Published in final edited form as:

Semin Cancer Biol. 2013 December ; 23(0): . doi:10.1016/j.semcancer.2013.09.002.

Microbiota regulation of inflammatory bowel disease and colorectal cancer

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Abstract

The host and microbiota have evolved mechanisms for coexistence over millions of years. Accumulating evidence indicates that a dynamic mutualism between the host and the commensal microbiota has important implications for health, and microbial colonization contributes to the maintenance of intestinal immune homeostasis. However, alterations in communication between the mucosal immune system and gut microbial communities have been implicated as the core defect that leads to chronic intestinal inflammation and cancer development. We will discuss the recent progress on how gut microbiota regulates intestinal homeostasis and the pathogenesis of inflammatory bowel disease and colorectal cancer.

1. Introduction

The intestine harbors a massive and diverse microbiota which can reach a density of 10^{12} organisms/g and is comprised of more than 1000 species, including both anaerobes and aerobes, containing at least 100 times as many genes as within our own genome [1–3]. Gut microbiota provides huge benefits to their host, including the breakdown of indigestible food, the supply of energy for colonic epithelial cells, and a barrier against invasive pathogenic bacteria; they also have a major impact on many host systems, particularly on the development of the intestine and the immune system. Despite this enormous bacterial challenge, the intestine lives in harmony with the microbiota. Multiple host mechanisms have evolved to regulate this relationship, including both innate and adaptive immune systems [4–9]. Commensal bacteria shape both mucosal and systemic immune homeostasis in the host, and the host intestinal mucosal cells also modulate the commensal habitat and maintain a commensal composition that avoids excessive antigen signaling and activation of the immune system. Impaired regulation in the interaction between the microbiota and the host immune system may lead to intestinal inflammation and cancer. The importance of microbiota in the pathogenesis of inflammatory bowel diseases (IBD) and intestinal

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neoplasia has been clearly demonstrated in animal models, where animals develop colitis in conventional environments, but not when the commensal microbiota is absent in germ-free (GF) conditions [3, 6]. The carcinogenic agent azoxymethane can induce colon tumors in colitic IL-10 deficient mice monocolonized with certain commensal bacteria, but fails to induce tumors in germ-free (GF) IL-10-deficient mice [10, 11]. In this article, we will review the recent progress in the studies of the mechanisms into the molecular events that link intestinal microbiota and chronic inflammation and neoplasia.

2. Host-microbiota interaction

A complex dynamic relationship between the host and the gut microbiota occurs shortly after birth. Human microbiome projects have provided great insights into the composition and function of microbiota. The composition of intestinal microbiota differs among individuals, and several recent studies suggest that the microbial cohort remains relatively constant once adulthood is reached, however, the composition of the resident microbiota may alter as a result of environmental factors such as diet and antibiotic usage [12]. There is high interpersonal variation in species composition of the intestinal microbiota in humans and no single species is ubiquitously present in all humans. However, when the metagenome of the human intestinal microbiota has been analyzed, a core composition of genes is evident and comparable among humans [13–15]. A recent study reported that microbiota stability followed a power-law function, which when extrapolated suggests that most strains in an individual are residents for decades, indicating that the microbiota is quite stable during an individual's adult life [16]. Within the normal human microbiota, strict anaerobes, including *Bacteriodes*, *Eubacterium*, *Bifidobacterium*, *Fusobacterium*, *Peptostreptococcus*, and *Atopobium*, are dominant with the *Bacteroidetes* and the *Firmicutes* being the most abundant phyla, accounting for more than 90% of all the phylotypes. *Proteobacteria*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia* are relatively few in samples from the human distal gut [1, 17–19]. Members of *Bacteroidetes* and *Actinobacteria* are significantly more stable components than the population average [16]. Host and microbiota have established a mutualistic relationship, in which both microbes and their animal host depend on each other for optimal survival [7, 20]. The interactions of host and microbiota occur at multiple levels. Although direct contact between microbes and host mucosal cells is limited, the microbiota regulates host immunity through several different pathways and a range of bacterial metabolites [21–23]. Importantly, the signaling is bidirectional, and the host immunity influences also the microbiota which is evident in the setting of deficiencies of innate immunity. It is clear now that alterations in the host immunity lead to changes in the composition of the microbiota, and resetting microbial populations via fecal transplantation can transfer inflammatory or metabolic phenotypes in animals [24–26].

2.1 Microbiota regulation of mucosal immune responses

Comparative studies in GF and conventional animals demonstrate that the intestinal microflora is crucial for the development and function of the mucosal immune system, especially during early life, a process important to overall immunity in adults [27]. GF mice have underdeveloped mucosal and systemic immune systems with decreased cellularity of the Peyer's patches (PP), lamina propria (LP), mesenteric lymph nodes (MLN), and spleen as well as diminished mucosal immune function compared with animals housed under conventional environments. Interestingly, these developmental deficits can be restored by colonization with commensal bacteria [28, 29]. A recent study further demonstrated that the intestinal microbiota contributes to angiogenesis within the core of intestinal villi. Upon comparing the capillary networks of GF mice with those of ex-GF mice colonized during or after completion of postnatal intestine development, villi capillaries in GF mice developed poorly during weaning and remain poorly developed until adulthood. Further, the vascular developmental program can be restarted and completed within 10 days after colonization

with a complete microbiota harvested from conventionally-raised mice, indicating that development of villi capillaries also depends on intestinal microbiota [30]. In steady conditions, the microbiota also modulate the development and function of various immune cells, including T cells, IgA-producing B cells, T cells, invariant natural killer T (iNKT) cells, NK cells, dendritic cells, macrophages, as well as recently identified innate lymphoid cells (ILCs) [20, 23, 27, 31]. In GF mice, IgA⁺ B cells in gut lymphoid tissues and lamina propria as well as IgA levels in the intestinal lumen are greatly decreased compared to mice housed under conventional conditions [27, 32]. Foxp3-expressing regulatory T (Treg) cells and IL-17-producing Th17 cells are enriched in the lamina propria of mice under conventional conditions, however, levels of Treg and Th17 cells are greatly decreased in GF mice. Transplantation of intestinal microbiota from conventionally raised mice restores Treg and Th17 cells levels, as well as the expression of the immune-suppressive cytokine IL-10 in Treg cells, demonstrating that the intestinal microbiota regulates the development and function of Treg and Th17 cells in the intestinal mucosa [33, 34].

Accumulating evidence indicates that different commensal bacteria differentially regulate the development and function of different immune cells in the mucosa. While *Bacteroides fragilis* and Clostridia species promote Treg cells, thus functioning as immunosuppressing commensals, segmented filamentous bacteria (SFB) stimulate Th17 cell development, which function either by protecting the intestine against infection from pathogenic microbes or promoting harmful inflammatory responses [33–35]. In addition, colonization of GF mice with SFB also stimulates IgA⁺ blasts in the germinal centers of PP and IgA production in intestinal lumen [27]. Thus, the microbiota shapes the immune landscape in the gut and provides both proinflammatory and anti-inflammatory signaling. These signals are balanced, and both types are required to support homeostasis.

2.1.1 Microbiota regulation of Treg cells—Multiple levels of regulation have been implicated in maintaining the intestinal immune homeostasis and controlling the immune responses to microbiota antigens. Among the multi-mechanisms, CD4⁺Foxp3⁺ Treg cells play a crucial role. Treg cells constitutively express high levels of the transcription factor Foxp3, which is considered to confer their development as well as their regulatory activity. Microbiota greatly influence Treg cells in the intestines as GF mice demonstrate a reduced level of intestinal Treg cells. However, the intestinal microbiota do not function uniformly to regulate T cell development and function. In fact, different commensal bacteria differentially influence proinflammatory as well as anti-inflammatory T cell development and function. The well-studied commensal bacteria that promote Treg cells in the intestines are *Bacteroides fragilis* and Cluster IV and XIVa Clostridia. *B. fragilis* is the first commensal to be implicated in affecting mucosal T cell homeostasis by promoting Treg cell function [35]. *B. fragilis* is a Gram-negative member of the phylum Bacteroidetes. The genus *Bacteroides* is well represented in the human gut and is able to utilize the nutrients in the gut microenvironment, although it is not a very abundant member of the gut microbiota [36]. *B. fragilis* is not normally present in conventionally-raised SPF mice, and colonization with *B. fragilis* protects mice from colitis induced by adoptive transfer of CD4⁺CD45RB^{hi} T cells into immunodeficient RAG^{−/−} mice as well as colitis induced by administering wild-type mice with 2,4,6-trinitrobenzene sulfonic acid (TNBS) intrarectally [37]. Colonization of SPF or GF mice with *B. fragilis* leads to an induction of IL-10 producing Foxp3⁺ Treg cells [35, 38]. Blockade of Foxp3⁺ Treg cells and/or IL-10 abrogates the inhibitory function in mice colonized with *B. fragilis*, leaving them more susceptible to inflammation, indicating that *B. fragilis* colonization modulates intestinal immune homeostasis by promoting Treg cell development and function. Expression of bacterial capsular polysaccharide A (PSA) is required for the induction of Treg cells and anti-inflammatory effects of *B. fragilis*. PSA-deficient *B. fragilis* mutants lose the ability to induce IL-10 producing Foxp3⁺ Treg cells and do not protect the intestines from inflammation [35, 37]. Treatment of mice with

purified PSA induces Treg cells through stimulation of TLR2, and reduces intestinal inflammation by promoting the colonization of the gut by *B. fragilis*. PSA is thus able to replicate the effects of *B. fragilis* colonization, including the induction of IL-10 producing Foxp3⁺ Treg cells and protection from intestinal inflammation by suppressing effector Th17 cell production in animal models [35, 37, 38]. Interestingly, lack of PSA expression results in Th17 cell expansion and loss of the mutualistic ability of *B. fragilis* to colonize host tissues [38], furthering the importance of PSA in *B. fragilis* regulation of intestinal immune responses.

Certain clusters of Clostridia have recently been shown to be able to induce Treg cell differentiation in the intestines. Clostridia are Gram-positive, rod-shaped, endospore-forming bacteria, and intestinal Clostridia are a heterogeneous group that forms the core of Firmicutes of the normal commensal microbiota. Most gut commensal Clostridia are nontoxigenic members of clusters XIVa and IV, which constitute 10%–40% of the total microbiota [39]. Interestingly, the levels of Clostridia clusters IV and XIVa in adult mouse colon microbiota are found to correlate with the numbers of colonic Treg cells [33]. Colonization with 46 strains of Clostridia clusters XIVa and IV, originally isolated from the sporulating microbiota fraction in conventional mice and able to normalize the enlarged ceca of GF mice [40], increases the Treg cells in the intestines [33]. Importantly, these Clostridia were found to replenish near steady-state numbers of colonic Treg cells as well as high levels of IL-10 production by Treg cells in GF mice. Consistently, colonization of GF mice with altered Schaedler flora (ASF), a defined bacterial cocktail containing eight enteric species that includes *Clostridium clostridioforme*, induces the accumulation of Treg cells in the colon [41].

The host relies on bacteria to breakdown undigestible dietary components, such as fibers [1]. Recent studies demonstrate that gut microbiota-derived bacterial fermentation products, such as short chain fatty acids (SCFA), also regulate the size and function of the colonic Treg cell pool and protect against colitis [42]. GF mice had reduced concentrations of the three most abundant luminal SCFA: acetic acid, propionic acid and butyric acid. Feeding SCFA in drinking water to GF mice increased colonic Treg cell frequency and number but did not increase the number or frequency of splenic, MLN, or thymic Treg cells. SCFA increased CD4⁺ T cell frequency and number but did not alter colonic Th1 or Th17 cell numbers. Treatment with SCFA significantly increased Foxp3 and IL-10 expression in Treg cells both in vivo and in vitro, suggesting that SCFA specifically induce Foxp3⁺ IL-10-producing Treg cells. SCFA-induction of Treg cells is mediated by G protein-coupled receptor (GPR) 43 which binds SCFA and through its expression on innate immune cells mediates resolution of inflammatory responses. Intestinal Treg cells, particularly colonic Treg cells, which were largely dependent upon microbiota-derived signals, had significantly higher levels of Ffar2, which encodes GPR43, than Treg cells isolated from spleen or MLN. When Ffar2^{-/-} mice and Ffar2^{+/+} littermates were treated with propionate, which has the highest affinity for Ffar2 [43], propionate enhanced colonic Treg cell frequency and number in Ffar2^{+/+} but not Ffar2^{-/-} mice. In addition, propionate enhanced Foxp3 and IL-10 expression in Ffar2^{+/+}, but not in Ffar2^{-/-} colonic Treg cells. SCFA-mediated, enhanced colonic Treg cell suppressive capacity was also dependent on Ffar2 [42]. Thus, certain commensal bacteria selectively induce intestinal Treg cells under certain conditions via products of themselves or their fermentation products. Of note, most studies reported so far indicate that such commensal bacteria-induced Treg cells produce high levels of anti-inflammatory cytokine IL-10 but not TGF β , another signature cytokine of Treg cells. As high levels of TGF β is present in the intestines, it raises an important question of whether certain commensal bacteria can selectively induce Treg cell production of TGF β , or if TGF β is induced through common pathway(s) shared by all commensal bacteria in the intestines.

2.1.2 Microbiota regulation of Th17 cells—Th17 cells, which produce the signature cytokines IL-17A, IL-17F, IL-21, IL-22, and GM-CSF, have been shown to protect the intestines from infection as well as to promote chronic intestine inflammation [44–48]. In contrast to secondary lymphoid tissues, Th17 cells are enriched in the intestinal lamina propria in steady conditions in the absence of infection or overt inflammation, indicating a role of microbiota in the induction of Th17 cell development [49]. Consistently, Th17 cells are not present in GF mice but are induced upon colonization with the full complement of gut bacteria from conventional mice [50, 51]. However, not all commensal bacteria are able to induce Th17 cells, as C57BL/6 mice from a colony at the Jackson Laboratory do not contain intestinal Th17 cells and total microbiota from these mice do not induce Th17 cells in GF animals. In contrast, colonization with microbiota from C57BL/6 mice from a colony at Taconic Farms does induce intestinal Th17 cells in GF mice, indicating that only certain commensal bacteria are able to induce intestinal Th17 cells. In search of Th17-inducing commensal bacteria, microbiota are compared between the colonies at Jackson Laboratory and at Taconic Farms. It reveals that SFB is highly enriched in Taconic but are absent from Jackson C57BL/6 mice [34]. Monocolonization of GF mice with SFB or introduction of SFB into Jackson C57BL/6 mice induces Th17 cell differentiation in intestinal lamina propria [34, 52], an indicative of SFB as Th17-cell-inducing commensal bacteria. Importantly, Th17 cells induced by SFB are functional in the intestines, in that SFB colonization leads to improved protection against infections with intestinal pathogens, as shown for *Citrobacter rodentium* in mice [34]. How SFB promote intestinal Th17 cell development is still unknown, although the close interaction of SFB with epithelial cells and Peyer's patches could be involved in such effects.

The delicate balance between Treg cells and Th17/Th1 effector cells contributes to the intestinal immune homeostasis, which is regulated by both commensal bacteria and host factors. Although *B. fragilis*, unbalanced Clostridia flora, and ASF species preferably induce intestinal Treg cells in steady conditions, they also can induce Th17/Th1 cells in the absence of Treg cells. While wild-type *B. fragilis* induces intestinal Treg cells, PSA-mutated *B. fragilis* induces Th17 cells in the intestines [38]. In IL-10 deficient mice, in which Treg cell function is impaired, cluster Clostridia and ASF species induce Th17/Th1 cells [33, 41], indicating that although certain commensal bacteria play an immunosuppressive role under steady conditions, they can also induce a proinflammatory response under certain context. The existence of multiple species of the commensal bacteria that differentially regulate host immune homeostasis suggests that differences in the composition of microbiota may contribute to individual differences in immune responses during infection, autoimmunity, cancer, or other immunological conditions.

2.1.3 Microbiota and Innate Immune System—The innate immune system is the first line of host mucosal response to intestinal microbiota, which is tightly controlled and critical for maintaining tissue integrity and homeostasis within the intestinal mucosa. However, many mucosal insults in the intestine may disrupt this finely tuned system. Cross talk between the intestinal innate immune system and microbiota favors a mutual growth, survival and inflammatory control of the intestinal ecosystem. Intestinal microbiota contribute to host defense by outcompeting and limiting the growth of potentially harmful enteric pathogens, and control intestinal inflammation by producing symbiotic factors [37]. Through crosstalk with the innate immune system, the microbiota also regulates intestinal homeostasis by providing instructive signals that drive intestinal epithelial cell (IEC) turnover and maturation, and the conditioning of the immune system both at mucosal sites and systemically [53, 54]. A typical feature of innate immunity is the ability to distinguish between potentially pathogenic microbial components and harmless antigens by cell surface or cytosolic pattern recognition receptor (PRR) families and their interconnected signaling platforms. Toll-like receptors (TLR) and NOD-like receptors (NLR) enable innate cells to

recognize conserved characteristic molecules present on microorganisms and described as pathogen-associated molecular patterns (PAMPs), including lipopolysaccharides, peptidoglycans, flagellin, formylated peptides among others [55, 56]. As those molecules are also present in commensal bacteria, they have also been called as microbe-associated molecular patterns (MAMPs). PRR expression is not limited to innate immune cells, but can also be found in cancer cells and nearby stromal cells. Both TLRs and NLRs are widely expressed by various cell types of the gut mucosa. Microbiota can regulate the intestinal innate immune system by activation of these bacterial receptors, which stimulates central signaling cascades that include nuclear factor- κ B (NF- κ B) pathway. This pathway is negatively regulated by commensal bacterial induction of inhibitory molecules, including A20, peroxisomal proliferator-activated receptor (PPAR γ), I κ B, IL-10, TGF β , and blockade of I κ B polyubiquitination and degradation [3, 57, 58]. Thus innate cell expression of TLRs and NLR is crucial for response to microbiota as well as for the establishment of intestinal homeostasis by the microbiota. In nonhematopoietic cells, aberrant PRR signaling may induce hyperproliferative and anti-apoptotic responses, whereas impaired PRR signaling in hematopoietic cells may drive and maintain the chronically inflamed environment in the lamina propria [24, 59, 60]. It has been shown that activation of TLRs by commensals inhibits immune activation IEC through various mechanisms [61], whereas deficiency of TLR expression or impaired TLR signaling negatively regulates IEC regeneration after injury and aggravates intestinal inflammation [60].

Colonization of GF animals with *Enterococcus faecalis* or *Bacteroides vulgatus* transiently activate NF- κ B signaling and induce chemokine expression in colonic epithelial cells through TLR2 and TLR4 signaling, respectively, which are inhibited by IL-10 and TGF β produced by lamina propria lymphocytes [62–64]. Recent studies demonstrated a critical role of commensal bacteria-induced NF- κ B dependent mucosal homeostatic responses by epithelial cells, in that dextran sodium sulfate (DSS)-induced colitis is enhanced in GF mice, TLR9-deficient mice, TLR4-deficient mice, and mice deficient in MyD88, an adaptor protein required for TLR and IL-1 signaling [65–68]. Further, conditional ablation of NEMO (I κ B kinase) in IEC causes spontaneous severe colitis [69]. Blockade of epithelial NF- κ B signaling leads to increased bacterial translocation across the injured epithelium, similar to TLR4-deficient mice treated with DSS, and activation of pathogenic CD4⁺ T cells [69, 70]. Interestingly, regulatory vs inductive TLR signaling is polarized; basolateral stimulation of TLR9 by synthetic bacterial DNA activated NF- κ B, whereas physiologic apical stimulation of epithelial cell lines inhibited NF- κ B signaling by blocking degradation of ubiquitinated I κ B [67]. Thus the activation of TLR and downstream pathway is likely to be involved in mediating immunomodulatory effects of commensals in general, and innate immune malfunction may trigger inflammation-associated mucosal barrier rearrangement and neoplastic growth.

2.2 Host regulation of microbiota

Through coevolution, the gut microbiota and host has established the mutualistic relationship, in which both microbes and their host depend on each other for optimal survival and function. Multiple factors contribute to the development and composition of the intestinal microbiota, including maternal transmission, early life exposures, diet, and genetics [71]. However, the relative roles of environmental vs. genetic influences are not yet well understood.

2.2.1 Genetic background on microbiota—Although it has suggested a primary role for early exposure rather than genetic direction of microbial composition, several studies also demonstrate a role for host genetic background in shaping the microbiota composition. Monoassociation of 23 inbred mouse strains with the 8 defined ASF populations indicated

important genetic effects, in that quantitative and reproducible strain-specific variations in the frequencies of the 8 ASF members were observed across 23 different barrier-housed inbred mouse strains [72, 73]. The microbiota of two different mouse strains were implanted into a foster mother, with a resounding lack of differences demonstrated the lasting effects of maternal microbiota transmission [72, 73]. The gut microbiotas of zebrafish and mice share six bacterial divisions, although the specific bacteria within these divisions differ. It has been reported that when reciprocal transplantations of these microbiotas into GF zebrafish and mouse recipients were performed, the microbiota communities were assembled in predictable ways. The transplanted microbiota resembled its community of origin from the host in terms of the lineages present in the recipient animals, although the relative abundance of the lineages changed to resemble the normal gut microbial community composition of the recipient host [74].

Intestinal cells secrete an array of peptides with anti-microbial properties, including defensins, lysozyme, and secretory IgA, which regulate the colonization of pathogens as well as microbiota in the gut. Defensins are synthesized in Paneth cells and released from the intestinal crypts and at the epithelial surface and become trapped in the mucus layer to provide a bactericidal barrier. When GF C57BL/6 and 129/SvEv mice were colonized with identical commensal bacterial sources, their resultant ileal and fecal bacteria populations demonstrated that bacterial communities clustered based on the background strain of the recipient mice, regardless of bacterial source, indicating that genetic background shapes the microbiota composition. This could be due to the difference in Paneth cells between the strains, as 129/SvEv mice have fewer Paneth cells than C57BL/6 mice, and C57BL/6 and 129/SvEv mice display distinct patterns of ileal antimicrobial peptides (AMP) and defensin expression, which have been shown to regulate the microbiota [75].

2.2.2 Innate response regulation of microbiota—Host genetic variations also affect the composition of microbiota. Mutations in specific innate immune sensors and related pathways modulate cell priming of the intestinal mucosa and handling of the intestinal microbiota, driving inflammation, and carcinogenesis. It has been shown that deficiencies in PRR signaling control homeostasis at the level of microbiota composition. Deficiencies of Nod1, Nod2, Nlrp3, and Nlrp6 have all been shown to affect microbiota homeostasis, which leads to increased disease susceptibility [24, 76–78]. Importantly, the increased susceptibility to colitis observed in Nlrp6 deficient mice can be passed on to other mice housed in the same cage, thus the deficiency in Nlrp6 predisposes mice to a communicable colitogenic microbiota, in which the presence of Prevotellaceae and candidate phylum TM7 are dominated [24]. Of note, a recent comprehensive study demonstrated that the distinct microbiota composition of TLR-deficient mice is due to housing isolation rather than defective innate immunity, and loss of TLR signaling does not affect microbiota composition, thus challenging the argument of TLR regulation of microbiota composition [79].

2.2.3 Intestinal IgA regulation of microbiota—Among the multiple regulation mechanisms of intestinal homeostasis, intestinal IgA plays a crucial role in regulation of host response to microbiota. Secretory IgA is a specialized antibody type which is released into the intestinal lumen through polymeric Ig receptor (pIgR) expressed on epithelial cells, where it has a prolonged lifespan compared to regular antibodies and forms an immunological barrier against luminal microorganisms. Although IgA also plays a role in host resistance to infection, SPF mice that have had no pathogen exposure have abundant IgA levels whereas GF animals do not, arguing that the major role of IgA is in maintaining the balance between the host and its microbiota. Intestinal IgA production in mice deficient for α and μ chains of the T cell receptor, which lack both α T cells and μ T cells, is reduced to about a quarter of the level compared to wild-type mice but not completely lost [32, 80],

indicating that both T cell-dependent and -independent pathways contribute to intestinal IgA production. Foxp3⁺ Treg cells and Th17 cells are important for T cell-dependent induction of intestinal IgA, in that microbiota antigen-specific Foxp3⁺ Treg cells promote IgA production via production of TGF β whereas Th17 cells stimulate IEC pIgR expression via production of IL-17, thus promoting intestinal IgA responses [80–82]. Lamina propria dendritic cells and IECs can also directly initiate B-cell IgA class switching and IgA production through secretion of cytokine B-cell activation factor belonging to the TNF family (BAFF) and a proliferation inducing ligand (APRIL) following ligation of TLR and activation of NF- κ B [83–87]. The production of IgA is clearly dependent on the microbiota and changes in its composition alter the IgA pattern. Colonization with SFB not only induces intestinal Th17 cells in GF mice, but also induces intestinal IgA production.

Intestinal IgA contributes to protection of the host from systemic translocation of bacteria or bacterial products. Intestinal IgA, but not serum IgA, blocks mucosal flagellin uptake and systemic T cell activation in mice [80]. Mice deficient for IgA or pIgR demonstrate an increased bacterial translocation. In addition, intestinal IgA regulates the balance of commensal bacteria and consequently the composition of the intestinal microflora. In mice deficient for activation-induced cytidine deaminase (AID), which lack IgA-producing plasma cells and intestinal IgA production, aerobic bacteria, particularly SFB, are selectively expanded. Administration of IgA reverses these effects, indicating that intestinal IgA regulates aerobic bacterial colonization [88]. IgA can also regulate bacterial gene expression. Colonization with commensal bacteria *B. thetaiotaomicron* induces strong immune responses in GF RAG^{-/-} mice, which lack T cells, B cells, and IgA. This response is inhibited in the presence of IgA, even though it does not affect the growth rate of *B. thetaiotaomicron*. In turn, IgA modulated bacterial gene expression, inhibiting the immune response [89].

3. Microbiota regulation of IBD

Crohn's disease (CD) and ulcerative colitis (UC) are the two major disease entities of IBD. CD is characterized by deep inflammation with granulomas, and may affect any part of the GI tract, although it most commonly affects the distal ileum and caecum. UC is largely limited to the colon, particularly the distal colon and the rectum, and causes more superficial ulceration. Great progress has been made in the last decade on the pathogenesis of IBD, which appears to be disorders of the host immune response to microbiota manifested by a state of local immune hyper-reactivity. This is supported by data from induced gene mutations in mice, and, more recently, by the identification of gene variants in humans that result in IBD or IBD susceptibility [6, 9, 71, 90–92]. A variety of mouse models that develop chronic intestinal inflammation resembling IBD have provided strong support for the hypothesis that IBD is due to a dysregulated mucosal CD4⁺ T cell response to enteric bacterial antigens initiated by an abnormal innate response in a genetically susceptible host [6].

3.1 Microbiota in animal models of colitis

Despite the mutualism between the microbiota and the host, alterations in the composition of the intestinal microbiota may change intestinal homeostasis and the nature of the immune response and lead to development of colitis. Over the past decade, many models for colitis have been developed, either induced by manipulating the genome of experimental animals or by environmental insults. Almost every immune gene has either been deleted or expressed as a transgene and only a small subset of such mutants are found to develop IBD. These models have shown that multiple pathways are involved, that CD4 T cells are the major effector cells mediating inflammation in most models, and that multiple hits are required in the innate, adaptive and regulatory compartments for disease expression [6, 93].

In almost every instance, such as IL-10 deficient mice and CD45RB^{hi} T cell transfer model, disease develops in a SPF or conventional environment but not in a GF environment, and in some instances colitis is ameliorated when the mice are treated with antibiotics [94–98], indicating that the microbiota play a crucial role in disease pathogenesis. There appears to be a complex three-way interaction in the intestine between the microbiota, the epithelium, and mucosal immune cells, the latter involving cells of both the innate and acquired immune system. Some microbial products likely target innate immune cells and serve an adjuvant function, by interacting with TLRs. Others microbial products are able to stimulate T cells and B cells [99].

Both Th1 and Th17 cells have been implicated as important mediators of inflammation in IBD. IL-12 and IL-23, two key cytokines driving Th1 and Th17 cell development, are increased in CD, and MLN DCs from CD patients induce both Th1 and Th17 immune responses [100–104]. Purified Th1 and Th17 cells reactive to microbiota antigens induce colitis in an adoptive transfer model [45]. Anti-IL-12/IL-23p40 antibody therapy, which targets both Th1 and Th17 cells, is effective in CD patients [105, 106]. T cells respond to specific antigens but little is known about which microbiota antigens are driving the pathogenic T-cell response in IBD. It has been reported that in C3H/HeJBir mice that develop spontaneous colitis, CD4⁺ T cells develop a strong T cell response to a small set of selected enteric bacterial antigens, and these T cells can transfer disease to immune deficient SCID mice once activated by enteric bacterial antigens in vitro [107]. Serologic expression cloning has identified a limited set of some 60 microbiota antigens, among which flagellins from Clostridia, particularly *Lachnospiraceae*, are the largest cluster. Transfer of T cells reactive to such commensal flagellins induces colitis in SCID mice and multiple models of colitis and half of CD patients have serum IgG to this cluster of flagellins. Thus, those commensal flagellins are identified as immunodominant antigens in multiple models of colitis as well as in CD patients [108]. Flagellin possesses the properties of both a ligand for TLR5 that stimulates innate responses and a potent antigen for adaptive responses [109]. Thus, flagellin can bridge innate and adaptive responses, and provides a window into the normal host immune response to its microbiota and abnormal responses resulting in colitis development.

It has been demonstrated that the composition of microbiota shifts during experimental colitis, although the consequences of these shifts in microbiota are unclear, particularly whether they are cause or effect. In T-bet^{-/-} Rag2^{-/-} ulcerative colitis (TRUC) mice, in which spontaneous colitis and subsequent colorectal cancer develop because of alterations in both innate and adaptive immune responses, an expansion of proteobacteria has been demonstrated as a feature of dysbiosis. This dysbiosis is marked by expansion of enterobacteriaceae, particularly of *Klebsiella pneumonia* and *Proteus mirabilis*, and this dysbiotic microbiota can transmit colitis to wild-type hosts [25, 110]. Studies in GF animals monocolonized with individual bacterial species show both bacterial- and host-specific responses. Not all strains of commensal bacteria are able to induce colitis, and the same commensal bacteria can induce colitis in certain genetic background but not in others. When monoassociated with HLA B27 transgenic rats, *Bacteroides vulgatus* induce colitis but *E. coli*, *Enterococcus faecalis*, and multiple other bacterial species do not [111, 112]. In contrast, *E. coli* and *E. faecalis*, but not *B. vulgatus*, induce colitis in monocolonized IL-10 deficient mice. However, all these 3 bacterial species do not induce colitis in monocolonized bone marrow-transplanted CD3 transgenic mice. In a single inbred host, two different bacterial strains caused different phenotypes of disease and show additive effects. When monoassociated with IL-10 deficient mice, *E. coli* induces relatively mild cecal-predominant colitis, whereas *E. faecalis* causes more severe, late-onset distal colonic inflammation. Dual association with both species causes more aggressive, early-onset pancolitis [3, 113, 114].

Thus, both bacterial- and host-specific responses are involved in the pathogenesis of colitis induced by commensal bacterial strains.

3.2 Microbiota in human IBD

Clinical, experimental, and therapeutic studies have demonstrated the role for the microbiota and microbial agents in the pathogenesis of IBD. Recent studies on the gut microbiota in patients with IBD reveal quantitative and qualitative changes in composition, that is characterized by low diversity of species in the gut microbial communities, but increased density of mucosal surface colonization and epithelial invasion in areas with active disease. Microbial communities in individuals with CD differed from those in healthy individuals, and profiles from individuals with CD that predominantly involved the ileum differed from those with CD that predominantly involved the colon. In IBD patients, there is a decrease in strict anaerobes, particular Firmicutes, and a bloom of proteobacteria [39]. Changes specific to patients with ileal CD included the disappearance of core genera, such as *Faecalibacterium* and *Roseburia*, and increased amounts of *Enterobacteriaceae* and *Ruminococcus gnavus* [115]. Some potentially anti-inflammatory species, such as *Faecalibacterium prausnitzii*, are also reduced [116]. By contrast, a greater abundance in *Enterobacteriaceae*, particularly *E. coli* species, has been observed in CD patients, and the change is more remarkable in mucosal tissue specimens than in fecal samples [117]. Functional alterations are most evident in adherent, invasive *Escherichia coli* (AIEC) that colonize the ileum of CD patients [118], [119]. Tissue invasion by such AIEC may be due to defects in autophagy, which has been shown to be an important defense mechanism against this microbe [120]. Although it is still unclear the role for compositional changes of the microbiota in IBD subsets, it may contribute to disease severity, since abnormal microbiota populations are correlated with the occurrence of abscesses in CD patients.

4. Microbiota regulation of colorectal cancer

Chronic inflammation is one of the hallmarks of cancer [121]. Individuals with IBD have a considerably increased risk for colorectal cancer (CRC) than the general population [122, 123], depending on the site of inflammation and the length of time with associated colitis. Chronic inflammatory responses can create a tumor-supporting microenvironment through activated immune cells that secrete multiple mediators, which may influence neoplastic development, invasion, metastasis, and angiogenesis. As we have discussed, a healthy balance between the intestinal immune system and the commensal microbiota is necessary for maintaining intestinal homeostasis in any host, and the dysregulation of intestinal immune responses, or in the commensal microbial population can result in rampant intestinal inflammation and injury. Here, we discuss the mechanisms by which severe inflammation, as seen in individuals with colitis, can alter the physiology of the intestinal environment and influence the development of colorectal cancer, as well as the role of the microbiota in shaping these malignancies.

Given the chronic pathogenesis of IBD, a number of mouse models have been presented using genetic deficiencies to establish colitis in mice. With some deficiencies, the induction of CRC via the procarcinogen azoxymethane (AOM) is greatly exacerbated in these mice with severe inflammation. Deficiency in the downstream TLR-signaling molecule SIGIRR results in uncontrolled TLR activation after exposure to microbiota, and disrupts intestinal homeostasis. Exaggerated TLR signaling in SIGIRR^{-/-} mice results in the persistent survival of IECs, and increases in proinflammatory cytokines. Notably, SIGIRR^{-/-} mice have an increased susceptibility to CRC with AOM treatment after colitis has been established [124]. While excessive TLR activation is pathogenic, the lack of TLR signaling also leaves the host subject to disrupted intestinal homeostasis. Mice that are deficient in MyD88 are unable to signal through TLRs, and have significant defects in the intestinal

mucosa, with increased numbers of proliferating cells in the intestinal crypts, and have low levels of a number of cytokines necessary for preserving intestinal homeostasis [125]. These defects result in the inability to heal and repair the intestinal barrier after intestinal injury, and leave the mice more susceptible to colitis and CRC.

IL-10 is a pleiotropic cytokine that is necessary for immune regulation and control of inflammatory responses. Deficiency in IL-10 results in spontaneous colitis in mice. The enhanced inflammation in IL-10^{-/-} mice results in increased carcinogenesis after AOM treatment than conventional mice. The use of the IL-10^{-/-} AOM model highlights the importance of the microbiota and intestinal inflammation in the development of CRC, as GF IL-10^{-/-} mice, devoid of microbiota, exhibit no intestinal inflammation and tumors [10, 11]. As discussed above, this observation has remained consistent across a number of other models of CRC that are characterized by excessive inflammation[126].

Approximately 15%–20% of cancers arise from patients with underlying chronic inflammation [122]. Consistent inflammation and its damaging effects on local tissue, as well as the constant efforts to repair tissue, greatly influence the possibility of tumorigenesis. This is particularly accordant in patients with IBD, as cumulative risk for developing CRC increases significantly with the number of years diagnosed with IBD. Colitis is characterized by chronic inflammation that is typically mediated by Th1 and Th17 cells. Recently, the effects of Th17 cytokines in driving CRC have been described [127]. Initial specimens of patients with CRC indicated that levels of IL-17, the signature proinflammatory cytokine of Th17 cells, and IL-23, which positively regulates Th17 cell growth and maintenance, are increased in CRC tumors. Analysis of Apc^{min} mice (common model of murine CRC) further indicated that IL-17 and IL-23 continued to be increased in colonic tumors. Notably, with the use of a Cre-driven inducible loss of APC, IL-23 was induced early on during tumorigenesis, and IL-23 levels were markedly high before APC loss, and did not increase after APC deletion. However, the size and number of tumors present decreased after ablation of IL-23 or IL-23 receptor. Further, Apc^{min} mice deficient in IL-17 signaling (IL-17ra^{-/-}) also exhibited a decrease in size and number of tumors, indicating that inflammation can greatly influence tumorigenesis, whereas events in advanced CRC do not increase inflammation.

Inflammatory factors often induce tumorigenesis by acting upon IECs, leading to malignant proliferation and resulting adenomas. Treatment of mice with dextran sulfate sodium (DSS) leads to severe intestinal barrier disruption as a result of its toxic effects on epithelial cells. Prolonged use of DSS, coupled with doses of AOM results in murine CRC. Increased tumoral IL-6 and subsequent epithelial STAT3 activation have been identified as the clear promoters of tumor progression, as STAT3 signaling promotes the survival, growth, and progression of premalignant cells [128, 129]. While IL-6 and STAT3 are important for the healing and regeneration of the mucosal barrier during acute injury, chronic epithelial turnover and regeneration appears to favor tumor progression. IL-6 can also indirectly promote tumorigenesis since IL-6 is a key promoter of Th17 cell induction. As such, it is conceivable that the induction of IL-6 after AOM treatment can also regulate a number of other proinflammatory processes that contribute to tumorigenesis through the upregulation of Th17 cell functions. A key product of Th17 cells is tumor necrosis factor (TNF), a hallmark inflammatory molecule. As its name suggests anti-oncogenic properties, TNF can control tumorigenesis by inducing apoptosis in epithelial cells. However, TNF also exacerbates inflammation by recruiting inflammatory monocytes and neutrophils, and increasing the production of reactive oxygen species (ROS) [130]. By contributing to an environment of chronic inflammation, TNF promotes conditions that are ripe for chromosomal damage and genetic instability as a result of ROS induction. Indicative of this observation is the use of monoclonal antibody therapy to neutralize TNF after DSS and

AOM exposure [130]. As a result of decreased colonic inflammation in the absence of TNF, tumor size and number decreased. The reduction in tumor formation was attributed to decreased numbers of infiltrating macrophages and neutrophils in the absence of TNF. Further observation that nuclear β -catenin was decreased in the absence of TNF lends support to the notion that the byproducts of inflammatory monocytes can set off the colon carcinogenesis [130]. Therefore, further investigation into IBD patients under anti-TNF treatment and their associated rates of CRC incidence may be warranted.

The microbiota has been implicated in the pathogenesis of IBD-associated inflammation-induced CRC. As mentioned previously, IL-10^{-/-} mice develop colitis and also colon tumors with AOM under SPF conditions, but not under GF conditions. When GF IL-10^{-/-} mice are transferred into SPF conditions, 100% of IL-10^{-/-} mice develop colitis, and 60% to 80% of mice develop colon tumors with the addition of AOM [10, 11], demonstrating a key role of microbiota in the development of colon tumors. Shifts in the composition of the microflora have been also linked to a propensity for intestinal tumorigenesis. Several bacterial strains, including *Streptococcus bovis/gallolyticus*, enterotoxigenic *Bacteroides fragilis*, and *Escherichia coli* NC101 have been shown as risk factors for CRC for both IBD patients and the general population [10, 131, 132]. *Bacteroides vulgatus* and *Bacteroides stercoris* have also been associated with an increased risk for CRC, whereas *Lactobacillus acidophilus*, *Lactobacillus* S06, and *Eubacterium aerofaciens* species are associated with low risk in humans [133]. Mice deficient in the NOD-like receptor family pyrin domain containing 6 (NLRP6) inflammasome demonstrate an enhanced inflammation-induced CRC formation. Wild-type mice cohoused with NLRP6^{-/-} mice develop a remarkably enhanced tendency for inflammation-induced CRC formation, which is mediated through IL-18-induced alterations in the microflora, indicating that inflammation-altered elements in the microbiota of inflammasome-deficient mice drive inflammation-induced CRC [134]. Consistently, a recent study identifies the intestinal microbiota as a target of inflammation that regulates the progression of CRC. In colitic IL-10^{-/-} mice, inflammation modifies gut microbial composition, which is associated with a significantly increase of luminal *Verrucomicrobia*, *Bacteroidetes*, and *Proteobacteria* as compared with that of wild-type mice. Within *Proteobacteria*, the *Gammaproteobacteria* class, *Enterobacteriales* order including *E. coli*, and *Enterobacteriaceae* family are all significantly more abundant in IL-10^{-/-} mice. Monocolonization with the commensal *Escherichia coli* NC101, but not *Enterococcus faecalis*, promotes invasive carcinoma in AOM-treated IL10^{-/-} mice, although both *E. coli* NC101 and *E. faecalis* induce similar levels of colitis. Key to the carcinogenic role of *E. coli* NC101 may be the polyketide synthase (pks) genotoxic island, which is present in *E. coli* NC101, but not in *E. faecalis*. Deletion of the (pks) genotoxic island from *E. coli* NC101 decreases tumor multiplicity and invasion in AOM/IL10^{-/-} mice, without altering intestinal inflammation, suggesting that intestinal inflammation can promote tumorigenesis by altering microbial composition and inducing the expansion of microorganisms with genotoxic capabilities. Thus, both inflammation and bacteria-specific factors are required for the development of colitis-associated CRC [10].

In addition to *E. coli* NC101 and *Bacteroides fragilis* which promote colitis-associated CRC, *Fusobacterium nucleatum*, an opportunistic commensal anaerobe in the oral cavity, has recently been shown to promote CRC that are not associated with colitis [135, 136]. *Fusobacterium* species are enriched in human colorectal CRC relative to adjacent normal tissue [137, 138]. When human isolates of *F. nucleatum* or control *Streptococcus* species are introduced to Apc^{Min/+} mice, the onset of colonic tumors in mice with *F. nucleatum* is accelerated as Apc^{Min/+} mice fed with *F. nucleatum* develop a significantly higher numbers of colonic tumors as compared to Apc^{Min/+} mice fed with *Streptococcus* spp.. Importantly, colonic tumors from Apc^{Min/+} mice exposed to *F. nucleatum* exhibit a proinflammatory expression signature that is shared with human fusobacteria-positive colorectal carcinomas

[135]. FadA, an adhesion molecule produced by *F. nucleatum*, interacts with E-cadherin on host cells and triggers proliferation of colorectal-cancer cells by modulating E-cadherin and activating β -catenin signaling, thus leading to increased expression of transcription factors, oncogenes, Wnt genes, and inflammatory genes, as well as growth stimulation of CRC cells [136].

5. Summary

The host and microbiota have evolved mechanisms for coexistence over millions of years. Accumulating evidence indicates that a dynamic mutualism between the host and the commensal microbiota has important implications for health, and microbial colonization contributes to the maintenance of intestinal immune homeostasis. However, alterations in communication between the mucosal immune system and gut microbial communities have been implicated as the core defect that leads to chronic intestinal inflammation and cancer development. Although great progress has been made in last decade on the mechanisms involved in microbiota regulation of intestinal homeostasis as well as intestinal inflammation and tumorigenesis, more questions have been raised and need to be investigated. For examples, although several commensal bacteria have been implicated in regulation of Treg and Th17 cells, it is still unclear the mechanisms involved and what are the roles they play in intestinal inflammation and tumorigenesis in humans. It is also unknown why certain commensal bacteria, but not others, regulate intestinal homeostasis and the pathogenesis of IBD and cancer through interaction with TLRs and inflammasomes, although all commensal bacteria have the potential to do so. Eventually, can we identify the commensal bacteria which either induce or inhibit IBD and CRC, and isolate them for clinical application? The advance in technologies and methodologies will greatly help our efforts to answer all those questions.

Acknowledgments

This work was supported by grants from NIH DK079918, AI083484, John Sealy Memorial Research fund, the National Natural Science Foundation of China (81061120521, 81270470), and the Shanghai Science and Technology Commission (12XD1404000).

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. Mackie RI, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. *Am J Clin Nutr*. 1999; 69:1035S–1045S. [PubMed: 10232646]
3. Sartor RB. Microbial influences in inflammatory bowel diseases. *Gastroenterology*. 2008; 134:577–594. [PubMed: 18242222]
4. Belkaid Y, Naik S. Compartmentalized and systemic control of tissue immunity by commensals. *Nat Immunol*. 2013; 14:646–653. [PubMed: 23778791]
5. Elinav E, Henao-Mejia J, Flavell RA. Integrative inflammasome activity in the regulation of intestinal mucosal immune responses. *Mucosal Immunol*. 2013; 6:4–13. [PubMed: 23212196]
6. Elson CO, Cong Y, McCracken VJ, Dimmitt RA, Lorenz RG, Weaver CT. Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. *Immunol Rev*. 2005; 206:260–276. [PubMed: 16048554]
7. Macpherson AJ. The immune geography of IgA induction and function. *Mucosal Immunology*. 2007; 1:12–22.
8. Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature*. 2011; 474:298–306. [PubMed: 21677746]
9. Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. *J Clin Invest*. 2007; 117:514–521. [PubMed: 17332878]

10. Arthur JC, Perez-Chanona E, Muhlbauer M, Tomkovich S, Uronis JM, Fan TJ, Campbell BJ, Abujamel T, Dogan B, Rogers AB, Rhodes JM, Stintzi A, Simpson KW, Hansen JJ, Keku TO, Fodor AA, Jobin C. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science*. 2012; 338:120–123. [PubMed: 22903521]
11. Uronis JM, Muhlbauer M, Herfarth HH, Rubinas TC, Jones GS, Jobin C. Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. *PLoS One*. 2009; 4:e6026. [PubMed: 19551144]
12. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature*. 2012; 489:220–230. [PubMed: 22972295]
13. 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]
14. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. 2010; 464:59–65. [PubMed: 20203603]
15. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. *Nature*. 2009; 457:480–484. [PubMed: 19043404]
16. Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman AL, Clemente JC, Knight R, Heath AC, Leibel RL, Rosenbaum M, Gordon JI. The long-term stability of the human gut microbiota. *Science*. 2013; 341:1237439. [PubMed: 23828941]
17. Caporaso JG, Lauber CL, Costello EK, Berg-Lyons D, Gonzalez A, Stombaugh J, Knights D, Gajer P, Ravel J, Fierer N, Gordon JI, Knight R. Moving pictures of the human microbiome. *Genome Biol*. 2011; 12:R50. [PubMed: 21624126]
18. 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]
19. Lagier JC, Armougom F, Million M, Hugon P, Pagnier I, Robert C, Bittar F, Fournous G, Gimenez G, Maraninchi M, Trape JF, Koonin EV, La Scola B, Raoult D. Microbial culturomics: paradigm shift in the human gut microbiome study. *Clin Microbiol Infect*. 2012; 18:1185–1193. [PubMed: 23033984]
20. Ivanov II, Honda K. Intestinal commensal microbes as immune modulators. *Cell Host Microbe*. 2012; 12:496–508. [PubMed: 23084918]
21. Backhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, Versalovic J, Young V, Finlay BB. Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. *Cell Host Microbe*. 2012; 12:611–622. [PubMed: 23159051]
22. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. *Cell*. 2012; 148:1258–1270. [PubMed: 22424233]
23. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science*. 2012; 336:1268–1273. [PubMed: 22674334]
24. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, Peaper DR, Bertin J, Eisenbarth SC, Gordon JI, Flavell RA. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell*. 2011; 145:745–757. [PubMed: 21565393]
25. 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]
26. Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, Sitaraman SV, Knight R, Ley RE, Gewirtz AT. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science*. 2010; 328:228–231. [PubMed: 20203013]
27. Cebra JJ, Periwal SB, Lee G, Lee F, Shroff KE. Development and maintenance of the gut-associated lymphoid tissue (GALT): the roles of enteric bacteria and viruses. *Dev Immunol*. 1998; 6:13–18. [PubMed: 9716901]
28. Cebra JJ. Influences of microbiota on intestinal immune system development. *Am J Clin Nutr*. 1999; 69:1046S–1051S. [PubMed: 10232647]

29. Jiang HQ, Thurnheer MC, Zuercher AW, Boiko NV, Bos NA, Cebra JJ. Interactions of commensal gut microbes with subsets of B- and T-cells in the murine host. *Vaccine*. 2004; 22:805–811. [PubMed: 15040931]
30. 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]
31. Honda K, Littman DR. The microbiome in infectious disease and inflammation. *Annu Rev Immunol*. 2012; 30:759–795. [PubMed: 22224764]
32. Macpherson AJ, Uhr T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science*. 2004; 303:1662–1665. [PubMed: 15016999]
33. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y, Taniguchi T, Takeda K, Hori S, Ivanov II, Umesaki Y, Itoh K, Honda K. Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science*. 2011; 331:337–341. [PubMed: 21205640]
34. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*. 2009; 139:485–498. [PubMed: 19836068]
35. Round JL, Mazmanian SK. Inducible Foxp3⁺ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci U S A*. 2010; 107:12204–12209. [PubMed: 20566854]
36. Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat Rev Microbiol*. 2008; 6:121–131. [PubMed: 18180751]
37. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature*. 2008; 453:620–625. [PubMed: 18509436]
38. 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]
39. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA*. 2007; 104:13780–13785. [PubMed: 17699621]
40. Itoh K, Mitsuoka T. Characterization of clostridia isolated from faeces of limited flora mice and their effect on caecal size when associated with germ-free mice. *Lab Anim*. 1985; 19:111–118. [PubMed: 3889493]
41. Geuking MB, Cahenzli J, Lawson MA, Ng DC, Slack E, Hapfelmeier S, McCoy KD, Macpherson AJ. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity*. 2011; 34:794–806. [PubMed: 21596591]
42. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, Glickman JN, Garrett WS. The Microbial Metabolites, Short-Chain Fatty Acids, Regulate Colonic Treg Cell Homeostasis. *Science*. 2013 Epub July 4, 2013.
43. Le Poul E, Loison C, Struyf S, Springael JY, Lannoy V, Decobecq ME, Brezillon S, Dupriez V, Vassart G, Van Damme J, Parmentier M, Detheux M. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J Biol Chem*. 2003; 278:25481–25489. [PubMed: 12711604]
44. Elson CO, Cong Y, Weaver CT, Schoeb TR, McClanahan TK, Fick RB, Kastelein RA. Monoclonal anti-interleukin 23 reverses active colitis in a T cell-mediated model in mice. *Gastroenterology*. 2007; 132:2359–2370. [PubMed: 17570211]
45. 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]
46. Kullberg MC, Jankovic D, Feng CG, Hue S, Gorelick PL, McKenzie BS, Cua DJ, Powrie F, Cheever AW, Maloy KJ, Sher A. IL-23 plays a key role in *Helicobacter hepaticus*-induced T cell-dependent colitis. *J Exp Med*. 2006; 203:2485–2494. [PubMed: 17030948]

47. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature*. 2006; 441:231–234. [PubMed: 16648837]
48. Yen D, Cheung J, Scheerens H, Poulet F, McClanahan T, McKenzie B, Kleinschek MA, Owyang A, Mattson J, Blumenschein W, Murphy E, Sathe M, Cua DJ, Kastelein RA, Rennick D. IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. *J Clin Invest*. 2006; 116:1310–1316. [PubMed: 16670770]
49. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR. The orphan nuclear receptor ROR γ directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell*. 2006; 126:1121–1133. [PubMed: 16990136]
50. Atarashi K, Nishimura J, Shima T, Umesaki Y, Yamamoto M, Onoue M, Yagita H, Ishii N, Evans R, Honda K, Takeda K. ATP drives lamina propria T(H)17 cell differentiation. *Nature*. 2008; 455:808–812. [PubMed: 18716618]
51. Ivanov II, de Frutos RL, 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]
52. Gaboriau-Routhiau V, Rakotobe S, Lecuyer E, Mulder I, Lan A, Bridonneau C, Rochet V, Pisi A, De Paepe M, Brandi G, Eberl G, Snel J, Kelly D, Cerf-Bensussan N. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity*. 2009; 31:677–689. [PubMed: 19833089]
53. Clarke TB, Davis KM, Lysenko ES, Zhou AY, Yu Y, Weiser JN. Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nat Med*. 2010; 16:228–231. [PubMed: 20081863]
54. Hand T, Belkaid Y. Microbial control of regulatory and effector T cell responses in the gut. *Curr Opin Immunol*. 2010; 22:63–72. [PubMed: 20171861]
55. Kanneganti TD, Lamkanfi M, Nunez G. Intracellular NOD-like receptors in host defense and disease. *Immunity*. 2007; 27:549–559. [PubMed: 17967410]
56. Kawai T, Akira S. TLR signaling. *Semin Immunol*. 2007; 19:24–32. [PubMed: 17275323]
57. Neish AS, Gewirtz AT, Zeng H, Young AN, Hobert ME, Karmali V, Rao AS, Madara JL. Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. *Science*. 2000; 289:1560–1563. [PubMed: 10968793]
58. Wertz IE, O'Rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S, Wu P, Wiesmann C, Baker R, Boone DL, Ma A, Koonin EV, Dixit VM. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. *Nature*. 2004; 430:694–699. [PubMed: 15258597]
59. Normand S, Delanoye-Crespin A, Bressenot A, Huot L, Grandjean T, Peyrin-Biroulet L, Lemoine Y, Hot D, Chamaillard M. Nod-like receptor pyrin domain-containing protein 6 (NLRP6) controls epithelial self-renewal and colorectal carcinogenesis upon injury. *Proc Natl Acad Sci U S A*. 2011; 108:9601–9606. [PubMed: 21593405]
60. Rakoff-Nahoum S, Medzhitov R. Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. *Science*. 2007; 317:124–127. [PubMed: 17615359]
61. Cerf-Bensussan N, Gaboriau-Routhiau V. The immune system and the gut microbiota: friends or foes? *Nat Rev Immunol*. 2010; 10:735–744. [PubMed: 20865020]
62. Haller D, Holt L, Kim SC, Schwabe RF, Sartor RB, Jobin C. Transforming growth factor-beta 1 inhibits non-pathogenic Gram negative bacteria-induced NF-kappa B recruitment to the interleukin-6 gene promoter in intestinal epithelial cells through modulation of histone acetylation. *J Biol Chem*. 2003; 278:23851–23860. [PubMed: 12672795]
63. Karrasch T, Kim JS, Muhlbauer M, Magness ST, Jobin C. Gnotobiotic IL-10-/-; NF-kappa B(EGFP) mice reveal the critical role of TLR/NF-kappa B signaling in commensal bacteria-induced colitis. *J Immunol*. 2007; 178:6522–6532. [PubMed: 17475882]
64. Ruiz PA, Shkoda A, Kim SC, Sartor RB, Haller D. IL-10 gene-deficient mice lack TGF-beta/Smad-mediated TLR2 degradation and fail to inhibit proinflammatory gene expression in intestinal epithelial cells under conditions of chronic inflammation. *Ann N Y Acad Sci*. 2006; 1072:389–394. [PubMed: 17057220]

65. Fukata M, Michelsen KS, Eri R, Thomas LS, Hu B, Lukasek K, Nast CC, Lechago J, Xu R, Naiki Y, Soliman A, Arditi M, Abreu MT. Toll-like receptor-4 is required for intestinal response to epithelial injury and limiting bacterial translocation in a murine model of acute colitis. *Am J Physiol Gastrointest Liver Physiol*. 2005; 288:G1055–1065. [PubMed: 15826931]
66. Kitajima S, Morimoto M, Sagara E, Shimizu C, Ikeda Y. Dextran sodium sulfate-induced colitis in germ-free IQI/Jic mice. *Exp Anim*. 2001; 50:387–395. [PubMed: 11769541]
67. Lee J, Mo JH, Katakura K, Alkalay I, Rucker AN, Liu YT, Lee HK, Shen C, Cojocaru G, Shenouda S, Kagnoff M, Eckmann L, Ben-Neriah Y, Raz E. Maintenance of colonic homeostasis by distinctive apical TLR9 signalling in intestinal epithelial cells. *Nat Cell Biol*. 2006; 8:1327–1336. [PubMed: 17128265]
68. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell*. 2004; 118:229–241. [PubMed: 15260992]
69. Nenci A, Becker C, Wullaert A, Gareus R, van Loo G, Danese S, Huth M, Nikolaev A, Neufert C, Madison B, Gumucio D, Neurath MF, Pasparakis M. Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature*. 2007; 446:557–561. [PubMed: 17361131]
70. Fukata M, Chen A, Klepper A, Krishnareddy S, Vamadevan AS, Thomas LS, Xu R, Inoue H, Arditi M, Dannenberg AJ, Abreu MT. Cox-2 is regulated by Toll-like receptor-4 (TLR4) signaling: Role in proliferation and apoptosis in the intestine. *Gastroenterology*. 2006; 131:862–877. [PubMed: 16952555]
71. Sartor RB. Genetics and environmental interactions shape the intestinal microbiome to promote inflammatory bowel disease versus mucosal homeostasis. *Gastroenterology*. 2010; 139:1816–1819. [PubMed: 21029802]
72. Deloris Alexander A, Orcutt RP, Henry JC, Baker J Jr, Bissahoyo AC, Threadgill DW. Quantitative PCR assays for mouse enteric flora reveal strain-dependent differences in composition that are influenced by the microenvironment. *Mamm Genome*. 2006; 17:1093–1104. [PubMed: 17091319]
73. Friswell MK, Gika H, Stratford IJ, Theodoridis G, Telfer B, Wilson ID, McBain AJ. Site and strain-specific variation in gut microbiota profiles and metabolism in experimental mice. *PLoS One*. 2010; 5:e8584. [PubMed: 20052418]
74. Rawls JF, Mahowald MA, Ley RE, Gordon JI. Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. *Cell*. 2006; 127:423–433. [PubMed: 17055441]
75. Gulati AS, Shanahan MT, Arthur JC, Grossniklaus E, von Furstenberg RJ, Kreuk L, Henning SJ, Jobin C, Sartor RB. Mouse background strain profoundly influences Paneth cell function and intestinal microbial composition. *PLoS One*. 2012; 7:e32403. [PubMed: 22384242]
76. 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]
77. Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature*. 2012; 482:179–185. [PubMed: 22297845]
78. Petnicki-Ocwieja T, Hrnecir T, Liu YJ, Biswas A, Hudcovic T, Tlaskalova-Hogenova H, Kobayashi KS. Nod2 is required for the regulation of commensal microbiota in the intestine. *Proc Natl Acad Sci U S A*. 2009; 106:15813–15818. [PubMed: 19805227]
79. Ubeda C, Lipuma L, Gobourne A, Viale A, Leiner I, Equinda M, Khanin R, Pamer EG. Familial transmission rather than defective innate immunity shapes the distinct intestinal microbiota of TLR-deficient mice. *J Exp Med*. 2012; 209:1445–1456. [PubMed: 22826298]
80. 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]

81. Cao AT, Yao S, Gong B, Elson CO, Cong Y. Th17 cells upregulate polymeric Ig receptor and intestinal IgA and contribute to intestinal homeostasis. *J Immunol.* 2012; 189:4666–4673. [PubMed: 22993206]
82. 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]
83. Castigli E, Wilson SA, Scott S, Dedeoglu F, Xu S, Lam KP, Bram RJ, Jabara H, Geha RS. TACI and BAFF-R mediate isotype switching in B cells. *J Exp Med.* 2005; 201:35–39. [PubMed: 15630136]
84. He B, Xu W, Santini PA, Polydorides AD, Chiu A, Estrella J, Shan M, Chadburn A, Villanacci V, Plebani A, Knowles DM, Rescigno M, Cerutti A. 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]
85. Litinskiy MB, Nardelli B, Hilbert DM, He B, Schaffer A, Casali P, Cerutti A. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. *Nat Immunol.* 2002; 3:822–829. [PubMed: 12154359]
86. Tezuka H, Abe Y, Asano J, Sato T, Liu J, Iwata M, Ohteki T. Prominent role for plasmacytoid dendritic cells in mucosal T cell-independent IgA induction. *Immunity.* 2011; 34:247–257. [PubMed: 21333555]
87. von Bulow GU, van Deursen JM, Bram RJ. Regulation of the T-independent humoral response by TACI. *Immunity.* 2001; 14:573–582. [PubMed: 11371359]
88. Suzuki K, Meek B, Doi Y, Muramatsu M, Chiba T, Honjo T, Fagarasan S. Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. *Proc Natl Acad Sci U S A.* 2004; 101:1981–1986. [PubMed: 14766966]
89. Peterson DA, McNulty NP, Guruge JL, Gordon JI. IgA response to symbiotic bacteria as a mediator of gut homeostasis. *Cell Host Microbe.* 2007; 2:328–339. [PubMed: 18005754]
90. Blumberg RS, Saubermann LJ, Strober W. Animal models of mucosal inflammation and their relation to human inflammatory bowel disease. *Curr Opin Immunol.* 1999; 11:648–656. [PubMed: 10631550]
91. Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, Lees CW, Balschun T, Lee J, Roberts R, et al. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat Genet.* 2010; 42:1118–1125. [PubMed: 21102463]
92. McGovern DP, Gardet A, Torkvist L, Goyette P, Essers J, Taylor KD, Neale BM, Ong RT, Lagace C, Li C, Green T, et al. Genome-wide association identifies multiple ulcerative colitis susceptibility loci. *Nat Genet.* 2010; 42:332–337. [PubMed: 20228799]
93. Saleh M, Trinchieri G. Innate immune mechanisms of colitis and colitis-associated colorectal cancer. *Nat Rev Immunol.* 2011; 11:9–20. [PubMed: 21151034]
94. Aranda R, Sydora BC, McAllister PL, Binder SW, Yang HY, Targan SR, Kronenberg M. Analysis of intestinal lymphocytes in mouse colitis mediated by transfer of CD4+, CD45RBhigh T cells to SCID recipients. *J Immunol.* 1997; 158:3464–3473. [PubMed: 9120308]
95. Contractor NV, Bassiri H, Reya T, Park AY, Baumgart DC, Wasik MA, Emerson SG, Carding SR. Lymphoid hyperplasia, autoimmunity, and compromised intestinal intraepithelial lymphocyte development in colitis-free gnotobiotic IL-2-deficient mice. *J Immunol.* 1998; 160:385–394. [PubMed: 9551995]
96. Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell.* 1993; 75:263–274. [PubMed: 8402911]
97. Morrissey PJ, Charrier K, Braddy S, Liggitt D, Watson JD. CD4+ T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodeficient mice. Disease development is prevented by cotransfer of purified CD4+ T cells. *J Exp Med.* 1993; 178:237–244. [PubMed: 8100269]
98. Sadlack B, Merz H, Schorle H, Schimpl A, Feller AC, Horak I. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell.* 1993; 75:253–261. [PubMed: 8402910]

99. Feng T, Wang L, Schoeb TR, Elson CO, Cong Y. Microbiota innate stimulation is a prerequisite for T cell spontaneous proliferation and induction of experimental colitis. *J Exp Med*. 2010; 207:1321–1332. [PubMed: 20498021]
100. Fuss IJ, Becker C, Yang Z, Groden C, Hornung RL, Heller F, Neurath MF, Strober W, Mannon PJ. Both IL-12p70 and IL-23 are synthesized during active Crohn's disease and are down-regulated by treatment with anti-IL-12 p40 monoclonal antibody. *Inflamm Bowel Dis*. 2006; 12:9–15. [PubMed: 16374252]
101. Kobayashi T, Okamoto S, Hisamatsu T, Kamada N, Chinen H, Saito R, Kitazume MT, Nakazawa A, Sugita A, Koganei K, Isobe K, Hibi T. IL23 differentially regulates the Th1/Th17 balance in ulcerative colitis and Crohn's disease. *Gut*. 2008; 57:1682–1689. [PubMed: 18653729]
102. Monteleone G, Biancone L, Marasco R, Morrone G, Marasco O, Luzzza F, Pallone F. Interleukin 12 is expressed and actively released by Crohn's disease intestinal lamina propria mononuclear cells. *Gastroenterology*. 1997; 112:1169–1178. [PubMed: 9098000]
103. Sakuraba A, Sato T, Kamada N, Kitazume M, Sugita A, Hibi T. Th1/Th17 Immune Response is Induced by Mesenteric Lymph Node Dendritic Cells in Crohn's Disease. *Gastroenterology*. 2009
104. Schmidt C, Giese T, Ludwig B, Mueller-Molaian I, Marth T, Zeuzem S, Meuer SC, Stallmach A. Expression of interleukin-12-related cytokine transcripts in inflammatory bowel disease: elevated interleukin-23p19 and interleukin-27p28 in Crohn's disease but not in ulcerative colitis. *Inflamm Bowel Dis*. 2005; 11:16–23. [PubMed: 15674109]
105. Mannon PJ, Fuss IJ, Mayer L, Elson CO, Sandborn WJ, Present D, Dolin B, Goodman N, Groden C, Hornung RL, Quezado M, Yang Z, Neurath MF, Salfeld J, Veldman GM, Schwertschlag U, Strober W. Anti-interleukin-12 antibody for active Crohn's disease. *N Engl J Med*. 2004; 351:2069–2079. [PubMed: 15537905]
106. Sandborn WJ, Feagan BG, Fedorak RN, Scherl E, Fleisher MR, Katz S, Johanns J, Blank M, Rutgeerts P. A randomized trial of Ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with moderate-to-severe Crohn's disease. *Gastroenterology*. 2008; 135:1130–1141. [PubMed: 18706417]
107. Cong Y, Brandwein SL, McCabe RP, Lazenby A, Birkenmeier EH, Sundberg JP, Elson CO. CD4+ T cells reactive to enteric bacterial antigens in spontaneously colitic C3H/HeJBir mice: increased T helper cell type 1 response and ability to transfer disease. *J Exp Med*. 1998; 187:855–864. [PubMed: 9500788]
108. Lodes MJ, Cong Y, Elson CO, Mohamath R, Landers CJ, Targan SR, Fort M, Hershberg RM. Bacterial flagellin is a dominant antigen in Crohn disease. *J Clin Invest*. 2004; 113:1296–1306. [PubMed: 15124021]
109. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature*. 2001; 410:1099–1103. [PubMed: 11323673]
110. Garrett WS, Gallini CA, Yatsunenko T, Michaud M, DuBois A, Delaney ML, Punit S, Karlsson M, Bry L, Glickman JN, Gordon JI, Onderdonk AB, Glimcher LH. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. *Cell Host Microbe*. 8:292–300. [PubMed: 20833380]
111. Rath HC, Herfarth HH, Ikeda JS, Grenther WB, Hamm TE Jr, Balish E, Taurog JD, Hammer RE, Wilson KH, Sartor RB. Normal luminal bacteria, especially *Bacteroides* species, mediate chronic colitis, gastritis, and arthritis in HLA-B27/human beta2 microglobulin transgenic rats. *J Clin Invest*. 1996; 98:945–953. [PubMed: 8770866]
112. Rath HC, Wilson KH, Sartor RB. Differential induction of colitis and gastritis in HLA-B27 transgenic rats selectively colonized with *Bacteroides vulgatus* or *Escherichia coli*. *Infect Immun*. 1999; 67:2969–2974. [PubMed: 10338507]
113. Kim SC, Tonkonogy SL, Albright CA, Tsang J, Balish EJ, Braun J, Huycke MM, Sartor RB. Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria. *Gastroenterology*. 2005; 128:891–906. [PubMed: 15825073]
114. Kim SC, Tonkonogy SL, Karrasch T, Jobin C, Sartor RB. Dual-association of gnotobiotic IL-10-/- mice with 2 nonpathogenic commensal bacteria induces aggressive pancolitis. *Inflamm Bowel Dis*. 2007; 13:1457–1466. [PubMed: 17763473]

115. Willing BP, Dicksved J, Halfvarson J, Andersson AF, Lucio M, Zheng Z, Jarnerot G, Tysk C, Jansson JK, Engstrand L. A pyrosequencing study in twins shows that gastrointestinal microbial profiles vary with inflammatory bowel disease phenotypes. *Gastroenterology*. 2010; 139:1844–1854. e1841. [PubMed: 20816835]
116. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, Gratadoux JJ, Blugeon S, Bridonneau C, Furet JP, Corthier G, Grangette C, Vasquez N, Pochart P, Trugnan G, Thomas G, Blottiere HM, Dore J, Marteau P, Seksik P, Langella P. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci U S A*. 2008; 105:16731–16736. [PubMed: 18936492]
117. Chassaing B, Darfeuille-Michaud A. The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. *Gastroenterology*. 2011; 140:1720–1728. [PubMed: 21530738]
118. Barnich N, Darfeuille-Michaud A. Adherent-invasive *Escherichia coli* and Crohn's disease. *Curr Opin Gastroenterol*. 2007; 23:16–20. [PubMed: 17133079]
119. Darfeuille-Michaud A, Boudeau J, Bulois P, Neut C, Glasser AL, Barnich N, Bringer MA, Swidsinski A, Beaugerie L, Colombel JF. High prevalence of adherent-invasive *Escherichia coli* associated with ileal mucosa in Crohn's disease. *Gastroenterology*. 2004; 127:412–421. [PubMed: 15300573]
120. Lapaquette P, Glasser AL, Huett A, Xavier RJ, Darfeuille-Michaud A. Crohn's disease-associated adherent-invasive *E. coli* are selectively favoured by impaired autophagy to replicate intracellularly. *Cell Microbiol*. 2010; 12:99–113. [PubMed: 19747213]
121. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144:646–674. [PubMed: 21376230]
122. Wang K, Grivennikov SI, Karin M. Implications of anti-cytokine therapy in colorectal cancer and autoimmune diseases. *Ann Rheum Dis*. 2013; 72(Suppl 2):ii100–103. [PubMed: 23253923]
123. Wilson JA. Tumor necrosis factor alpha and colitis-associated colon cancer. *N Engl J Med*. 2008; 358:2733–2734. [PubMed: 18565868]
124. Xiao H, Gulen MF, Qin J, Yao J, Bulek K, Kish D, Altuntas CZ, Wald D, Ma C, Zhou H, Tuohy VK, Fairchild RL, de la Motte C, Cua D, Vallance BA, Li X. The Toll-interleukin-1 receptor member SIGIRR regulates colonic epithelial homeostasis, inflammation, and tumorigenesis. *Immunity*. 2007; 26:461–475. [PubMed: 17398123]
125. Clevers H. At the crossroads of inflammation and cancer. *Cell*. 2004; 118:671–674. [PubMed: 15369667]
126. 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]
127. Grivennikov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, Taniguchi K, Yu GY, Osterreicher CH, Hung KE, Datz C, Feng Y, Fearon ER, Oukka M, Tessarollo L, Coppola V, Yarovinsky F, Cheroutre H, Eckmann L, Trinchieri G, Karin M. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature*. 2012; 491:254–258. [PubMed: 23034650]
128. Grivennikov S, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, Scheller J, Rose-John S, Cheroutre H, Eckmann L, Karin M. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell*. 2009; 15:103–113. [PubMed: 19185845]
129. Li Y, de Haar C, Chen M, Deuring J, Gerrits MM, Smits R, Xia B, Kuipers EJ, van der Woude CJ. Disease-related expression of the IL6/STAT3/SOCS3 signalling pathway in ulcerative colitis and ulcerative colitis-related carcinogenesis. *Gut*. 2010; 59:227–235. [PubMed: 19926618]
130. Yan B, Wang H, Rabbani ZN, Zhao Y, Li W, Yuan Y, Li F, Dewhirst MW, Li CY. Tumor necrosis factor- α is a potent endogenous mutagen that promotes cellular transformation. *Cancer Res*. 2006; 66:11565–11570. [PubMed: 17178846]
131. Abdulamir AS, Hafidh RR, Abu Bakar F. The association of *Streptococcus bovis/gallolyticus* with colorectal tumors: the nature and the underlying mechanisms of its etiological role. *J Exp Clin Cancer Res*. 30:11. [PubMed: 21247505]

132. Goodwin AC, Destefano Shields CE, Wu S, Huso DL, Wu X, Murray-Stewart TR, Hacker-Prietz A, Rabizadeh S, Woster PM, Sears CL, Casero RA Jr. Polyamine catabolism contributes to enterotoxigenic *Bacteroides fragilis*-induced colon tumorigenesis. *Proc Natl Acad Sci U S A*. 2011; 108:15354–15359. [PubMed: 21876161]
133. Reddy BS, el-Bayoumy K, Louis YM. Germfree animal as a tool to study role of gut microflora and nutrition in cancer. *Prog Clin Biol Res*. 1985; 181:293–296. [PubMed: 4022986]
134. Hu B, Elinav E, Huber S, Strowig T, Hao L, Hafemann A, Jin C, Eisenbarth SC, Flavell RA. Microbiota-induced activation of epithelial IL-6 signaling links inflammasome-driven inflammation with transmissible cancer. *Proc Natl Acad Sci U S A*. 2013; 110:9862–9867. [PubMed: 23696660]
135. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, Clancy TE, Chung DC, Lochhead P, Hold GL, El-Omar EM, Brenner D, Fuchs CS, Meyerson M, Garrett WS. *Fusobacterium nucleatum* Potentiates Intestinal Tumorigenesis and Modulates the Tumor-Immune Microenvironment. *Cell Host Microbe*. 2013; 14:207–215. [PubMed: 23954159]
136. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. *Fusobacterium nucleatum* Promotes Colorectal Carcinogenesis by Modulating E-Cadherin/beta-Catenin Signaling via its FadA Adhesin. *Cell Host Microbe*. 2013; 14:195–206. [PubMed: 23954158]
137. Castellarin M, Warren RL, Freeman JD, Dreolini L, Krzywinski M, Strauss J, Barnes R, Watson P, Allen-Vercos E, Moore RA, Holt RA. *Fusobacterium nucleatum* infection is prevalent in human colorectal carcinoma. *Genome Res*. 2012; 22:299–306. [PubMed: 22009989]
138. Kostic AD, Gevers D, Pedamallu CS, Michaud M, Duke F, Earl AM, Ojesina AI, Jung J, Bass AJ, Tabernero J, Baselga J, Liu C, Shivdasani RA, Ogino S, Birren BW, Huttenhower C, Garrett WS, Meyerson M. Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma. *Genome Res*. 2012; 22:292–298. [PubMed: 22009990]