestinal microbial signals in shaping immune responses. These signals are recognized by innate immune cells and influence the ability of these cells to modulate both the local and systemic immune response.

Keywords

commensal bacteria; intestinal epithelial cells; intestinal immune homeostasis; intestinal microbiota; microbial signals; TLR; TLR ligands

Introduction

The bacteria, fungi, protozoa, and viruses that inhabit the gastrointestinal tract combine to comprise the host's intestinal microbiota and are integral to digestive and metabolic processes necessary for general health. The bacterial component of the microbiota is the most well defined and studied. A large degree of diversity of bacterial species is found

throughout the intestine, with the lowest total concentration of microbes found in the duodenum and steadily increasing until the peak density of 10^{11} – 10^{12} cfu/ml of luminal content in the proximal large intestine [1,2]. Nonculture-based methods have expanded the ability to identify individual bacterial species with current estimates of total diversity between 15 000 and 36 000 individual species inhabiting the human intestine [3]. The majority of these bacteria belong to the Bacteroidetes (~64%) and Firmicutes (~23%) phyla, with other communities from the Proteobacteria and Actinobacteria phyla.

Alterations in the composition of the intestinal microbiota have been linked with several human diseases. The occurrence of obesity in both mice and humans has been associated with a decrease in the frequency of Bacteroidetes and an increase among Firmicutes. The shift in the Bacteroidetes/Firmicutes ratio increases the ability of the microbiota to break down indigestible fiber into short-chain fatty acids (SCFAs), thus providing an additional energy source that is capable of being converted into fat [4–6]. In addition to obesity, clinical evidence has linked the microbiota to the unregulated immune responses observed in diseases such Crohn's disease, ulcerative colitis, and coeliac disease [7–13]. These chronic inflammatory diseases are associated with decreased species diversity within the patient's microbiota and aberrant immune responses to bacteria found within the intestine, including lymphocyte activation and proinflammatory cytokine secretion [14–20]. Furthermore, reduction in the intestinal microbiota via antibiotics or diversion of the fecal stream away from the inflamed portion of the intestine has been moderately successful in treating Crohn's disease, thus strengthening the link between the microbiota and intestinal inflammation [21–24].

Perturbations of the intestinal microbiota have also been linked to dysregulated immune responses and inflammatory diseases at peripheral tissue sites distal from the intestine [25]. For example, there is a strong correlation between treating infants with multiple rounds of antibiotics and the development of asthma [26,27]. The presence of particular bacterial species such as *Bifidobacterium pseudocatenulatum*, *Clostridium difficile*, and *Escherichia coli* is associated with increased cases of eczema [25,28,29]. Because of the correlation between altered microbiota and inflammatory diseases, administration of 'probiotic' bacterial species has been employed to prevent or ameliorate certain atopic and intestinal inflammatory diseases [30–34]. Despite encouraging results, the full potential of probiotic therapy may not be realized without a clearer understanding of the mechanisms through which the microbiota interact with and shape the immune system. This review will focus on the progress that has been made in identifying specific microbial signals that shape mammalian immune cell homeostasis.

Altered microbiota influence immune cell homeostasis: lessons from murine model systems

Mouse models enable the manipulation of the intestinal microbiota and systematic investigation of how the microbiota influence immune responses of the host. Studies employing mice lacking microbial communities (germ-free) and mice that have had the composition of their intestinal microbiota altered via antibiotic treatment have revealed that the intestinal microbiota can shape the development, distribution, activation level,

differentiation status, and inflammatory profile of dendritic cells, macrophages, natural killer (NK) cells, B cells, CD4⁺ T cells, and CD8⁺ T cells, both within the gut-associated lymphoid tissue (GALT) and at peripheral sites within the host [35–45]. An example that illustrates the emerging paradigm of microbiota/immune cell interactions is the role the microbiota has in shaping CD4⁺ T-cell differentiation in the lamina propria. Germ-free mice have fewer T helper cell 17 (Th17) CD4⁺ T cells in the lamina propria of the small intestine compared with conventionally reared mice [46•,47••]. Further, manipulation of the microbiota by oral administration of a specific antibiotic (vancomycin) also results in a reduced percentage of Th17 cells in the lamina propria of the small intestine [48••]. Consistent with the role of microbiota influencing CD4⁺ T-cell differentiation, exposure of mice to a different microbial community known to promote skewing of CD4⁺ T cells to a Th17 cell lineage resulted in increased frequencies of CD4⁺ interleukin (IL)-17A⁺ T cells. This induction of Th17 cells was correlated with the increased presence of members of the Bacteroidetes phylum in the microbiota [48••]. These observations emphasize the possibility that signals from distinct bacterial species have a differential role in influencing the immune cell phenotype within the intestine (Fig. 1).

Use of the germ-free mouse system has also been important in demonstrating a necessary role for bacteria in the induction and propagation of intestinal inflammation. Several transgenic mouse strains that spontaneously develop intestinal inflammation do not experience disease onset when bred in a sterile, germ-free environment [49–51]. Furthermore, it has been demonstrated that colitis-inducing bacterial species from a genetically susceptible host can be transferred into wild-type recipients and initiate disease [52]. Thus, certain microbial species are capable of inducing intestinal inflammation in otherwise healthy hosts.

The effects of the intestinal microbiota on the immune system appear to go beyond the intestine and have been shown to influence immune responses at peripheral sites. For example, germ-free mice have a diminished proinflammatory cytokine response to subcutaneous lipopolysaccharide (LPS) injection or ischemic perfusion [53•,54]. In addition, mice depleted of their intestinal microbiota via antibiotic treatment exhibit an exacerbated allergic response to intranasal ovalbumin (OVA) challenge driven by the Th2 cytokine IL-13 [53•,54,55]. Furthermore, it was demonstrated that an altered intestinal microbiota was responsible for ameliorating the severity of spontaneous diabetes in MyD88^{-/-} non-obese diabetic (NOD) mice [56]. This body of literature has been crucial in demonstrating that the intestinal microbiota can influence the immune response in peripheral tissues as well as the intestine.

Microbial recognition in the intestine

The immune system within the GALT must be able to initiate an appropriate immune response against invading pathogens while remaining hyporesponsive when sampling microbial signals from the commensal flora of the intestine. The resulting balance between proinflammatory and immunoregulatory responses is thought to be dependent on the context in which the microbial signals are recognized. Dendritic cells are a key cell type that modulate the immune response within the intestine [57]. Dendritic cells within the intestine

are exposed to bacterial products either via M-cell antigen sampling within Peyer's patches or directly by extending dendrites between tight junctions of intestinal epithelial cells (IECs) and into the intestinal lumen [58–61]. Intestinal dendritic cells are capable of taking up live bacteria and migrating into the mesenteric lymph nodes, thereby exposing resident T and B cells to the microbiota [62]. Dendritic cells express a wide range of pattern recognition receptors (PRRs) that can be activated by microbial products. PRRs expressed by dendritic cells include members of the transmembrane toll-like receptor (TLR) family and the intracellularly residing nucleotide-binding oligomerization domain (NOD) like receptors (NLRs) [63,64]. Unique pathogen-associated molecular patterns (PAMPs) directly stimulate TLRs or NLRs, leading to activation of the conserved NF- κ B, mitogen-activated protein kinase (MAPK), and inflammasome signaling pathways resulting in the expression of a proinflammatory gene profile characterized by cytokines such as IL-12, IL-1 β , and type I interferons (IFNs) [65–68]. However, not all dendritic cell/PAMP interactions result in a proinflammatory response in the intestine. Stimulation of intestinal dendritic cells with the TLR-4 ligand LPS did not result in costimulatory molecule upregulation or IL-12p70 expression as is normally seen with dendritic cells derived from other tissues of the body. Instead, LPS stimulation resulted in elevated IL-10 production by intestinal dendritic cells [69,70,71]. In addition, NOD2 signaling has been shown to negatively regulate TLR-2 activation of the NF- κ B pathway and thereby attenuate expression of Th1 cytokines [72,73].

IECs are directly exposed to the intestinal microbiota and also express TLRs and NOD receptors [64,74,75]. Furthermore, microbial products act upon IECs via these PRRs and influence intestinal immune cell development and homeostasis [76,77,78,79]. Microbiota-derived signals acting upon IECs can induce an immunoregulatory environment characterized by cytokines such as IL-10, transforming growth factor beta (TGF β), thymic stromal lymphopoietin (TSLP), and IL-25, which modulate dendritic cell, macrophage, and T and B-cell activation (Fig. 1) [35,80,81,82–86]. Similar to dendritic cells, PRR activation in IECs by invading pathogens can also elicit a proinflammatory response that is important in initiating the intestinal immune response to infection [87]. Therefore, although cells within the intestinal compartment express TLR and NOD receptors and the ligands for these PRRs are found within intestinal microbiota, the outcome of PAMP/PRR interactions is dependent upon the context in which they occur. Recent work has elucidated several key microbiota-derived signals that can activate TLR or NOD signaling and influence immune cell homeostasis. These can be broadly categorized into signals that have an immunoregulatory effect or signals that augment the proinflammatory immune response.

Signals from the intestinal microbiota can induce an immunoregulatory environment

The intestinal microbiota appears to be integral in inducing an immunoregulatory environment within the intestine. Germ-free mice were reported to have reduced expression of the immunoregulatory cytokine IL-25 in the large intestine [81]. Decreased IL-25 was correlated with an increased IL-23 expression and a higher frequency of proinflammatory Th17 cells in the lamina propria of the large intestine. Therefore, although the Th17 cell pool was expanded in the large intestine of germ-free mice, the frequency of T-cell receptor

(TCR) β^+ Th17 cells was lower in the small intestine of germ-free mice [46•,47•]. Thus, it appears that the microbiota can differentially influence immune cells at distinct anatomical locations along the gastrointestinal tract. Compartment-specific influences of the microbiota are consistent with the differences in composition of the microbiota along the length of the intestine. Germ-free mice also exhibit a diminished regulatory T-cell population in the mesenteric lymph nodes that produced less TGF β upon TCR stimulation [37]. These studies suggest that signals from the intestinal microbiota help establish an immunoregulatory state in the intestine. Medzhitov [64] demonstrated that the microbiota-derived signals LPS or lipoteichoic acid (LTA) could influence intestinal immune homeostasis via TLR-4 and TLR-2 signaling, respectively. In this study, mice depleted of their microbiota by antibiotics display exacerbated intestinal inflammation and increased mortality in response to dextran sodium sulfate-induced colitis. However, antibiotic-treated mice, subsequently treated with LPS or LTA, were able to limit intestinal inflammation and recover from chemically induced colitis [88]. The mechanisms by which these TLR ligands had this immunoregulatory effect remain unclear. However, these results indicate that the microbiota can modulate intestinal inflammation, and that a specific microbial signal can orchestrate these immunoregulatory effects in the absence of a living microbiota.

Although TLR ligands have predominantly been shown to be a powerful stimulus to activate a proinflammatory response, under certain conditions, TLR activation is capable of dampening an inflammatory response. In a series of studies, Mazmanian *et al.* [89,90•] demonstrated that signaling through TLR-2 by polysaccharide A (PSA) could induce an environment refractive to inflammation. A unique form of PSA, particular to *Bacteroides fragilis*, was found to induce an immunoregulatory effect on CD4 $^+$ T cells that was highlighted by an elevated production of the immunoregulatory cytokine IL-10 [91]. Treatment with PSA isolated from *B. fragilis* prevents the induction of *Helicobacter hepaticus*-induced colitis, indicating that a single microbiota-derived molecule can influence the immune response within the intestinal environment.

In addition to TLR ligands, the microbiota appears to provide other signaling molecules that modulate the intestinal immune network and limit proinflammatory responses. For example, the NOD2 ligand muramyl dipeptide (MDP) was found to negatively regulate the production of IL-12p40, IL-6 and tumor necrosis factor alpha (TNF α) by dendritic cells *in vitro* [92]. Furthermore, in-vivo administration of MDP resulted in a diminished proinflammatory cytokine profile of colonic lymphocytes and was able to protect against trinitrobenzene sulfonic acid (TNBS)-induced colitis. Konstantinov *et al.* [93] have recently described a specific surface layer A protein (SlpA) present on the intestinal bacterial species *Lactobacillus acidophilus* NCFM that enables bacterial binding to dendritic cells, resulting in the production of IL-10 and inhibition of IL-12p70. Also, butyrate, a SCFA by-product of intestinal bacteria fermentation, has been shown to be the major energy source of colonic epithelial cells and can reduce the expression of TNF α , TNF β , IL-6, and IL-1 β in lamina propria lymphocytes potentially via inhibition of the NF- κ B pathway [94–96]. Each of these microbiota-derived molecules is a potential signal through which the intestinal microbiota can induce immune regulation.

Proinflammatory signals from the intestinal microbiota

Although signals from the intestinal microbiota can help establish an immunoregulatory environment, there is also evidence from human and mouse studies linking the microbiota to intestinal inflammatory diseases. As mentioned previously, germ-free and antibiotic-treated mouse studies [46•,47••,48••] have demonstrated the importance of the microbiota in dictating the balance between proinflammatory Th17 cells and regulatory T cells populating the GALT. Atarashi *et al.* [47••] attributed this capability to bacterial-derived ATP that preferentially induced Th17 CD4⁺ T cells. ATP was shown to act on a population of CD70^{high} CD11c^{low} accessory cells that expressed P₂X receptors, a family of ATP-binding receptors. ATP stimulation induced these cells to express IL-6 and IL-23p19, cytokines that can promote Th17 cell development and survival. These findings raise the possibility that, in addition to the influence of distinct anatomical sites along the intestine, microbial signals can have differential immunologic effects depending on whether they are directly recognized by dendritic cells or other cell populations such as IECs.

The microbiota can also augment the immune response and aid in protective immunity against pathogenic infection. For example, Hall *et al.* [97••] reported that mice depleted of their intestinal microbiota by antibiotics had diminished IFN γ and IL-17 production in the small intestine following infection with the intestinal parasite *Encephalitozoon cuniculi*. This diminished immune response was correlated with a higher parasite burden compared with wild-type mice. Here, oral administration of the specific TLR ligand CpG, but not LPS, was sufficient to recapitulate the immune response in the antibiotic-treated mice and resulted in parasite clearance in a TLR-9-dependent manner [97••]. Studies [98–100] conducted with another intestinal pathogen, *Salmonella enterica*, found that oral antibiotic treatment increases host susceptibility to *Salmonella* infection, characterized by increased bacterial translocation and intestinal inflammation. Susceptibility to infection-induced colitis persisted in mice for up to 2 weeks after termination of antibiotic treatment, suggesting that enduring alterations in the composition of the microbiota may be responsible for the inability to limit inflammation [98]. Despite these findings, the mechanisms by which microbial signals can act as an adjuvant during an immune response remain to be elucidated.

Although these studies have uncovered several key molecules that influence intestinal immune cell populations, there is still the question as to how these signals, which are located in the intestine, can influence the systemic immune response. A possible explanation comes from the field of HIV research in which LPS and bacterial DNA have been detected in the serum of HIV-infected patients [101,102•]. It has been suggested that depletion of CD4⁺ T cells in the intestine following HIV infection eventually leads to intestinal barrier breakdown and microbial translocation of the intestinal microbiota, resulting in microbial signals such as LPS entering the bloodstream. Elevated levels of LPS in the serum of HIV-positive patients correlates with an increased activation phenotype in peripheral blood T cells indicating that the microbial signals may influence the immune response throughout the host [102•]. In addition, work done by Paulos *et al.* [103] found that microbial translocation of the intestinal microbiota had an adjuvant effect on the cytotoxic capability of tumor-specific CD8⁺ T cells. Combined, these studies suggest that microbial signals

derived from bacteria within the intestine can influence immune cell function and susceptibility to immunologic diseases throughout the body.

Conclusion

It is becoming increasingly apparent that the intestinal microbiota can shape immune cell development and function. The impact of the microbiota on the immune system is not uniform as unique signals derived from different bacterial species can have either an adjuvant effect or induce a state of immune hyporesponsiveness. As intestinal microbial signals are capable of promoting such a broad range of immunological outcomes, future studies are likely to focus on identifying the influence of specific microbial signals on immune homeostasis and the molecular mechanism through which these signals elicit their pleiotropic effect. Gaining a better understanding of microbiota/immune system interaction could offer the prospect of manipulating intestinal microbiota to successfully alleviate multiple inflammatory diseases in humans.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- • of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 579–580).

1. Savage DC. Microbial ecology of the gastrointestinal tract. *Annu Rev Microbiol.* 1977; 31:107–133. [PubMed: 334036]
2. Marteau P, Pochart P, Dore J, et al. Comparative study of bacterial groups within the human cecal and fecal microbiota. *Appl Environ Microbiol.* 2001; 67:4939–4942. [PubMed: 11571208]
3. Frank DN, Amand AL St, Feldman RA, et al. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA.* 2007; 104:13780–13785. [PubMed: 17699621]
4. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature.* 2006; 444:1022–1023. [PubMed: 17183309]
5. Turnbaugh PJ, Ley RE, Mahowald MA, et al. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature.* 2006; 444:1027–1031. [PubMed: 17183312]
6. Schwiertz A, Deubel S, Birringer M. Bioactivation of selenocysteine derivatives by beta-lyases present in common gastrointestinal bacterial species. *Int J Vitam Nutr Res.* 2008; 78:169–174. [PubMed: 19326339]

7. Karin M, Lawrence T, Nizet V. Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. *Cell*. 2006; 124:823–835. [PubMed: 16497591]
8. Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. *Nat Rev Immunol*. 2003; 3:521–533. [PubMed: 12876555]
9. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med*. 2005; 353:2462–2476. [PubMed: 16339095]
10. Dicksved J, Halfvarson J, Rosenquist M, et al. Molecular analysis of the gut microbiota of identical twins with Crohn's disease. *ISME J*. 2008; 2:716–727. [PubMed: 18401439]
11. Ott SJ, Musfeldt M, Wenderoth DF, et al. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut*. 2004; 53:685–693. [PubMed: 15082587]
12. De Hertogh G, Aerssens J, de Hoogt R, et al. Validation of 16S rDNA sequencing in microdissected bowel biopsies from Crohn's disease patients to assess bacterial flora diversity. *J Pathol*. 2006; 209:532–539. [PubMed: 16739113]
13. Tlaskalova-Hogenova H, Stepankova R, Hudcovic T, et al. Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. *Immunol Lett*. 2004; 93:97–108. [PubMed: 15158604]
14. Manichanh C, Rigottier-Gois L, Bonnaud E, et al. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut*. 2006; 55:205–211. [PubMed: 16188921]
15. Swidsinski A, Ladhoff A, Pernthaler A, et al. Mucosal flora in inflammatory bowel disease. *Gastroenterology*. 2002; 122:44–54. [PubMed: 11781279]
16. Macpherson A, Khoo UY, Forgacs I, et al. Mucosal antibodies in inflammatory bowel disease are directed against intestinal bacteria. *Gut*. 1996; 38:365–375. [PubMed: 8675088]
17. Cong Y, Weaver CT, Lazenby A, Elson CO. Bacterial-reactive T regulatory cells inhibit pathogenic immune responses to the enteric flora. *J Immunol*. 2002; 169:6112–6119. [PubMed: 12444113]
18. Sartor RB, Rath HC, Lichtman SN, van Tol EA. Animal models of intestinal and joint inflammation. *Baillieres Clin Rheumatol*. 1996; 10:55–76. [PubMed: 8674149]
19. Medina M, De Palma G, Ribes-Koninckx C, et al. Bifidobacterium strains suppress in vitro the pro-inflammatory milieu triggered by the large intestinal microbiota of coeliac patients. *J Inflamm (Lond)*. 2008; 5:19. [PubMed: 18980693]
20. Duchmann R, Kaiser I, Hermann E, et al. Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD). *Clin Exp Immunol*. 1995; 102:448–455. [PubMed: 8536356]
21. D'Haens GR, Geboes K, Peeters M, et al. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. *Gastroenterology*. 1998; 114:262–267. [PubMed: 9453485]
22. Sartor RB. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. *Gastroenterology*. 2004; 126:1620–1633. [PubMed: 15168372]
23. Casellas F, Borruel N, Papo M, et al. Antiinflammatory effects of enterically coated amoxicillin-clavulanic acid in active ulcerative colitis. *Inflamm Bowel Dis*. 1998; 4:1–5. [PubMed: 9552221]
24. Rutgeerts P, Goobes K, Peeters M, et al. Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. *Lancet*. 1991; 338:771–774. [PubMed: 1681159]
25. Penders J, Stobberingh EE, van den Brandt PA, Thijs C. The role of the intestinal microbiota in the development of atopic disorders. *Allergy*. 2007; 62:1223–1236. [PubMed: 17711557]
26. Marra F, Marra CA, Richardson K, et al. Antibiotic use in children is associated with increased risk of asthma. *Pediatrics*. 2009; 123:1003–1010. [PubMed: 19255032]
27. Kozyskyj AL, Ernst P, Becker AB. Increased risk of childhood asthma from antibiotic use in early life. *Chest*. 2007; 131:1753–1759. [PubMed: 17413050]
28. Gore C, Munro K, Lay C, et al. Bifidobacterium pseudocatenulatum is associated with atopic eczema: a nested case-control study investigating the fecal microbiota of infants. *J Allergy Clin Immunol*. 2008; 121:135–140. [PubMed: 17900682]

29. Penders J, Thijs C, van den Brandt PA, et al. Gut microbiota composition and development of atopic manifestations in infancy: the KOALA Birth Cohort Study. *Gut*. 2007; 56:661–667. [PubMed: 17047098]
30. Kukkonen K, Savilahti E, Haahtela T, et al. Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol*. 2007; 119:192–198. [PubMed: 17208601]
31. Hart AL, Stagg AJ, Kamm MA. Use of probiotics in the treatment of inflammatory bowel disease. *J Clin Gastroenterol*. 2003; 36:111–119. [PubMed: 12544192]
32. Hedin C, Whelan K, Lindsay JO. Evidence for the use of probiotics and prebiotics in inflammatory bowel disease: a review of clinical trials. *Proc Nutr Soc*. 2007; 66:307–315. [PubMed: 17637082]
33. Gionchetti P, Rizzello F, Venturi A, et al. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterology*. 2000; 119:305–309. [PubMed: 10930365]
34. Mimura T, Rizzello F, Helwig U, et al. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. *Gut*. 2004; 53:108–114. [PubMed: 14684584]
35. He B, Xu W, Santini PA, et al. Intestinal bacteria trigger T cell-independent immunoglobulin A(2) class switching by inducing epithelial-cell secretion of the cytokine APRIL. *Immunity*. 2007; 26:812–826. [PubMed: 17570691]
36. Fujiwara D, Wei B, Presley LL, et al. Systemic control of plasmacytoid dendritic cells by CD8⁺ T cells and commensal microbiota. *J Immunol*. 2008; 180:5843–5852. [PubMed: 18424703]
37. Ishikawa H, Tanaka K, Maeda Y, et al. Effect of intestinal microbiota on the induction of regulatory CD25⁺ CD4⁺ T cells. *Clin Exp Immunol*. 2008; 153:127–135. [PubMed: 18460018]
38. Kawaguchi M, Nanno M, Umesaki Y, et al. Cytolytic activity of intestinal intraepithelial lymphocytes in germ-free mice is strain dependent and determined by T cells expressing gamma delta T-cell antigen receptors. *Proc Natl Acad Sci U S A*. 1993; 90:8591–8594. [PubMed: 8378333]
39. Imaoka A, Matsumoto S, Setoyama H, et al. Proliferative recruitment of intestinal intraepithelial lymphocytes after microbial colonization of germ-free mice. *Eur J Immunol*. 1996; 26:945–948. [PubMed: 8625993]
40. Mikkelsen HB, Garbarsch C, Trantum-Jensen J, Thuneberg L. Macrophages in the small intestinal muscularis externa of embryos, newborn and adult germ-free mice. *J Mol Histol*. 2004; 35:377–387. [PubMed: 15503811]
41. Helgeland L, Dissen E, Dai KZ, et al. Microbial colonization induces oligo-clonal expansions of intraepithelial CD8 T cells in the gut. *Eur J Immunol*. 2004; 34:3389–3400. [PubMed: 15517613]
42. Oliveira MR, Tafuri WL, Afonso LC, et al. Germ-free mice produce high levels of interferon-gamma in response to infection with *Leishmania major* but fail to heal lesions. *Parasitology*. 2005; 131:477–488. [PubMed: 16174412]
43. Sanos SL, Bui VL, Mortha A, et al. RORgammat and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46⁺ cells. *Nat Immunol*. 2009; 10:83–91. [PubMed: 19029903]
44. Satoh-Takayama N, Vosschenrich CA, Lesjean-Pottier S, et al. Microbial flora drives interleukin 22 production in intestinal NKp46⁺ cells that provide innate mucosal immune defense. *Immunity*. 2008; 29:958–970. [PubMed: 19084435]
45. Macpherson AJ. IgA adaptation to the presence of commensal bacteria in the intestine. *Curr Top Microbiol Immunol*. 2006; 308:117–136. [PubMed: 16922088]
46. Niess JH, Leithauser F, Adler G, Reimann J. Commensal gut flora drives the expansion of proinflammatory CD4 T cells in the colonic lamina propria under normal and inflammatory conditions. *J Immunol*. 2008; 180:559–568. This reference was the first to demonstrate an altered Th17 cell population at a steady state in germ-free mice. [PubMed: 18097058]
47. Atarashi K, Nishimura J, Shima T, et al. ATP drives lamina propria T(H)17 cell differentiation. *Nature*. 2008; 455:808–812. This study demonstrates that a non-TLR/NOD ligand derived from the microbiota can influence immune cell differentiation. [PubMed: 18716618]

- 48••. Ivanov II, Frutos Rde L, Manel N, et al. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe*. 2008; 4:337–349. The authors demonstrate altered microbiota influence on CD4⁺ T-cell differentiation. Immune cell homeostasis is influenced by the composition of the intestinal microbiota not merely the presence or absence of microbes. [PubMed: 18854238]
49. Schultz M, Tonkonogy SL, Sellon RK, et al. IL-2-deficient mice raised under germfree conditions develop delayed mild focal intestinal inflammation. *Am J Physiol*. 1999; 276:G1461–G1472. [PubMed: 10362650]
50. Sellon RK, Tonkonogy S, Schultz M, et al. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun*. 1998; 66:5224–5231. [PubMed: 9784526]
51. Dianda L, Hanby AM, Wright NA, et al. T cell receptor-alpha beta-deficient mice fail to develop colitis in the absence of a microbial environment. *Am J Pathol*. 1997; 150:91–97. [PubMed: 9006326]
52. Garrett WS, Lord GM, Punit S, et al. Communicable ulcerative colitis induced by T-beta deficiency in the innate immune system. *Cell*. 2007; 131:33–45. [PubMed: 17923086]
- 53•. Amaral FA, Sachs D, Costa VV, et al. Commensal microbiota is fundamental for the development of inflammatory pain. *Proc Natl Acad Sci USA*. 2008; 105:2193–2197. References [53•,56] show that the intestinal microbiota can alter the proinflammatory cytokine environment and immune response in tissues separate from the GALT G10. [PubMed: 18268332]
54. Souza DG, Vieira AT, Soares AC, et al. The essential role of the intestinal microbiota in facilitating acute inflammatory responses. *J Immunol*. 2004; 173:4137–4146. [PubMed: 15356164]
55. Noverr MC, Falkowski NR, McDonald RA, et al. Development of allergic airway disease in mice following antibiotic therapy and fungal microbiota increase: role of host genetics, antigen, and interleukin-13. *Infect Immun*. 2005; 73:30–38. [PubMed: 15618138]
56. Wen L, Ley RE, Volchkov PY, et al. Innate immunity and intestinal microbiota in the development of type 1 diabetes. *Nature*. 2008; 455:1109–1113. [PubMed: 18806780]
57. Coombes JL, Powrie F. Dendritic cells in intestinal immune regulation. *Nat Rev Immunol*. 2008; 8:435–446. [PubMed: 18500229]
58. Rescigno M, Urbano M, Valzasina B, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol*. 2001; 2:361–367. [PubMed: 11276208]
59. Niess JH, Brand S, Gu X, et al. CX3CR1 -mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science*. 2005; 307:254–258. [PubMed: 15653504]
60. Mach J, Hsieh T, Hsieh D, et al. Development of intestinal M cells. *Immunol Rev*. 2005; 206:177–189. [PubMed: 16048549]
61. Milling SW, Cousins L, MacPherson GG. How do DCs interact with intestinal antigens? *Trends Immunol*. 2005; 26:349–352. [PubMed: 15978521]
62. Macpherson AJ, UhrT. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science*. 2004; 303:1662–1665. [PubMed: 15016999]
63. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. *Nat Immunol*. 2004; 5:987–995. [PubMed: 15454922]
64. Medzhitov R. Recognition of microorganisms and activation of the immune response. *Nature*. 2007; 449:819–826. [PubMed: 17943118]
65. Strober W, Murray PJ, Kitani A, Watanabe T. Signalling pathways and molecular interactions of NOD1 and NOD2. *Nat Rev Immunol*. 2006; 6:9–20. [PubMed: 16493424]
66. Sartor RB. Microbial influences in inflammatory bowel diseases. *Gastroenterology*. 2008; 134:577–594. [PubMed: 18242222]
67. Girardin SE, Boneca IG, Carneiro LA, et al. Nod1 detects a unique mur-o-peptide from Gram-negative bacterial peptidoglycan. *Science*. 2003; 300:1584–1587. [PubMed: 12791997]
68. Girardin SE, Boneca IG, Viala J, et al. Nod2 is a general sensor of pepti-doglycan through muramyl dipeptide (MDP) detection. *J Biol Chem*. 2003; 278:8869–8872. [PubMed: 12527755]

69. Cerovic V, Jenkins CD, Barnes AG, et al. Hyporesponsiveness of intestinal dendritic cells to TLR stimulation is limited to TLR4. *J Immunol.* 2009; 182:2405–2415. The authors show that intestinal dendritic cells have unique properties and respond differently to TLR stimulation compared with dendritic cells found in other tissues of the body. [PubMed: 19201895]
70. Monteleone I, Platt AM, Jaensson E, et al. IL-10-dependent partial refractoriness to toll-like receptor stimulation modulates gut mucosal dendritic cell function. *Eur J Immunol.* 2008; 38:1533–1547. [PubMed: 18461564]
71. Takenaka S, Safroneeva E, Xing Z, Gauldie J. Dendritic cells derived from murine colonic mucosa have unique functional and phenotypic characteristics. *J Immunol.* 2007; 178:7984–7993. [PubMed: 17548635]
72. Watanabe T, Kitani A, Murray PJ, Strober W. NOD2 is a negative regulator of toll-like receptor 2-mediated T helper type 1 responses. *Nat Immunol.* 2004; 5:800–808. [PubMed: 15220916]
73. Yang Z, Fuss IJ, Watanabe T, et al. NOD2 transgenic mice exhibit enhanced MDP-mediated down-regulation of TLR2 responses and resistance to colitis induction. *Gastroenterology.* 2007; 133:1510–1521. [PubMed: 17915219]
74. Hisamatsu T, Suzuki M, Reinecker HC, et al. CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. *Gastroenterology.* 2003; 124:993–1000. [PubMed: 12671896]
75. Fritz JH, Ferrero RL, Philpott DJ, Girardin SE. Nod-like proteins in immunity, inflammation and disease. *Nat Immunol.* 2006; 7:1250–1257. [PubMed: 17110941]
76. Hasegawa M, Yang K, Hashimoto M, et al. Differential release and distribution of Nod1 and Nod2 immunostimulatory molecules among bacterial species and environments. *J Biol Chem.* 2006; 281:29054–29063. [PubMed: 16870615]
77. Bouskra D, Brezillon C, Berard M, et al. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature.* 2008; 456:507–510. The authors demonstrate that a NOD ligand originating from the intestinal microbiota can stimulate IECs. Further, IECs can shape immune cell development and function within the intestine. [PubMed: 18987631]
78. Zaph C, Troy AE, Taylor BC, et al. Epithelial-cell-intrinsic IKK-beta expression regulates intestinal immune homeostasis. *Nature.* 2007; 446:552–556. [PubMed: 17322906]
79. Nenci A, Becker C, Wullaert A, et al. Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature.* 2007; 446:557–561. [PubMed: 17361131]
80. Taylor BC, Zaph C, Troy AE, et al. TSLP regulates intestinal immunity and inflammation in mouse models of helminth infection and colitis. *J Exp Med.* 2009; 206:655–667. [PubMed: 19273626]
81. Zaph C, Du Y, Saenz SA, et al. Commensal-dependent expression of IL-25 regulates the IL-23-IL-17 axis in the intestine. *J Exp Med.* 2008; 205:2191–2198. This reference raises the possibility that microbial signals can differentially influence immune cells at distinct locations along the gastrointestinal tract. [PubMed: 18762568]
82. Dignass AU, Podolsky DK. Cytokine modulation of intestinal epithelial cell restitution: central role of transforming growth factor beta. *Gastroenterology.* 1993; 105:1323–1332. [PubMed: 8224636]
83. Brown SL, Riehl TE, Walker MR, et al. Myd88-dependent positioning of Ptg2-expressing stromal cells maintains colonic epithelial proliferation during injury. *J Clin Invest.* 2007; 117:258–269. [PubMed: 17200722]
84. Rimoldi M, Chieppa M, Salucci V, et al. Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nat Immunol.* 2005; 6:507–514. [PubMed: 15821737]
85. Rimoldi M, Chieppa M, Larghi P, et al. Monocyte-derived dendritic cells activated by bacteria or by bacteria-stimulated epithelial cells are functionally different. *Blood.* 2005; 106:2818–2826. [PubMed: 16030185]
86. Zeuthen LH, Fink LN, Frokiaer H. Epithelial cells prime the immune response to an array of gut-derived commensals towards a tolerogenic phenotype through distinct actions of thymic stromal lymphopoietin and transforming growth factor-beta. *Immunology.* 2008; 123:197–208. [PubMed: 17655740]

87. Zilbauer M, Dorrell N, Elmi A, et al. A major role for intestinal epithelial nucleotide oligomerization domain 1 (NOD1) in eliciting host bactericidal immune responses to *Campylobacter jejuni*. *Cell Microbiol*. 2007; 9:2404–2416. [PubMed: 17521327]
88. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, et al. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell*. 2004; 118:229–241. [PubMed: 15260992]
89. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell*. 2005; 122:107–118. [PubMed: 16009137]
- 90•. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature*. 2008; 453:620–625. The authors demonstrate that administration of a unique microbiota-derived product is sufficient to dampen the inflammatory immune response and protect against experimentally induced colitis. [PubMed: 18509436]
91. Wang Q, McLoughlin RM, Cobb BA, et al. A bacterial carbohydrate links innate and adaptive responses through toll-like receptor 2. *J Exp Med*. 2006; 203:2853–2863. [PubMed: 17178920]
92. Watanabe T, Asano N, Murray PJ, et al. Muramyl dipeptide activation of nucleotide-binding oligomerization domain 2 protects mice from experimental colitis. *J Clin Invest*. 2008; 118:545–559. [PubMed: 18188453]
93. Konstantinov SR, Smidt H, de Vos WM, et al. S layer protein A of *Lactobacillus acidophilus* NCFM regulates immature dendritic cell and T cell functions. *Proc Natl Acad Sci USA*. 2008; 105:19474–19479. [PubMed: 19047644]
94. Roediger WE. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut*. 1980; 21:793–798. [PubMed: 7429343]
95. Segain JP, Raingeard de la Bletiere D, Bourreille A, et al. Butyrate inhibits inflammatory responses through NF-kappaB inhibition: implications for Crohn's disease. *Gut*. 2000; 47:397–403. [PubMed: 10940278]
96. Luhrs H, Gerke T, Muller JG, et al. Butyrate inhibits NF-kappaB activation in lamina propria macrophages of patients with ulcerative colitis. *Scand J Gastroenterol*. 2002; 37:458–466. [PubMed: 11989838]
- 97•. Hall JA, Bouladoux N, Sun CM, et al. Commensal DNA limits regulatory T cell conversion and is a natural adjuvant of intestinal immune responses. *Immunity*. 2008; 29:637–649. The authors demonstrate that a specific microbial product can act as an adjuvant and augment the immune response against infection. [PubMed: 18835196]
98. Croswell A, Amir E, Tegatz P, et al. Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric *Salmonella* infection. *Infect Immun*. 2009; 77:2741–2753. [PubMed: 19380465]
99. Sekirov I, Tam NM, Jogova M, et al. Antibiotic-induced perturbations of the intestinal microbiota alter host susceptibility to enteric infection. *Infect Immun*. 2008; 76:4726–4736. [PubMed: 18678663]
100. Garner CD, Antonopoulos DA, Wagner B, et al. Perturbation of the small intestine microbial ecology by streptomycin alters pathology in a *Salmonella enterica* serovar typhimurium murine model of infection. *Infect Immun*. 2009; 77:2691–2702. [PubMed: 19433544]
101. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med*. 2006; 12:1365–1371. [PubMed: 17115046]
- 102•. Jiang W, Lederman MM, Hunt P, et al. Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. *J Infect Dis*. 2009; 199:1177–1185. The authors show that signals from the microbiota can be detected in the serum and detection correlates with T-cell activation. Thus, signals from the microbiota can have an influence on immune activation. [PubMed: 19265479]
103. Paulos CM, Wrzesinski C, Kaiser A, et al. Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8⁺ T cells via TLR4 signaling. *J Clin Invest*. 2007; 117:2197–2204. [PubMed: 17657310]

Figure 1. Specific signals derived from the intestinal microbiota stimulate different cell types within the intestine (e.g. dendritic cells and intestinal epithelial cells)

Microbial signals influence DCs and IECs to induce either an immunoregulatory or proinflammatory cytokine environment. This cytokine environment can condition antigen-presenting cells and influence CD4⁺ T-cell differentiation within the intestine. DC, dendritic cells; IEC, intestinal epithelial cells; IL, interleukin; MDP, muramyl dipeptide; NOD, nucleotide-binding oligomerization domain; PSA, polysaccharide A; SCFA, short-chain fatty acids; TGFβ, transforming growth factor beta; Th, T helper cell; TLR, toll-like receptor; TNFα, tumor necrosis factor alpha; Treg, T regulatory cells; TSLP, thymic stromal lymphopoietin.