Published in final edited form as:

Semin Immunol. 2012 February ; 24(1): 58–66. doi:10.1016/j.smim.2011.11.008.

Intestinal Microbiota: Shaping local and systemic immune responses

Michael Molloy, Nicolas Bouladoux, and Yasmine Belkaid

Mucosal Immunology Unit, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 4 Center Drive, Room 4/126, Bethesda, MD 20892, USA ybelkaid@niaid.nih.gov

Abstract

Recent studies have highlighted the fundamental role of commensal microbes in the maintenance of host homeostasis. For instances, commensals can play a major role in the control of host defense, metabolism and tissue development. Over the past few years, abundant experimental data also support their central role in the induction and control of both innate and adaptive responses. It is now clearly established that commensals are not equal in their capacity to trigger control regulatory or effector responses, however, the molecular basis of these differences has only recently begun to be explored. This review will discuss recent findings evaluating how commensals shape both effector and regulatory responses at steady state and during infections and the consequence of this effect on local and systemic protective and inflammatory responses.

The human intestine harbors and is in constant contact with 1000 trillion microbes, composed of an estimated 4000 strains (1, 2). Recent studies have changed our perspective of commensal microbes from benign passengers, to active participants in both the post-natal development of mucosal and systemic immunity, and in its long-term steady-state function. To maintain its interaction with commensals and sustain its function as a digestive organ, the gastrointestinal tract environment requires the constant induction and maintenance of various classes of regulatory responses. However, immune tolerance does not represent the only fate of immune responses at mucosal sites as a certain degree of constitutive effector responses and inflammation is beneficial for the host, not only to reinforce the barrier, but also to allow for the development of protective responses when required. This implies that the regulation of this environment is more complex than initially thought and relies on the maintenance of a constant balance of antagonistic signals. This review will discuss recent findings evaluating how commensals shape both effector and regulatory responses at steady state and during infections and the consequence of this regulation on protective responses against pathogens.

Commensals control the first line of defense

Various aspects of host protective structures and innate immunity develop extensively after birth, due in large part to the interaction with the recently acquired microbiota (3). Studies performed in germ free (GF) animals revealed that the microbiota plays a critical role in secondary lymphoid structure development (4, 5). This also includes fortification of the intestinal barrier through epithelial cell maturation and angiogenesis of a capillary network

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

that facilitates transport of white blood cells (6, 7). The molecular mechanism responsible for this development remains incompletely understood, but at least in part involves a variety of pattern recognition receptors (PRRs) which are capable of detecting microbe-associated molecular patterns (MAMPs) including toll-like receptors (TLRs), NOD-like receptors (NLRs) and RIG-like receptors (RLRs) (8). Several lines of evidence indicate that microbial signals are also responsible for the induction and development of isolated lymphoid follicles (ILFs) from cryptopatches in the intestinal tract. Notably, ILFs appear within the first weeks after birth of mice and can eventually number in the hundreds (9). The current model hypothesizes that gram-negative bacterial derived peptidoglycans are sensed by NOD1 expressed on intestinal epithelial cells (IECs) (10). The IECs subsequently express CCL20 and β -defensin 3 which activate LT α i cells leading to the formation of ILF (11-13). An alternative but not exclusive model proposes that the activated LT α i cells would engage lymphoid tissue organizer (LT α o) cells, which are mesenchymal in origin, to express CCL20 for the recruitment for B cells.

Other critical components of host defense are represented by the mucus layer and antimicrobial peptides both under the tight control of the flora (14-16). Engagement of PRRs by commensally derived products induces expression of a variety of anti-microbial peptides, which are critical in preventing translocation of bacteria through the rest of the host tissue (17). One of the best-characterized mucosal anti-microbial peptides is RegIII γ , which is expressed soon after birth or following colonization of GF mice (18). Production of this lectin is tightly controlled by the flora in an MyD88 dependent manner and has a direct microbicidal effect on Gram-positive bacteria (18, 19) (20). Similarly, the PRR NOD2 controls expression of a subset of α -defensins and cryptdins by Paneth cells (21). Thus, by virtue of favoring structural development and innate immune responses at the intestinal interface, the flora plays a dominant role in controlling primary encounter with pathogens.

The homeostasis of the GI tract: balance of inflammatory and regulatory signals

Although mucosal surfaces have to constitutively integrate a multitude of microbial derived signals, new evidence suggest that defined bacteria or microbial products can play a dominant role in the induction of distinct class of immune responses. At steady state, the gut is home to a large number of lymphocytes, a large fraction of them with the potentiality to produce cytokines such as IL-17, IL-22, FN- γ and / or IL-10 (22, 23). The flora tightly controls this constitutive production of cytokines, as GF mice show extensive deficiencies in basal cytokine production (22, 24). Further, in absence of flora, the CD4⁺ T cell population is diminished, disproportionately affecting Th1 and Th17 cells, although, Treg frequencies are maintained or increased in the small intestine (22, 23). Colonization of GF mice with complex microbiota orchestrates a broad spectrum of T helper (Th1, Th17) and regulatory T cell responses (25). Some of the factors that govern the induction of constitutive effector and regulatory responses in the GI tract and how such conditioning affect subsequent effector responses against pathogens will be discussed in this review.

Regulatory responses in the GI tract

A complex regulatory network including specialized population of antigen presenting cells, lymphocytes and innate cytokines, controls GI tract homeostasis and converge to favor the induction of regulatory responses toward antigens present at mucosal sites. Early reports suggested that commensals played a central role in maintaining such regulation. Indeed, oral tolerance (26) - the active suppression of inflammatory responses to food and other orally ingested antigens - could not be induced in the absence of gut flora or gut flora derived signals (27, 28) and feeding germ free mice with LPS was sufficient to restore this process

(27). The presence of commensals has been also associated with the suppression of IgE and Th2 responses following antigen feeding (29). Oral tolerance could also be rescued by reconstitution of germ free mice with *Bifidobacterium infantis*, a dominant commensal bacteria (29). Some of this effect of the flora has been associated with the cross talk between commensal derived LPS and TLR4 (30, 31). Intriguingly, recent evidence demonstrate that conditioning of naïve T cells with LPS, a phenomenon that would occur in gut associated lymphoid structure, provides a tonic inhibitory role for TLR4 signaling on subsequent-dependent CD4⁺T cell responses (32). Such phenomenon may account for the limitation of aberrant responses to orally derived antigen. Although LPS has been associated with the acquisition of oral tolerance, the precise molecular mechanism accounting for such phenomenon and the targets of microbial derived signals remains incompletely understood.

Induction of Treg cells at mucosal sites

Although immunological tolerance is likely to be achieved via multiple and redundant mechanisms (26), over the past few years, several actors including TGF- β , IL-10 and in particular Foxp3 regulatory T (Treg) cell have taken central stage in our understanding of this process. Treg cells maintain both peripheral and mucosal homeostasis throughout the lifespan of the host (33). Treg cells typically develop during thymic selection processes; however they can also develop extra-thymically in response to chronic antigen stimulation or exposure to environmental and food antigen at mucosal sites (34). In particular, the gut-associated lymphoid tissue is a preferential site for the peripheral induction of Treg cells (35-38). Development of inducible Treg (iTreg) cells requires transcription factor binding to the intronic enhancer element (enhancer-1) of the foxp3 locus, also known as conserved non-coding sequence 1 (CNS1) and is dependent on several soluble mediators, including: TGF- β , IL-2 and the vitamin A metabolite retinoic acid (RA) (35-37, 39-42). This process is tightly controlled at steady state by the capacity of a specialized population of gut tropic DCs expressing CD103 to produce RA (35, 36, 43). In addition to supporting iTreg differentiation, RA derived traffic signals are required for a sustained expansion of iTreg cells in the gut (44). This expansion is propagated through IL-10 mediated interactions with lamina propria resident CX3CR1⁺ macrophages (44). In previous studies, similar interactions were shown to contribute to both the induction and maintenance of Treg cells (43, 45). Recent findings demonstrated that the physiological relevance of iTreg induction at mucosal sites is associated with their central role in oral tolerance (46, 47). Further, RA is also required to elicit pro-inflammatory helper T cell responses to infection and mucosal vaccination (42, 48). Antagonism of RA receptor (RAR) signaling results in a cell-autonomous CD4 T cell activation defect, which impairs intermediate signaling events, including calcium mobilization. Altogether, these findings reveal a fundamental role for the RA-RAR axis in the development of both regulatory and inflammatory arms of adaptive immunity (49). The precise factors that govern the activation of enzymes involved in the metabolism of RA as well as how commensals or pathogens modify the metabolism of vitamin A remains poorly understood. However, interaction with microbial products and in particular via TLR2 can promote vitamin A metabolism (50). A reciprocal regulation between the flora and vitamin A metabolism is further supported by the observation that vitamin A deficiency leads to dramatic shift in commensal populations (51). Of note, some of this alteration in communal population may result from the role of RA in controlling IgA responses and or homing receptors of on effector cells required for the proper establishment of the mucosal firewall (52, 53). An important future area of study will be to understand the complex interplay between vitamin A and commensal populations in the induction of regulatory and effector responses to pathogens. Another mean by which commensals may control oral tolerance is associated with the role of the flora in the control of antigen sampling of luminal contents by DCs from the underlying lamina propria compartment (54). Recent reports also indicate that the gut flora can directly contribute to the expansion of

lamina propria resident CX3CR1 macrophages that have been associated with local expansion of Treg cells (55). Nevertheless the role of commensals on mucosal APC function at steady state remains poorly understood. Studies indicate a role for commensals in controlling liver resident DCs as gut-derived bacterial products, by stimulating hepatic IL-6/STAT3 signaling, inhibit hepatic DC activation and or maturation, and thus elevate the threshold needed for translating triggers of innate immunity into adaptive immune responses (56). Such control appears to be tissue specific as *ex vivo* analysis of DC status of activation revealed a similar pattern of activation between GF and conventionally raised mice in secondary lymphoid organs including mesenteric lymph nodes (57, 58). However, it is worth noting that the diet of GF mice contains endotoxins that can provide surrogate signals to the ones normally provided by the flora. Coupled with activation induced by tissue dissociation, this is likely to blunt any potential differences resulting from the absence of commensals. Based on the known role for gut microbiota in promoting both regulatory and effector populations, a better understanding of the role of commensals in shaping tissue resident and peripheral DC activation level, antigen uptake, half life and migratory capacity is clearly needed.

Although, various lines of research have established that the presence of Treg cells is necessary to limit exuberant response to commensals during inflammatory settings (59), how commensals themselves contribute to Treg cell function and or induction, has, until recently, remained unclear. Among the most significant advance in this line of research is the recent finding that the microbiota can promote the induction and / or activation of Treg cells at mucosal sites. The first demonstration that a single symbiont molecule could promote regulatory responses was provided by the identification of the polysaccharide A (PSA) which is produced by a prominent human symbiont *Bacteroides fragilis* (5). Colonization of GF mice with *B. fragilis* or treatment with purified PSA directs the development of the immune system (5). Furthermore, via PSA expression, *B. fragilis* can protect mice from experimental colitis induced by *Helicobacter hepaticus*, a commensal bacterium with pathogenic potential (60). This protective activity was associated with the capacity of PSA to induce or expand IL-10 producing CD4⁺T cells (60-62). Additionally, *B. Fragilis* was able to promote Treg cell function via TLR2, a phenomenon associated with their capacity to limit IL-17 responses (63). Induction of Treg cells is not restricted to *B. fragilis* as the presence of an indigenous Clostridium species also promotes Treg cell accumulation via, at least in part, its capacity to create a TGF- β rich environment (64). This induction of Treg function requires TCR mediated signals, MyD88 and Ticam-1, as well as signaling via IL-10R (64). Based on the fundamental role of Treg cells in maintaining mucosal homeostasis, it is likely that rather than being restricted to defined bacteria, most indigenous flora have evolved to favor this aspect of the regulatory network.

Promising results have been obtained with probiotics - bacteria known to confer a health benefit to the host - in the treatment of human inflammatory diseases of the intestine and in the prevention and treatment of atopic eczema in neonates and infants, and some of the effect of probiotics is believed to be associated with the induction or expansion of Treg cells. For instances, in mice, treatment with probiotic Lactobacillus and/or Bifidobacterium suppressed TNBS-induced colitis (65, 66), as well as allergic responses due possibly to the induction of TGF- β production (67) and stimulation of Treg cells that are able to suppress allergic responses (68). There is also good evidence of probiotic modulation of DCs towards a pro-regulatory function (69, 70). Multiple reports indicate that bacteria with known anti-inflammatory properties such as bifidobacteria, lactobacilli and *Streptococcus salivarius* are capable of promoting both Treg and IL-10 producing, FoxP3-Tr1 cells (61, 70-74).

In addition of the capacity of commensals to co-opt Treg cells and potentate their function, recent evidence support the idea that immune tolerance toward commensals is also

controlled by the nature of the antigen recognized by Treg cells. Indeed, colonic Treg cells use TCR distinct from those used by those from other locations and that a fraction of them can react preferentially to antigens derived from the commensal population (75). These results suggest a major role for the microbiota in shaping the repertoire of tissue resident Treg cells and in the maintenance of host-microbe mutualism at barrier sites. Based on the abundance and complexity of the flora, one could speculate that opportunity for cross reactivity between commensals and pathogenic organism derived antigen is high. Thus, microbial pressure in the gut could lead to the induction and maintenance of a pool of activated Treg cells (both natural and inducible) that may not only maintain a mutualistic relationship with the microbiota, but also via cross reactive responses, promote pathogen expansion and maintain heterologous chronic infections (76).

Promotion of infection via Microbiota induced regulatory responses

Several studies highlighted a fundamental role for the microbiota in promoting pathogen transmission and recent evidence suggests that this effect may, in some instances, rely on the appropriate induction of regulatory responses. One of the first illustrations of the positive effect of the flora on pathogen development and survival was revealed in a model of *Trichuris muris* nematode infection (77). In this model of infection, egg hatching in the large intestine only occurred upon tight contact with bacteria suggesting that the microflora provides critical cues for the appropriate establishment of the life cycle of gut tropic nematodes (77). More recently, two studies using viral models revealed that such a role might represent a novel paradigm for the transmission of various pathogens including viruses. Poliovirus relies on the microbiota for efficient replication, an effect that can be at least in part associated with the capacity of the virus to bind to cardinal microbial products such as LPS (78). Similarly the capacity of MMTV to bind to commensally derived LPS favors mucosal transmission of the virus via the induction of the regulatory cytokine IL-10. Such effect leads to a state of immunological unresponsiveness toward viral antigen that favors transmission of the virus (79). Of note, in these studies the elements accounting for the promoting effect of the flora on pathogen transmission and / or virulence are highly represented microbial products, suggesting that both virus and nematodes may have evolved to bypass commensal population shift by thriving on ubiquitous microbial derived components. This would suggest that although manipulating the flora may represent an efficient way of altering immunity to pathogens, this strategy is unlikely to have global consequences on pathogen transmission. Based on the pleiotropic effect of the flora in induction of regulatory pathways, host metabolism and tissue resident function, one would expect that a high number of pathogens transmitted via mucosa or using commensal rich habitats have evolved to benefit from the complex interaction of the host with its microbiota.

Shaping effector responses

Induction of immunological tolerance and Treg cells does not represent a universal fate for commensally derived antigens. Indeed, the GI tract is clearly home to a large fraction of cells with effector potential for which antigenic specificities remain unclear. For instances, the gut contains the highest frequencies of cells able to produce IL-17 under steady state conditions (80). Recent evidence demonstrates that Th17 cells develop normally in mice expressing a single TCR in the absence of Ag (81). However even under this setting, the flora is essential for their development (81). Although these results strongly support the idea that the flora controls the tissue derived cues required for the induction and or maintenance of IL-17, they do not preclude that under less contrived settings, a significant portion of mucosal Th17 cells recognize commensal antigens. Further, a significant fraction of T cells with Th1 characteristics reside in the GI tract with frequencies that are also tightly controlled by commensal flora (22, 24, 25). The current view is that constitutive sensing of

commensal plays an important homeostatic role while active responses against the flora is believed to be associated to pathogenesis. However, this distinction is clearly not absolute and need to be revisited in light of the observation that healthy human serum normally contains antibodies against commensals (82) suggesting that a certain degree of commensal recognition is a common occurrence and in most circumstances, is not associated with pathogenic responses. We could speculate that in a symmetrical manner to what is now proposed for Treg cells, antigenic specificities of tissue resident effector cells are likely highly enriched for commensal antigens. How much mucosal Th1 and Th17 cells contribute to the tone of the tissue and local APC function remains to be further addressed.

Independently of inflammatory settings, various lines of research propose a major role for the microbiota, and in particular specifically defined groups of bacteria, in influencing immune system development and skewing under steady state condition. For instance, mice lacking a key group of microbes (since identified as Segmented Filamentous Bacteria (SFB)) have severely decreased numbers of mucosal Th17 cells in the small intestine, although the Treg compartment is expanded in this environment (22) (25, 83). Remarkably, SFB in association with a specific pathogen free (SPF) flora, can trigger intestinal inflammation in lymphopenic host (84). As shown by scanning electron microscopy, SFB adhere tightly to Peyer's Patches and epithelial cells of the small intestine and concomitantly induce local expression of IFN- γ , IL-10 and IL-17. SFB colonization of GF mice leads to increased Treg cells as well as IFN- γ and IL-17 producing CD4 T cells in the small intestine and colon (22) (25, 83). However, absolute numbers of these cells are not restored to the level of those mice with a complete microbiota suggesting that as expected, SFB may be sufficient for such changes but need to synergize with other microorganisms to coordinate the full maturation of the intestinal immune system. As such, based on the complexity of the flora under less contrived situations it remains unlikely that a single group of bacteria could account for all immunologic functions of the GI tract. The challenge over the next few years will be to understand the appropriate composition and / or location of commensal populations that are optimal for the establishment of balanced responses in the GI tract.

Some of this balance can be favored by defined dominant microbial derived signals. Indeed, commensals and pathogenic microbes interact with the host immune system through conserved ligands that are cardinal features of microorganisms (85). Many of these ligands signal through the Toll-like family of receptors (85). TLRs are widely expressed by cells of hematopoietic origin, as well as non-hematopoietic cells, including the epithelial cells lining the intestinal tract (86).

Bacterial flagellin is a structural protein that forms the main portion of flagella and promotes bacterial chemotaxis, adhesion and invasion of host tissue in the context of pathogenic bacteria. In addition, many commensal bacteria also have flagella and several lines of evidence indicate that interaction of commensal flagellin with TLR5 plays an important role in the GI tract. Unlike other TLR family members, TLR5 is not expressed on macrophages or conventional DCs in mice and poorly expressed by intestinal epithelial cells. Instead TLR5 is highly expressed by lamina propria DCs (LpDCs) from the small intestine and in particular on CD11c^{hi}CD11b^{hi} dendritic cells (53, 87). Flagellin stimulated LpDCs do not produce IL-10 and TNF α , but instead express chemokines, prostaglandin and antimicrobial peptides (87). In addition, LpDCs stimulated with flagellin produce both IL-6 and IL-12 (87). Whereas DCs from non-GALT tissues induce Th1 cells in response to TLR ligands, LpDCs induce ROR γ T functional Th17 cells as well as Th1 cells from naïve CD4⁺T cells in response to flagellin *in vitro* (53). In addition, these DCs induce the generation of both Th17 cells and Th1 cells in an antigen specific manner *in vivo* (53). Of note, flagellin also represents an immunodominant antigen in Crohn's disease patients (88). On the other hand, several lines of evidence support the idea that commensal derived flagellin plays a

regulatory role in the GI tract. Indeed TLR5 ligands are clearly sensed at steady state condition as evidenced by the observation that mice lacking TLR5 spontaneously develop colitis (89). Furthermore, mice lacking TLR5 over-express genes associated with innate and adaptive immunity contributing not only to colitis development but also to their enhanced protection against infection to enteric pathogens such as salmonella (90).

TLR9 recognizes unmethylated cytosine phosphate guanosine (CpG) dinucleotides, which are abundant in prokaryotic DNA found in intestinal flora. Using synthesized sequences containing CpG, previous studies have shown that engagement of TLR9 expressed on DCs, Treg and conventional T cells can limit Treg cell suppressive function (91, 92). Previous work identified an association between Crohn's disease and a promoter polymorphism in the TLR9 gene in humans (93). Such an association supports the idea of a role for gut floral DNA (gDNA) sensing in the pathophysiology of inflammatory bowel diseases (IBD). In mice, gDNA plays a major role in intestinal homeostasis through TLR9 engagement (94) and constitutive interaction between gDNA and TLR9 in the gut can act as an immunological adjuvant and critically controls the balance between Treg and effector T cells (94). Naïve TLR9 deficient mice displayed a striking increase in the frequency of Foxp3+ Treg cells within intestinal effector sites, accompanied by a significant reduction in constitutive IL-17 and IFN- γ production by effector T cells. Complementing this, gDNA, strongly constrained the capacity of lamina propria DCs to induce Treg cell conversion *in vitro* and promoted effector responses to oral pathogen (94). Previous work showed that, *in vitro*, gut flora bacteria are not equal in their capacity to stimulate TLR9 and do so with varying levels of efficiency that correlate with the frequency of [CG] dinucleotides (94, 95). Thus, it is tempting to speculate that alteration of Treg / Teff cell homeostasis mediated by TLR9 signaling may be differentially regulated by specific gut flora species. In contrast to TLR9 deficient mice, mice lacking TLR2 have decreased frequencies of Treg cells (96). Surprisingly, Myd88/Trif double deficient mice have normal numbers of Th17 cells in both the small and large intestine indicating that individual TLRs potentially influence the differentiation of Th17 cells in either a positive or negative manner (22, 97). Conversely, the alteration in the ability to sense microbial signals might affect the composition of bacterial species, which could subsequently lead to alterations in Th17 cell induction or maintenance. It has also been noted that bacterially infected apoptotic cells trigger dendritic cell production of IL-6 and TGF- β , which are critical for Th17 induction in mice, through recognition of phosphatidylserine exposed on the apoptotic cells (98). How much this process is controlled by resident bacteria and is responsible for steady state IL-17 production in the GI tract would be important to evaluate.

Adenosine 5'-triphosphate (ATP), can modulate immune cell function by means of activation of the ATP sensor P2X and P2Y receptors and commensal bacteria have been shown to generate large amounts of ATP (97). Consistent with this observation, germ free animals have reduced ATP in their feces compared to conventional mice. ATP derived from commensal bacteria can activate a unique subset of lamina propria, CD70^{high}CD11c^{low} cells, leading to the differentiation of Th17 cells (97). Of note, the precise nature of this subset remains to be fully characterized. Systemic or rectal administration of ATP into these germ-free mice results in a marked increase in the number of lamina propria Th-17 cells. The CD70^{high}CD11c^{low} subset of the lamina propria cells expresses IL-6, IL-23p19 and TGF- β -activating integrin- α V and - β 8, in response to ATP stimulation, and preferentially induces Th17 differentiation. The critical role of ATP is further underscored by the observation that administration of ATP exacerbates a T-cell-mediated colitis model with enhanced Th17 differentiation (97). These data provided evidence that commensal-derived metabolites could direct effector T cell polarization.

Microbiota-B cells cross regulation

IgA are a dominant feature of the host-commensal dialogue as IgA are critically important in shaping the microbiota and mediating pathogen clearance. For instances, IgA production has also been shown to exclude *Shigella flexneri* during infection by binding bacteria at the mucosal layer (99). Mice lacking IgA have expansion of SFB and other Clostridium related species in the intestinal tract (100). IgA responses appear to lack typical memory characteristics and respond rapidly to change in the gut flora composition. This point was elegantly demonstrated using reversible colonization of mice with a strain of auxotrophic *E.coli* that required nutrients unavailable by mammalian host metabolites (101). IgA responses persisted in the absence of bacteria, however introduction of additional species of bacteria caused a rapid decline in IgA specific for the auxotrophic bacteria. This suggests that in colonized animals, the persistence of IgA would be limited by the presence of additional bacterial species. One advantage of this system would be the ability to adapt IgA production specifically to the commensals present at a given time (101).

The discovery that RA is critical for the generation of immunoglobulin A (IgA)-secreting B cells offers further evidence of a multifactorial role for RA in mucosal immunity (52, 53). Notably, microbial induced cytokines such as IL-6 are also integral cofactors in this process (52, 53). Evidence indicates that Treg cells can also provide help in the generation of IgA producing B cells (102). Analysis of human population revealed that in the context of Crohn's Disease, flagellins are immunodominant antigens of the microbiota (88). An explanation of how the mucosal immune system prevents harmful responses against flagellin was provided by a recent study demonstrating that Intestinal IgA can regulate the activation of peripheral flagellin-specific CD4⁺T cells (103). Importantly, Treg cells control such antigen IgA responses in an antigen specific manner via production of TGF- β . This study uncovered a new role for Treg cells as a major helper T cell for the induction and maintenance of intestinal IgA responses and prevention of responses against major flora antigens under steady state condition (103). Additionally, transfer of Treg cells into T cell deficient mice induces formation of germinal centers in PP and dramatically increases IgA production (102, 104). In the germinal centers, Treg cells down-regulate expression of FoxP3 and instead express markers of T follicular helper cells (105). Follicular dendritic cells in the PP are critical for the induction of IgA responses as they express TGF- β , which is crucial for IgA class switching (105). The follicular dendritic cells appear to respond directly to TLR and RA signaling by inducing the recruitment and differentiation of lymphocytes into germinal centers (105). In addition to the role of the flora in IgA responses and specificities, an area of research that has been clearly understudied is the capacity of commensals to control B cell responses at large in the context of infection and vaccination. Based on the prominent role of the flora in controlling various arms of the immune network and the known roles of various TLR receptors in controlling B cell function (106), this area of research is likely to yield results of high clinical relevance.

Role of commensals in pathogenic responses

A role for the gut microbiota is now well established in the pathophysiology of mucosal inflammatory diseases. Crohn's disease and ulcerative colitis are two major disease entities of inflammatory bowel disease (IBD)(107). Despite differences in the target site and inflammatory processes associated with these two forms of IBD, compelling evidence generated from studies of human patients and experimental models indicate that both disorders depend upon the presence of the microbiota (reviewed in (108)). Early studies demonstrated that the critical effector cell in most models of experimental colitis that involve the adaptive immune system is the CD4 T cell (59, 108, 109). One striking experimental example of the influence of the flora on mucosal disorder is that of TRUC

mice that develop a spontaneous and transferrable form of ulcerative colitis (110). Deficiency of T-bet in the innate immune system leads to exaggerated tumor necrosis factor (TNF) production by dendritic cells, which together with the absence of Treg cells creates a chronic inflammatory state that modulates the composition of the microflora and eventually leads to the development of colorectal cancer (110, 111). Colitis in the TRUC mice is caused by increased production of TNF- α by colonic DCs as restoration of T-bet expression specifically in CD11c+ populations allowed for reduced TNF- α production and prevented neoplasia and excessive inflammation (111). Interestingly, transfer of the microbiota from TRUC mice into wild-type recipients also transfers the colitis indicating that microbiota shifts are likely the cause of disease. Two species, *Proteus mirabilis* and *Klebsiella pneumoniae* are found at increased frequency in TRUC mice and can induce colitis in SPF wild-type mice (112).

A recent study revealed that the inflammasomes - complexes that function as sensors of endogenous or exogenous damage-associated molecular patterns – can also play a role in controlling the delicate homeostatic relationship with the flora (113). Deficiency of NLRP6 inflammasome from colonic cells results in reduced IL-18 levels and shift in microbiota composition characterized by expansion of the bacterial phyla Bacteroidetes (Prevotellaceae) and TM7. Further, these mice spontaneously develop colitis and were more susceptible to chemically induced colitis (113). As for the TRUC mice, colitogenic potential of this microbiota is transferable to neonatal or adult wild-type mice. Such “infectious” microbiota shaped by innate deficiency are likely to represent a dominant mechanism for the initiation of mucosal inflammatory disorders.

The gut also represents one of the primary sites of exposure to pathogenic microbes. In this environment, the pro-inflammatory properties of commensals can directly contribute to the pathogenesis of mucosal infection. One of the first examples of this scenario was demonstrated in an oral model of *Toxoplasma gondii* infection in which pathology is associated with exuberant sensing of commensals via TLR receptors (114, 115). This infection is also characterized by a reduction of the flora complexity and increase in gram-negative bacteria that in turn exacerbates the pathological process (114). Disruption of the microbiota composition has also been documented in the context of other enteric infections such as *Citrobacter rodentium* or *Salmonella typhimurium* (116, 117). In addition to microbial changes induced by infection, as previously discussed, accumulating evidence indicates that host genotypes reciprocally affect microbiota composition, which in turn alters host responses. Thus acute mucosal infections may represent an important initiatory event in the triggering of subsequent GI disorder.

Another important sets of question will be aimed at understanding how deviation of regulatory pathways under inflammatory settings could modify the immunological tone of mucosal tissues, and in some cases, lead to inflammatory disorders. Indeed, under highly inflammatory settings, Treg cells including commensal specific Treg cells can express T-bet and acquire effector function (118-120). Further, it is becoming clear that inflammatory mediators modulate the capacity of DCs to induce Treg cells in favor of the induction of effector responses (121-126). For instance, when activated with commensal derived DNA, LpDCs that under steady state are poised to induce Treg cells, produce large amount of IL-6 and consequently are impaired in their capacity to induce Treg cells (23). Based on recent findings revealing an enrichment of Treg cells specific for the flora at mucosal site, we could speculate that a deviation of commensal specific Treg cells toward an effector phenotype could lead to severe inflammatory disorders. Indeed, recent experimental evidences using commensal specific transgenic mice support this notion (75, 127). Thus, at barrier sites, responses against pathogens occur in the context of commensal shifts and reactivity to the flora that in turn amplifies the inflammatory process. This could lead to

aberrant responses against innocuous antigens. The long-term consequences of these responses remain to be fully understood.

Bystander effect of gut commensals on peripheral responses

One consequence of the immune system's reliance on microflora for optimal development and immunoregulation, is that antibiotic therapies may result in unintended activation of immune effector mechanisms. In experimental models, antibiotic treatment renders mice more susceptible to induction of food allergy (128) as well as allergic airway inflammation (129). For the human population, antibiotics are seen as major modifiers of beneficial human-microbe interactions (130) superimposed on alterations caused by other exogenous factors including urbanization, global travel, and dietary changes (131). The acute effects of antibiotic treatment on the native gut microbiota range from self-limiting diarrhea to life-threatening pseudomembranous colitis induced by bacteria filling the niches provided by the reduction in bacterial diversity (132). The long-term consequences of such perturbations for the human-microbial symbiosis are more difficult to discern, but chronic conditions such as asthma and atopic disease have been associated with childhood antibiotic use and an altered intestinal microbiota (133-135).

More direct evidence of the systemic influence of the microbiota on peripheral immune responses were recently provided. Indeed, recent experimental evidence suggests that, despite being contained by mucosal immunity, gut microbiota can impact responses at distal sites. Interestingly, a recent study demonstrated that peptidoglycan from radio-labeled *E.coli* could be found in the serum and improved killing of *Streptococcus pneumoniae* and *Staphylococcus aureus* by bone-marrow derived neutrophils in a Nod1 dependent manner (136). Importantly, detection of MAMPs is not limited to the intestinal mucosa but also has been shown to occur in the bone marrow where signaling can alter hematopoiesis. Further, bacterial products can induce monocyte emigration in response to very low levels of toll-like receptor ligands (137). Whether the diffusion of commensal products into the blood stream contributes to steady-state monocyte egress from the bone marrow remains to be determined. The microbiota also has the ability to regulate immune responses against various infections as mice given broad spectrum antibiotics mounted a severely blunted T and B cell response against an intranasal infection with the A/PR8 strain of influenza, resulting in elevated viral titers (138). Interestingly, no differences in immune responses were observed when mice were infected with HSV-2 or *Legionella pneumophila* (138). This differential requirement was attributed to the influenza specific response requiring inflammasome-mediated induction of IL-1 β and IL-18 secretion. Interestingly, administration of TLR agonists rectally was capable of restoring the immune response in antibiotic treated mice indicating that either the microbial products are capable of diffusing to distinctly distal sites, or that inflammasome activation does not need to occur in the same location as the local infection (138).

In addition to protective immunity, gut commensals can also alter autoimmune conditions. Mice lacking intestinal microbiota develop less-severe disease in models of arthritis and experimental autoimmune encephalomyelitis (EAE) (62, 139). On the other hand, colonization with SFB promotes autoimmune arthritis through the induction of antigen specific Th17 cells, which promote auto-antibody production via B cell expansion in germinal centers. A recent study further showed that recruitment and activation of autoantibody-producing B cells from the endogenous immune repertoire depends on availability of the target autoantigen and commensal microbiota (140). The commensal microbiota can also help to reduce inflammation as colonization of mice with *B.fragilis* results in the expansion of IL-10 producing Treg cells which limit the pro-inflammatory mechanisms of EAE in a TLR2 deficient manner (62). As well, diabetes in the Non-Obese

Diabetes (NOD) mouse model has been related to their housing conditions and presumably their commensal flora. When NOD mice were made deficient for MyD88, induction of disease was delayed and connected with a distinct flora from MyD88 intact controls (141). Further, a recent study indicates that NOD mice naturally colonized with SFB have a correlative protection from diabetes (142). Finally, single chain fatty acids (SCFA), such as acetate, are one of the most important metabolites provided by commensal organisms (143). It has been shown that the recognition of these SCFA by innate immune cells is critical for the regulation of inflammation in response to not only intestinal injury but also in models of arthritis and allergy (144). Therefore, the innate immune response is shaped not only by interaction with microbial products but also commensally-derived metabolites. Thus, while it is readily accepted that shifts in microbiota composition and density can affect local immune responses, it is becoming readily apparent that changes in bacterial species in the gut can also alter immunity and inflammation in distal organs from the intestine. Nevertheless, despite our growing understanding of the ramification of the host-microbe alliance, the degree to which the gut flora acts as the dominant source of commensal signals and directly contributes to immunity at distal sites remains unclear. The body is comprised of various tissue microenvironments with finely tuned local immunosurveillance systems, many of which are in close apposition with distinct commensal niches (145). Outside of the GI tract, how resident commensals control these unique physiological niches remains unknown.

At this point, it remains unclear to what extent any recalibration either locally or systemically is purely induced by perturbation of the commensal population and / or infection, or is a result of endogenous controls within the immune system itself. On the basis of our current knowledge, it seems likely that all three components play an essential role in reaching a stable and nonpathogenic steady state for the longer term. The challenge over the next few years will be to develop novel experimental approaches allowing the exploration of these complexes and constantly evolving interactions.

References

1. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*. 2006; 124:837–848. [PubMed: 16497592]
2. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. *Science*. 2005; 308:1635–1638. [PubMed: 15831718]
3. Salzman NH, Underwood MA, Bevins CL. Paneth cells, defensins, and the commensal microbiota: a hypothesis on intimate interplay at the intestinal mucosa. *Semin Immunol*. 2007; 19:70–83. [PubMed: 17485224]
4. Talham GL, Jiang HQ, Bos NA, Cebra JJ. Segmented filamentous bacteria are potent stimuli of a physiologically normal state of the murine gut mucosal immune system. *Infect Immun*. 1999; 67:1992–2000. [PubMed: 10085047]
5. 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]
6. Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI. Molecular analysis of commensal host-microbial relationships in the intestine. *Science*. 2001; 291:881–884. [PubMed: 11157169]
7. Stappenbeck TS, Hooper LV, Gordon JI. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. *Proc Natl Acad Sci U S A*. 2002; 99:15451–15455. [PubMed: 12432102]
8. Lavelle EC, Murphy C, O'Neill LA, Creagh EM. The role of TLRs, NLRs, and RLRs in mucosal innate immunity and homeostasis. *Mucosal Immunol*. 2010; 3:17–28. [PubMed: 19890268]

9. Hamada H, Hiroi T, Nishiyama Y, Takahashi H, Masunaga Y, Hachimura S, Kaminogawa S, Takahashi-Iwanaga H, Iwanaga T, Kiyono H, et al. Identification of multiple isolated lymphoid follicles on the antimesenteric wall of the mouse small intestine. *J Immunol*. 2002; 168:57–64. [PubMed: 11751946]
10. Fritz JH, Ferrero RL, Philpott DJ, Girardin SE. Nod-like proteins in immunity, inflammation and disease. *Nature immunology*. 2006; 7:1250–1257. [PubMed: 17110941]
11. Eberl G, Lochner M. The development of intestinal lymphoid tissues at the interface of self and microbiota. *Mucosal immunology*. 2009; 2:478–485. [PubMed: 19741595]
12. Bouskra D, Brezillon C, Berard M, Werts C, Varona R, Boneca IG, Eberl G. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature*. 2008; 456:507–510. [PubMed: 18987631]
13. Yang D, Chertov O, Bykovskaia SN, Chen Q, Buffo MJ, Shogan J, Anderson M, Schroder JM, Wang JM, Howard OM, et al. Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. *Science*. 1999; 286:525–528. [PubMed: 10521347]
14. Johansson ME, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:15064–15069. [PubMed: 18806221]
15. Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA. Probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression. *The American journal of physiology*. 1999; 276:G941–950. [PubMed: 10198338]
16. Van der Sluis M, De Koning BA, De Bruijn AC, Velcich A, Meijerink JP, Van Goudoever JB, Buller HA, Dekker J, Van Seuningen I, Renes IB, et al. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. *Gastroenterology*. 2006; 131:117–129. [PubMed: 16831596]
17. Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat Rev Immunol*. 2010; 10:159–169. [PubMed: 20182457]
18. Cash HL, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science*. 2006; 313:1126–1130. [PubMed: 16931762]
19. Mukherjee S, Partch CL, Lehotzky RE, Whitham CV, Chu H, Bevins CL, Gardner KH, Hooper LV. Regulation of C-type lectin antimicrobial activity by a flexible N-terminal prosegment. *J Biol Chem*. 2009; 284:4881–4888. [PubMed: 19095652]
20. Ismail AS, Severson KM, Vaishnava S, Behrendt CL, Yu X, Benjamin JL, Ruhn KA, Hou B, DeFranco AL, Yarovsky F, et al. Gammadelta intraepithelial lymphocytes are essential mediators of host-microbial homeostasis at the intestinal mucosal surface. *Proc Natl Acad Sci U S A*. 2011; 108:8743–8748. [PubMed: 21555560]
21. Kobayashi KS, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nunez G, Flavell RA. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science*. 2005; 307:731–734. [PubMed: 15692051]
22. Ivanov II, Rde L, Frutos, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, Finlay BB, Littman DR. 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. [PubMed: 18854238]
23. Hall JA, Bouladoux N, Sun C, Wohlfert EA, Blank RB, Zhu Q, Grigg ME, Berzofsky JA, Belkaid Y. Commensal DNA limits regulatory T cell conversion and is a natural adjuvant of intestinal immune responses. *Immunity*. 2008
24. Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. *Nat Rev Immunol*. 2004; 4:478–485. [PubMed: 15173836]
25. Gaboriau-Routhiau V, Rakotobe S, Lecuyer E, Mulder I, Lan A, Bridonneau C, Rochet V, Pisi A, De Paepe M, Brandi G, et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity*. 2009; 31:677–689. [PubMed: 19833089]
26. Weiner HL, da Cunha AP, Quintana F, Wu H. Oral tolerance. *Immunol Rev*. 2011; 241:241–259. [PubMed: 21488901]

27. Wannemuehler MJ, Kiyono H, Babb JL, Michalek SM, McGhee JR. Lipopolysaccharide (LPS) regulation of the immune response: LPS converts germfree mice to sensitivity to oral tolerance induction. *J Immunol.* 1982; 129:959–965. [PubMed: 6980928]
28. Kiyono H, McGhee JR, Wannemuehler MJ, Michalek SM. Lack of oral tolerance in C3H/HeJ mice. *J Exp Med.* 1982; 155:605–610. [PubMed: 7035600]
29. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol.* 1997; 159:1739–1745. [PubMed: 9257835]
30. Michalek SM, Kiyono H, Wannemuehler MJ, Mosteller LM, McGhee JR. Lipopolysaccharide (LPS) regulation of the immune response: LPS influence on oral tolerance induction. *J Immunol.* 1982; 128:1992–1998. [PubMed: 6460815]
31. Bashir ME, Louie S, Shi HN, Nagler-Anderson C. Toll-like receptor 4 signaling by intestinal microbes influences susceptibility to food allergy. *J Immunol.* 2004; 172:6978–6987. [PubMed: 15153518]
32. Gonzalez-Navajas JM, Fine S, Law J, Datta SK, Nguyen KP, Yu M, Corr M, Katakura K, Eckman L, Lee J, et al. TLR4 signaling in effector CD4+ T cells regulates TCR activation and experimental colitis in mice. *J Clin Invest.* 2010; 120:570–581. [PubMed: 20051628]
33. Kim JM, Rasmussen JP, Rudensky AY. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nat Immunol.* 2007; 8:191–197. [PubMed: 17136045]
34. Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor? *Immunity.* 2009; 30:626–635. [PubMed: 19464985]
35. Sun CM, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, Belkaid Y. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J Exp Med.* 2007
36. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, Powrie F. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF- β - and retinoic acid-dependent mechanism. *J Exp Med.* 2007
37. Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, Cheroutre H. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science.* 2007; 317:256–260. [PubMed: 17569825]
38. Mucida D, Kutchukhidze N, Erazo A, Russo M, Lafaille JJ, Curotto de Lafaille MA. Oral tolerance in the absence of naturally occurring Tregs. *J Clin Invest.* 2005; 115:1923–1933. [PubMed: 15937545]
39. Tone Y, Furuuchi K, Kojima Y, Tykocinski ML, Greene MI, Tone M. Smad3 and NFAT cooperate to induce Foxp3 expression through its enhancer. *Nat Immunol.* 2008; 9:194–202. [PubMed: 18157133]
40. Zheng Y, Josefowicz S, Chaudhry A, Peng XP, Forbush K, Rudensky AY. Role of conserved non-coding DNA elements in the Foxp3 gene in regulatory T-cell fate. *Nature.* 2010; 463:808–812. [PubMed: 20072126]
41. Benson MJ, Pino-Lagos K, Roseblatt M, Noelle RJ. All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. *J Exp Med.* 2007; 204:1765–1774. [PubMed: 17620363]
42. Hall JA, Cannons JL, Grainger JR, Dos Santos LM, Hand TW, Naik S, Wohlfert EA, Chou DB, Oldenhove G, Robinson M, et al. Essential role for retinoic acid in the promotion of CD4(+) T cell effector responses via retinoic acid receptor alpha. *Immunity.* 2011; 34:435–447. [PubMed: 21419664]
43. Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. *Nat Immunol.* 2007; 8:1086–1094. [PubMed: 17873879]
44. Hadis U, Wahl B, Schulz O, Hardtke-Wolenski M, Schippers A, Wagner N, Muller W, Sparwasser T, Forster R, Pabst O. Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. *Immunity.* 2011; 34:237–246. [PubMed: 21333554]

45. Murai M, Turovskaya O, Kim G, Madan R, Karp CL, Cheroutre H, Kronenberg M. Interleukin 10 acts on regulatory T cells to maintain expression of the transcription factor Foxp3 and suppressive function in mice with colitis. *Nat Immunol.* 2009; 10:1178–1184. [PubMed: 19783988]
46. Curotto de Lafaille MA, Kutchukhidze N, Shen S, Ding Y, Yee H, Lafaille JJ. Adaptive Foxp3+ regulatory T cell-dependent and -independent control of allergic inflammation. *Immunity.* 2008; 29:114–126. [PubMed: 18617425]
47. Cassani B, Villablanca EJ, Quintana FJ, Love PE, Lacy-Hulbert A, Blaner WS, Sparwasser T, Snapper SB, Weiner HL, Mora JR. Gut-tropic T Cells that Express Integrin $\alpha 4\beta 7$ and CCR9 are Required for Induction of Oral Immune Tolerance in Mice. *Gastroenterology.* 2011
48. Pino-Lagos K, Guo Y, Brown C, Alexander MP, Elgueta R, Bennett KA, De Vries V, Nowak E, Blomhoff R, Sockanathan S, et al. A retinoic acid-dependent checkpoint in the development of CD4+ T cell-mediated immunity. *J Exp Med.* 2011; 208:1767–1775. [PubMed: 21859847]
49. Hall JA, Grainger JR, Spencer SP, Belkaid Y. The role of retinoic acid in tolerance and immunity. *Immunity.* 2011; 35:13–22. [PubMed: 21777796]
50. Manicassamy S, Ravindran R, Deng J, Oluoch H, Denning TL, Kasturi SP, Rosenthal KM, Evavold BD, Pulendran B. Toll-like receptor 2-dependent induction of vitamin A-metabolizing enzymes in dendritic cells promotes T regulatory responses and inhibits autoimmunity. *Nat Med.* 2009; 15:401–409. [PubMed: 19252500]
51. Cha HR, Chang SY, Chang JH, Kim JO, Yang JY, Kim CH, Kweon MN. Downregulation of Th17 cells in the small intestine by disruption of gut flora in the absence of retinoic acid. *J Immunol.* 2010; 184:6799–6806. [PubMed: 20488794]
52. Mora JR, Iwata M, Eksteen B, Song SY, Junt T, Senman B, Otipoby KL, Yokota A, Takeuchi H, Ricciardi-Castagnoli P, et al. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science.* 2006; 314:1157–1160. [PubMed: 17110582]
53. Uematsu S, Fujimoto K, Jang MH, Yang BG, Jung YJ, Nishiyama M, Sato S, Tsujimura T, Yamamoto M, Yokota Y, et al. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. *Nat Immunol.* 2008; 9:769–776. [PubMed: 18516037]
54. Chieppa M, Rescigno M, Huang AY, Germain RN. Dynamic imaging of dendritic cell extension into the small bowel lumen in response to epithelial cell TLR engagement. *J Exp Med.* 2006; 203:2841–2852. [PubMed: 17145958]
55. Niess JH, Adler G. Enteric flora expands gut lamina propria CX3CR1+ dendritic cells supporting inflammatory immune responses under normal and inflammatory conditions. *J Immunol.* 2010; 184:2026–2037. [PubMed: 20089703]
56. Lunz JG 3rd, Specht SM, Murase N, Isse K, Demetris AJ. Gut-derived commensal bacterial products inhibit liver dendritic cell maturation by stimulating hepatic interleukin-6/signal transducer and activator of transcription 3 activity. *Hepatology.* 2007; 46:1946–1959. [PubMed: 17935227]
57. Wilson NS, Young LJ, Kupresanin F, Naik SH, Vremec D, Heath WR, Akira S, Shortman K, Boyle J, Maraskovsky E, et al. Normal proportion and expression of maturation markers in migratory dendritic cells in the absence of germs or Toll-like receptor signaling. *Immunol Cell Biol.* 2008; 86:200–205. [PubMed: 18026177]
58. Walton KL, He J, Kelsall BL, Sartor RB, Fisher NC. Dendritic cells in germ-free and specific pathogen-free mice have similar phenotypes and in vitro antigen presenting function. *Immunol Lett.* 2006; 102:16–24. [PubMed: 16105690]
59. Izcue A, Coombes JL, Powrie F. Regulatory T cells suppress systemic and mucosal immune activation to control intestinal inflammation. *Immunol Rev.* 2006; 212:256–271. [PubMed: 16903919]
60. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature.* 2008; 453:620–625. [PubMed: 18509436]
61. O'Mahony C, Scully P, O'Mahony D, Murphy S, O'Brien F, Lyons A, Sherlock G, MacSharry J, Kiely B, Shanahan F, et al. Commensal-induced regulatory T cells mediate protection against pathogen-stimulated NF-kappaB activation. *PLoS Pathog.* 2008; 4:e1000112. [PubMed: 18670628]

62. Ochoa-Reparaz J, Mielcarz DW, Ditrio LE, Burroughs AR, Begum-Haque S, Dasgupta S, Kasper DL, Kasper LH. Central nervous system demyelinating disease protection by the human commensal *Bacteroides fragilis* depends on polysaccharide A expression. *J Immunol.* 2010; 185:4101–4108. [PubMed: 20817872]
63. Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, Mazmanian SK. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science.* 2011; 332:974–977. [PubMed: 21512004]
64. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y, et al. Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science.* 2011; 331:337–341. [PubMed: 21205640]
65. Di Giacinto C, Marinaro M, Sanchez M, Strober W, Boirivant M. Probiotics ameliorate recurrent Th1-mediated murine colitis by inducing IL-10 and IL-10-dependent TGF- β -bearing regulatory cells. *J Immunol.* 2005; 174:3237–3246. [PubMed: 15749854]
66. Zoumpopoulou G, Foligne B, Christodoulou K, Grangette C, Pot B, Tsakalidou E. *Lactobacillus fermentum* ACA-DC 179 displays probiotic potential in vitro and protects against trinitrobenzene sulfonic acid (TNBS)-induced colitis and *Salmonella* infection in murine models. *Int J Food Microbiol.* 2008; 121:18–26. [PubMed: 18077037]
67. Feleszko W, Jaworska J, Rha RD, Steinhausen S, Avagyan A, Jaudszus A, Ahrens B, Groneberg DA, Wahn U, Hamelmann E. Probiotic-induced suppression of allergic sensitization and airway inflammation is associated with an increase of T regulatory-dependent mechanisms in a murine model of asthma. *Clin Exp Allergy.* 2007; 37:498–505. [PubMed: 17430345]
68. Karimi K, Inman MD, Bienenstock J, Forsythe P. *Lactobacillus reuteri*-induced regulatory T cells protect against an allergic airway response in mice. *Am J Respir Crit Care Med.* 2009; 179:186–193. [PubMed: 19029003]
69. Smits HH, Engering A, van der Kleij D, de Jong EC, Schipper K, van Capel TM, Zaat BA, Yazdanbakhsh M, Wierenga EA, van Kooyk Y, et al. Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. *J Allergy Clin Immunol.* 2005; 115:1260–1267. [PubMed: 15940144]
70. Foligne B, Zoumpopoulou G, Dewulf J, Ben Younes A, Chareyre F, Sirard JC, Pot B, Grangette C. A key role of dendritic cells in probiotic functionality. *PLoS One.* 2007; 2:e313. [PubMed: 17375199]
71. Di Giacinto C, Marinaro M, Sanchez M, Strober W, Boirivant M. Probiotics ameliorate recurrent Th1-mediated murine colitis by inducing IL-10 and IL-10-dependent TGF- β -bearing regulatory cells. *J Immunol.* 2005; 174:3237–3246. [PubMed: 15749854]
72. Karimi K, Inman MD, Bienenstock J, Forsythe P. *Lactobacillus reuteri*-induced regulatory T cells protect against an allergic airway response in mice. *Am J Respir Crit Care Med.* 2009; 179:186–193. [PubMed: 19029003]
73. Ostman S, Rask C, Wold AE, Hultkrantz S, Telemo E. Impaired regulatory T cell function in germ-free mice. *Eur J Immunol.* 2006; 36:2336–2346. [PubMed: 16897813]
74. Torii A, Torii S, Fujiwara S, Tanaka H, Inagaki N, Nagai H. *Lactobacillus Acidophilus* strain L-92 regulates the production of Th1 cytokine as well as Th2 cytokines. *Allergol Int.* 2007; 56:293–301. [PubMed: 17646735]
75. Lathrop SK, Bloom SM, Rao SM, Nutsch K, Lio CW, Santacruz N, Peterson DA, Stappenbeck TS, Hsieh CS. Peripheral education of the immune system by colonic commensal microbiota. *Nature.* 2011; 478:250–254. [PubMed: 21937990]
76. Belkaid Y, Tarbell K. Regulatory T cells in the control of host-microorganism interactions (*). *Annu Rev Immunol.* 2009; 27:551–589. [PubMed: 19302048]
77. Hayes KS, Bancroft AJ, Goldrick M, Portsmouth C, Roberts IS, Grencis RK. Exploitation of the intestinal microflora by the parasitic nematode *Trichuris muris*. *Science.* 2011; 328:1391–1394. [PubMed: 20538949]
78. Kuss SK, Best GT, Etheredge CA, Pruijssers AJ, Frierson JM, Hooper LV, Dermody TS, Pfeiffer JK. Intestinal Microbiota promote enteric virus replication and systemic pathogenesis. *Science.* 2011

79. Kane M, Case LK, Kopaskie K, Kozlova A, MacDermid C, Chervonsky AV, Golovkina TV. Successful transmission of a retrovirus depends on the commensal microbiota. *Science*. 2011
80. Lochner M, Peduto L, Cherrier M, Sawa S, Langa F, Varona R, Riethmacher D, Si-Tahar M, Di Santo JP, Eberl G. In vivo equilibrium of proinflammatory IL-17+ and regulatory IL-10+ Foxp3+ RORgamma t+ T cells. *J Exp Med*. 2008; 205:1381–1393. [PubMed: 18504307]
81. Lochner M, Berard M, Sawa S, Hauer S, Gaboriau-Routhiau V, Fernandez TD, Snel J, Bousso P, Cerf-Bensussan N, Eberl G. Restricted microbiota and absence of cognate TCR antigen leads to an unbalanced generation of Th17 cells. *J Immunol*. 2011; 186:1531–1537. [PubMed: 21178008]
82. Macpherson A, Khoo UY, Forgacs I, Philpott-Howard J, Bjarnason I. Mucosal antibodies in inflammatory bowel disease are directed against intestinal bacteria. *Gut*. 1996; 38:365–375. [PubMed: 8675088]
83. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*. 2009; 139:485–498. [PubMed: 19836068]
84. Stepankova R, Powrie F, Kofronova O, Kozakova H, Hudcovic T, Hrnecir T, Uhlig H, Read S, Rehakova Z, Benada O, et al. Segmented filamentous bacteria in a defined bacterial cocktail induce intestinal inflammation in SCID mice reconstituted with CD45RBhigh CD4+ T cells. *Inflamm Bowel Dis*. 2007; 13:1202–1211. [PubMed: 17607724]
85. Sansonetti PJ, Di Santo JP. Debugging how bacteria manipulate the immune response. *Immunity*. 2007; 26:149–161. [PubMed: 17307704]
86. Takeda K, Kaisho T, Akira S. Toll-like receptors. *Annu Rev Immunol*. 2003; 21:335–376. [PubMed: 12524386]
87. Uematsu S, Jang MH, Chevrier N, Guo Z, Kumagai Y, Yamamoto M, Kato H, Sougawa N, Matsui H, Kuwata H, et al. Detection of pathogenic intestinal bacteria by Toll-like receptor 5 on intestinal CD11c+ lamina propria cells. *Nat Immunol*. 2006; 7:868–874. [PubMed: 16829963]
88. Lodes MJ, Cong Y, Elson CO, Mohamath R, Landers CJ, Targan SR, Fort M, Hersherberg RM. Bacterial flagellin is a dominant antigen in Crohn disease. *J Clin Invest*. 2004; 113:1296–1306. [PubMed: 15124021]
89. Vijay-Kumar M, Sanders CJ, Taylor RT, Kumar A, Aitken JD, Sitaraman SV, Neish AS, Uematsu S, Akira S, Williams IR, et al. Deletion of TLR5 results in spontaneous colitis in mice. *J Clin Invest*. 2007; 117:3909–3921. [PubMed: 18008007]
90. Vijay-Kumar M, Aitken JD, Kumar A, Neish AS, Uematsu S, Akira S, Gewirtz AT. Toll-like receptor 5-deficient mice have dysregulated intestinal gene expression and nonspecific resistance to Salmonella-induced typhoid-like disease. *Infect Immun*. 2008; 76:1276–1281. [PubMed: 18195036]
91. Pasare C, Medzhitov R. Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells.[comment]. *Science*. 2003; 299:1033–1036. [PubMed: 12532024]
92. Larosa DF, Gelman AE, Rahman AH, Zhang J, Turka LA, Walsh PT. CpG DNA inhibits CD4+CD25+ Treg suppression through direct MyD88-dependent costimulation of effector CD4+ T cells. *Immunol Lett*. 2007; 108:183–188. [PubMed: 17270282]
93. Torok HP, Glas J, Tonenchi L, Bruennler G, Folwaczny M, Folwaczny C. Crohn's disease is associated with a toll-like receptor-9 polymorphism. *Gastroenterology*. 2004; 127:365–366. [PubMed: 15236225]
94. Hall JA, Bouladoux N, Sun CM, Wohlfert EA, Blank RB, Zhu Q, Grigg ME, Berzofsky JA, Belkaid Y. Commensal DNA Limits Regulatory T Cell Conversion and Is a Natural Adjuvant of Intestinal Immune Responses. *Immunity*. 2008
95. Dalpke A, Frank J, Peter M, Heeg K. Activation of toll-like receptor 9 by DNA from different bacterial species. *Infect Immun*. 2006; 74:940–946. [PubMed: 16428738]
96. Liu H, Komai-Koma M, Xu D, Liew FY. Toll-like receptor 2 signaling modulates the functions of CD4+ CD25+ regulatory T cells. *Proc Natl Acad Sci U S A*. 2006; 103:7048–7053. [PubMed: 16632602]
97. Atarashi K, Nishimura J, Shima T, Umesaki Y, Yamamoto M, Onoue M, Yagita H, Ishii N, Evans R, Honda K, et al. ATP drives lamina propria T(H)17 cell differentiation. *Nature*. 2008; 455:808–812. [PubMed: 18716618]

98. Torchinsky MB, Garaude J, Martin AP, Blander JM. Innate immune recognition of infected apoptotic cells directs T(H)17 cell differentiation. *Nature*. 2009; 458:78–82. [PubMed: 19262671]
99. Boullier S, Tanguy M, Kadaoui KA, Caubet C, Sansonetti P, Corthesy B, Phalipon A. Secretory IgA-mediated neutralization of *Shigella flexneri* prevents intestinal tissue destruction by down-regulating inflammatory circuits. *Journal of immunology*. 2009; 183:5879–5885.
100. Suzuki K, Meek B, Doi Y, Muramatsu M, Chiba T, Honjo T, Fagarasan S. Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:1981–1986. [PubMed: 14766966]
101. Hapfelmeier S, Lawson MA, Slack E, Kirundi JK, Stoel M, Heikenwalder M, Cahenzli J, Velykoredko Y, Balmer ML, Endt K, et al. Reversible microbial colonization of germ-free mice reveals the dynamics of IgA immune responses. *Science*. 2010; 328:1705–1709. [PubMed: 20576892]
102. Cong Y, Feng T, Fujihashi K, Schoeb TR, Elson CO. A dominant, coordinated T regulatory cell-IgA response to the intestinal microbiota. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:19256–19261. [PubMed: 19889972]
103. Cong Y, Feng T, Fujihashi K, Schoeb TR, Elson CO. A dominant, coordinated T regulatory cell-IgA response to the intestinal microbiota. *Proc Natl Acad Sci U S A*. 2009; 106:19256–19261. [PubMed: 19889972]
104. Tsuji M, Komatsu N, Kawamoto S, Suzuki K, Kanagawa O, Honjo T, Hori S, Fagarasan S. Preferential generation of follicular B helper T cells from Foxp3+ T cells in gut Peyer's patches. *Science*. 2009; 323:1488–1492. [PubMed: 19286559]
105. Suzuki K, Maruya M, Kawamoto S, Sitnik K, Kitamura H, Agace WW, Fagarasan S. The sensing of environmental stimuli by follicular dendritic cells promotes immunoglobulin A generation in the gut. *Immunity*. 2010; 33:71–83. [PubMed: 20643338]
106. Green NM, Marshak-Rothstein A. Toll-like receptor driven B cell activation in the induction of systemic autoimmunity. *Semin Immunol*. 2011; 23:106–112. [PubMed: 21306913]
107. Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. *J Clin Invest*. 2007; 117:514–521. [PubMed: 17332878]
108. Saleh M, Elson CO. Experimental inflammatory bowel disease: insights into the host-microbiota dialog. *Immunity*. 34:293–302. [PubMed: 21435584]
109. Powrie F, Correa-Oliveira R, Mauze S, Coffman RL. Regulatory interactions between CD45RB^{high} and CD45RB^{low} CD4+ T cells are important for the balance between protective and pathogenic cell-mediated immunity. *J Exp Med*. 1994; 179:589–600. [PubMed: 7905019]
110. Garrett WS, Lord GM, Punit S, Lugo-Villarino G, Mazmanian SK, Ito S, Glickman JN, Glimcher LH. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell*. 2007; 131:33–45. [PubMed: 17923086]
111. Garrett WS, Punit S, Gallini CA, Michaud M, Zhang D, Sigrist KS, Lord GM, Glickman JN, Glimcher LH. Colitis-associated colorectal cancer driven by T-bet deficiency in dendritic cells. *Cancer cell*. 2009; 16:208–219. [PubMed: 19732721]
112. Garrett WS, Gallini CA, Yatsunenko T, Michaud M, DuBois A, Delaney ML, Punit S, Karlsson M, Bry L, Glickman JN, et al. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. *Cell host & microbe*. 2010; 8:292–300. [PubMed: 20833380]
113. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, Peaper DR, Bertin J, Eisenbarth SC, Gordon JI, et al. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell*. 2011; 145:745–757. [PubMed: 21565393]
114. Heimesaat MM, Bereswill S, Fischer A, Fuchs D, Struck D, Niebergall J, Jahn HK, Dunay IR, Moter A, Gescher DM, et al. Gram-negative bacteria aggravate murine small intestinal Th1-type immunopathology following oral infection with *Toxoplasma gondii*. *J Immunol*. 2006; 177:8785–8795. [PubMed: 17142781]
115. Benson A, Pifer R, Behrendt CL, Hooper LV, Yarovinsky F. Gut commensal bacteria direct a protective immune response against *Toxoplasma gondii*. *Cell Host Microbe*. 2009; 6:187–196. [PubMed: 19683684]

116. Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, Finlay BB. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. *Cell Host Microbe*. 2007; 2:204. [PubMed: 18030708]
117. Stecher B, Robbiani R, Walker AW, Westendorf AM, Barthel M, Kremer M, Chaffron S, Macpherson AJ, Buer J, Parkhill J, et al. *Salmonella enterica* serovar typhimurium exploits inflammation to compete with the intestinal microbiota. *PLoS Biol*. 2007; 5:2177–2189. [PubMed: 17760501]
118. Koch MA, Tucker-Heard G, Perdue NR, Killebrew JR, Urdahl KB, Campbell DJ. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. *Nature immunology*. 2009; 10:595–602. [PubMed: 19412181]
119. Oldenhove G, Bouladoux N, Wohlfert EA, Hall JA, Chou D, Dos Santos L, O'Brien S, Blank R, Lamb E, Natarajan S, et al. Decrease of Foxp3+ Treg cell number and acquisition of effector cell phenotype during lethal infection. *Immunity*. 2009; 31:772–786. [PubMed: 19896394]
120. Feng T, Cao AT, Weaver CT, Elson CO, Cong Y. Interleukin-12 converts Foxp3+ regulatory T cells to interferon-gamma-producing Foxp3+ T cells that inhibit colitis. *Gastroenterology*. 2011; 140:2031–2043. [PubMed: 21419767]
121. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity*. 2006; 24:179–189. [PubMed: 16473830]
122. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature*. 2006; 441:235–238. [PubMed: 16648838]
123. Korn T, Bettelli E, Gao W, Awasthi A, Jager A, Strom TB, Oukka M, Kuchroo VK. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature*. 2007; 448:484–487. [PubMed: 17581588]
124. Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, Schluns K, Tian Q, Watowich SS, Jetten AM, et al. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature*. 2007; 448:480–483. [PubMed: 17581589]
125. Stumhofer JS, Laurence A, Wilson EH, Huang E, Tato CM, Johnson LM, Villarino AV, Huang Q, Yoshimura A, Sehy D, et al. Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system. *Nat Immunol*. 2006; 7:937–945. [PubMed: 16906166]
126. Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol*. 2007; 8:967–974. [PubMed: 17581537]
127. Feng T, Qin H, Wang L, Benveniste EN, Elson CO, Cong Y. Th17 cells induce colitis and promote Th1 cell responses through IL-17 induction of innate IL-12 and IL-23 production. *J Immunol*. 2011; 186:6313–6318. [PubMed: 21531892]
128. Bashir ME, Louie S, Shi HN, Nagler-Anderson C. Toll-like receptor 4 signaling by intestinal microbes influences susceptibility to food allergy. *Journal of Immunology*. 2004; 172:6978–6987.
129. Noverr MC, Falkowski NR, McDonald RA, McKenzie AN, Huffnagle GB. 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]
130. Sullivan A, Edlund C, Nord CE. Effect of antimicrobial agents on the ecological balance of human microflora. *Lancet Infect Dis*. 2001; 1:101–114. [PubMed: 11871461]
131. Dethlefsen L, McFall-Ngai M, Relman DA. An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature*. 2007; 449:811–818. [PubMed: 17943117]
132. Beaugerie L, Petit JC. Microbial-gut interactions in health and disease. Antibiotic-associated diarrhoea. *Best Pract Res Clin Gastroenterol*. 2004; 18:337–352.
133. Noverr MC, Huffnagle GB. The 'microflora hypothesis' of allergic diseases. *Clin Exp Allergy*. 2005; 35:1511–1520. [PubMed: 16393316]

134. Prioult G, Nagler-Anderson C. Mucosal immunity and allergic responses: lack of regulation and/or lack of microbial stimulation? *Immunol Rev.* 2005; 206:204–218. [PubMed: 16048551]
135. Marra F, Lynd L, Coombes M, Richardson K, Legal M, Fitzgerald JM, Marra CA. Does antibiotic exposure during infancy lead to development of asthma?: a systematic review and metaanalysis. *Chest.* 2006; 129:610–618. [PubMed: 16537858]
136. Clarke TB, Davis KM, Lysenko ES, Zhou AY, Yu Y, Weiser JN. Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nature medicine.* 2010; 16:228–231.
137. Shi C, Jia T, Mendez-Ferrer S, Hohl TM, Serbina NV, Lipuma L, Leiner I, Li MO, Frenette PS, Pamer EG. Bone marrow mesenchymal stem and progenitor cells induce monocyte emigration in response to circulating toll-like receptor ligands. *Immunity.* 2011; 34:590–601. [PubMed: 21458307]
138. Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, Iwasaki A. Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proceedings of the National Academy of Sciences of the United States of America.* 2011; 108:5354–5359. [PubMed: 21402903]
139. Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, Umesaki Y, Littman DR, Benoist C, Mathis D. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity.* 2010; 32:815–827. [PubMed: 20620945]
140. Berer K, Mues M, Koutrolos M, Al Rasbi Z, Boziki M, Johner C, Wekerle H, Krishnamoorthy G. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. *Nature.* 2011
141. Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Bluestone JA, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature.* 2008; 455:1109–1113. [PubMed: 18806780]
142. Kriegel MA, Sefik E, Hill JA, Wu HJ, Benoist C, Mathis D. Naturally transmitted segmented filamentous bacteria segregate with diabetes protection in nonobese diabetic mice. *Proceedings of the National Academy of Sciences of the United States of America.* 2011; 108:11548–11553. [PubMed: 21709219]
143. Wolowczuk I, Verwaerde C, Viltart O, Delanoye A, Delacre M, Pot B, Grangette C. Feeding our immune system: impact on metabolism. *Clin Dev Immunol.* 2008; 2008:639803. [PubMed: 18350123]
144. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, Schilter HC, Rolph MS, Mackay F, Artis D, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature.* 2009; 461:1282–1286. [PubMed: 19865172]
145. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. *Science.* 2009; 326:1694–1697. [PubMed: 19892944]

- Commensals play a central role in promoting immune responses locally and systemically
- Defined bacteria can play a dominant role in the control of immune responses
- Commensals can also favor the transmission of pathogens and contribute to pathogenesis