Regulation of intestinal homeostasis by innate and adaptive immunity

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Abstract

The intestine is a unique tissue where an elaborate balance is maintained between tolerance and immune responses against a variety of environmental factors such as food and the microflora. In a healthy individual, the microflora stimulates innate and adaptive immune systems to maintain gut homeostasis. However, the interaction of environmental factors with particular genetic backgrounds can lead to dramatic changes in the composition of the microflora (i.e. dysbiosis). Many of the specific commensal-bacterial products and the signaling pathways they trigger have been characterized. The role of T1, T2, and T17 cells in inflammatory bowel disease has been widely investigated, as has the contribution of epithelial cells and subsets of dendritic cells and macrophages. To date, multiple regulatory cells in adaptive immunity, such as regulatory T cells and regulatory B cells, have been shown to maintain gut homeostasis by preventing inappropriate innate and adaptive immune responses to commensal bacteria. Additionally, regulatory myeloid cells have recently been identified that prevent intestinal inflammation by inhibiting T-cell proliferation. An increasing body of evidence has shown that multiple regulatory mechanisms contribute to the maintenance of gut homeostasis.

Keywords: adaptive immunity, inflammatory bowel disease (IBD), innate immunity, microflora

Introduction

The intestinal tract is constitutively exposed to environmental factors and the intestinal immune system must be balanced: activating pro-inflammatory pathways for host defense against pathogenic microorganisms while remaining unresponsive to symbiotic bacteria and food antigens (1–3). Excessive and constitutive activation of immune responses can cause gut tissue destruction. A great variety of immune cells therefore contribute to providing defense in response to invasion of pathogens and tolerance for the maintenance of gut homeostasis. To date, there is abundant evidence indicating the mutualism between the microflora (i.e. the collective population of nonpathogenic microbes in the gut) and intestinal immune cells. Mice bred under germ-free conditions possess underdeveloped gut-associated lymphoid tissue, few IgA+ plasma cells and CD4+ T cells in the lamina propria, reduced numbers of intraepithelial lymphocytes (IELs), and decreased MHC class II expression on antigen-presenting cells (4–7). Accordingly, mice bred in germ-free environments are unable to eradicate pathogens or to induce oral tolerance (4, 8–10). The presence of microflora in the gut is thus sufficient to develop an extensive and activated intestinal immune system.

Proper development of the mucosal immune system is responsible for the maintenance of gut homeostasis by eliminating invading pathogens. Overactivation of the mucosal system driven by the microflora and food antigens can, however, lead to the development of intestinal inflammation. Recently, multiple regulatory cells that control immune responses and maintain intestinal homeostasis and tolerance have been identified (11–14). This review focuses on the role of a variety of regulatory immune cells in gut homeostasis and inflammatory bowel disease (IBD).

Inflammatory bowel disease

There is a high prevalence of IBD, which comprises two distinct diseases, Crohn’s disease (CD) and ulcerative colitis (UC), in Europe and the USA. Note that other classifications of IBD include less common forms of colitis and Behçet's disease. Even
in Asian countries such as Japan and Korea, the numbers of patients with IBD have recently increased dramatically. Studies using animal models of IBD (induced genetically, chemically, or by transfer of CD4+CD45RBhigh naive T cells) have demonstrated that interactions of host genetic background and environmental factors including diet, lifestyle, hygiene, and the microflora can cause dysregulation of the immune system leading to the onset and/or progression of disease (15,16). Particularly, the microflora is strongly implicated in the pathogenesis of IBD and dramatic changes in the composition of the microflora have been demonstrated in IBD (17). In addition, the absence of a microflora leads to a reduction in gut inflammation observed in Tcra−/−, IL-2−/−, IL-10−/−, or MyD88−/− mice (16,18,19). Consistent with evidence from experimental mouse IBD models, antibiotics can provide therapeutic benefit in patients with CD (20,21).

Genome-wide mapping has identified the genes and genetic loci that contribute to IBD susceptibility, including 99 non-overlapping genetic risk loci and 28 that are shared between CD and UC (22,23). These data have demonstrated several pathways that are important for intestinal homeostasis, such as barrier function, regulation of innate immune responses, autophagy, endoplasmic reticulum stress, and adaptive immune regulation (24). Among these gene products, NOD2 (nucleotide-binding oligomerization domain-containing protein 2), which recognizes the core component of peptidoglycan, muramyl dipeptide, has been extensively studied recently. Muramyl dipeptide stimulation induces autophagy that regulates bacterial killing and antigen presentation through interactions with ATG16L1 (autophagy-related gene 16-like 1), which is also linked to CD susceptibility (25). In addition, NOD2 signaling may mediate immune tolerance by inhibiting TLR–NF-κB signaling (26). Patients with the CD-associated NOD2 mutation, 3020insC, show impaired inhibition of TLR–NF-κB signaling. Mutant human NOD2 has, furthermore, been shown to down-regulate the production of IL-10, an anti-inflammatory cytokine, by suppressing the activity of hnRNP A1 (heterogeneous nuclear ribonucleoprotein A1) (27).

Regulation of gut homeostasis by adaptive immune cells

T1/Th17 cells

It was commonly assumed that intestinal inflammation was caused by a disruption in the balance between Th1 and Th2 cytokine responses. Early evidence suggested a model in which CD might be Th1 mediated (via e.g. IFN-γ and IL-12) and UC might be Th2 mediated (via e.g. IL-4). However, the use of antibodies that block IFN-γ showed limited clinical effectiveness in patients with CD and a mouse colitis model, although treatment with antibodies that block IL-12p40, and thus neutralize IL-12 and IL-23, showed considerable efficacy in active CD.

The recent discovery of a third population of helper T cells, IL-17-producing Th17 cells, has resolved this discrepancy. Th17 cells induced by IL-6 and transforming growth factor (TGF)-β express the transcription factor RORγt (retinoic acid-related orphan receptor γt) as a ‘master regulator’ and expand in response to IL-23. The number of Th1 cells and Th17 cells increased in the lamina propria of patients with CD as compared with healthy individuals, suggesting that a Th17-associated IL-23–IL-17 axis contributes to the development of CD, in addition to the Th1 pathway (28).

Several studies have shown that accelerated Th2 responses profoundly contribute to pathogenesis of UC in Tcra−/− mice and the oxazolone-induced colitis model (29–31), although numbers of IL-4-producing T cells are reduced in patients with UC (32).

The recent identification of a subset of innate lymphoid cells, natural helper cells that are present in fat-associated lymphocyte clusters may suggest a Th2-cytokine-mediated etiology of UC, as natural helper cells constitutively produce large amounts of Th2 cytokines such as IL-13 and IL-5 that help the maintenance of B1 cells following IL-33, IL-2 and IL-25 stimulation (33).

Treg cells

In the steady state, the number and activity of pathogenic effector T cells are tightly regulated by an organized regulatory system consisting of multiple cell types that maintain intestinal homeostasis, since excessive Th1- and Th17-cell responses can result in gut inflammation. In the intestinal adaptive immune system, regulatory (Treg)–cell differentiation is preferentially induced rather than that of helper T cells (Fig. 1). About two decades ago, CD4+CD25+ Treg cells that contribute to the maintenance of self-tolerance by regulating immune responses to self and non-self antigens were identified (34). This cell subset has the ability to suppress inflammatory responses through the production of anti-inflammatory cytokines including IL-10 and TGF-β, in addition to other mechanisms that can inhibit the function of antigen-presenting cells via expression of CTLA-4, LAG-3 (lymphocyte-activation gene 3), CD39 and Nrp-1 (neuropilin 1) on Treg cells (35).

The transcription factor Foxp3 has been identified as a master regulator of Treg cells. Indeed, loss-of-function mutations in FOXP3 cause IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) (36). Intestinal Foxp3+ Treg cells produce large quantities of IL-10 as compared with those present in other tissues and may be important in the maintenance of gut homeostasis because IL-10-deficient Treg cells cannot prevent colitis in immunodeficient mice receiving CD4+CD45RBhigh naive T cells (37). In addition to this ability to prevent IBD, recent studies have demonstrated that Treg cells can reverse inflammation, depending on IL-10 (38,39). Treg-cell-derived IL-10 is thought to regulate the activity of intestinal myeloid cells.

Breg cells

Breg cells (regulatory B cells), which are characterized as CD1dhigh and which are present in the gut-associated lymphoid tissue, orchestrate the regulatory adaptive immune system (Fig. 1). CD1d, expressed on Breg cells, is required for IL-10 production, which can inhibit the inflammatory cascade (13). Accordingly, Breg cells defective in CD1d fail to suppress the exacerbation of chronic intestinal inflammation. These studies indicate that regulatory cells in the adaptive immune system help preserve the intestinal immune balance via IL-10 production resulting in the repression of enteric inflammation.
Role of the innate immune system in gut homeostasis

Epithelial cells

Innate immune cells and epithelial cells in the gut ‘sense’ pathogenic bacteria and some nonpathogenic bacteria via pattern-recognition receptors (PRRs) such as TLRs and NOD-like receptors (NLRs), which recognize conserved structures of microorganisms (3).

Intestinal epithelial cells function as the first barrier to luminal bacteria. Following the detection of microorganisms on the luminal side via innate immune receptors, epithelial cells secrete antibacterial peptides and cytokines (e.g. IL-10 and TGF-β) in the lamina propria, where they activate immune cells. However, it is unclear how the intestinal epithelium distinguishes between pathogenic microorganisms and microflora. One possibility is that TLRs have a restricted expression and localization in intestinal epithelial cells. For example, expression of TLR5, which recognizes flagellin, is restricted to the basolateral surface of the epithelial cells, thereby ensuring activation only by invading bacteria in the lamina propria (40). Additionally, subcellular localization of PRRs including TLR3, 8 and 9 in intracellular endosomal organelles and NLRs in the cytosolic compartment ensures that PRRs would not detect luminal commensal bacteria but recognize pathogenic bacteria invading into the epithelial cells.

Commensal bacteria have a major role in the maintenance of epithelial-cell integrity. Myd88−/− mice fail to express heat-shock protein 25 (HSP25) and HSP72 as well as cytokines, which mediate protection from intestinal epithelial cells from injury, resulting in high susceptibility to dextran
sulfate sodium-initiated colitis (41). Additionally, in Myd88−/− and Tlr4−/− mice, intestinal epithelial cell proliferation is markedly decreased while apoptosis is increased during dextran sulfate sodium-induced injury (41–43). The exposure of TLR4 to bacteria following injury would activate induction of growth factors, which promote proliferation and migration of epithelial cells. Furthermore, TLR2 maintains the expression of tight-junction protein ZO1 and protects against apoptosis, leading to an increase in barrier function of epithelial cells (44,45). These reports indicate that TLR/MyD88 signaling activated by commensal bacteria may play a crucial role in the maintenance of gut epithelial barrier integrity.

It is to be determined whether TLR expression on epithelial cells or other immune cell populations residing in the lamina propria is responsible for detection of commensal bacteria. The gut epithelial layers contain IELs. The exact functions of IELs remain unclear, although they may play crucial roles in epithelial tumor surveillance, reconstruction of gut tissue after injury or in the prevention of pathogenic bacteria penetration. 

Dendritic cells and macrophages

Patients with CD possess defective or inadequate innate immune responses, including cytokine production, pathogen clearance, and recruitment of neutrophils. Dendritic cells and macrophages are critical for initiation of innate immune responses during microbial invasion and inflammation. In the intestine, the activity of these innate-immune myeloid cells is finely regulated by several mechanisms because the excessive and inadequate initiation of innate immunity leads to the development of IBD.

Currently, intestinal innate-immune myeloid cells are divided into several subsets possessing different abilities to maintain gut immune homeostasis either through enhancing or suppressing T-cell responses (46–49). In particular, CXCR1+CD11b+CD11c− dendritic cells and CD103+ dendritic cells have been well characterized (50–52) (Figs 1 and 2). CXCR1+CD11b+CD11c− dendritic cells, which are also characterized by specific expression of CD70, express a series of ATP receptors that can induce T17-cell development (53). Administration of ATP to germ-free mice with few T17 cells enhances the activation of T17 cells in the lamina propria, indicating that ATP derived from commensal bacteria is responsible for initiation of T17-cell development (53). In contrast, CD103+ dendritic cells induce gut immune tolerance by promoting the differentiation of Foxp3+ Treg cells through the production of retinoic acid and TGF-β (48,54,55) (Fig. 1).

A recent study showed that TLR5-activated CD103+CD11c+ dendritic cells have T17-polarizing ability (56) (Fig. 2). In addition, CD103+ dendritic cells can stimulate CD4+ and CD8+ T-cell proliferation (57,58) and CD103+ dendritic cells activated by TLR ligands strongly induce cytotoxic T lymphocytes (57). Moreover, CD103+ dendritic cells contribute to flagellin-induced IL-23 production, leading to induction of IL-22 followed by sustained expression of the antimicrobial lectin RegIIIγ (regenerating islet-derived protein III γ) in gut epithelium (59) (Fig. 2). CD103+ dendritic cells can thus mediate multiple responses by maintaining intestinal immune tolerance to intestinal antigens and promoting protective immunity through induction of T-cell activation and antimicrobial defense. Apart from CXCR1+CD11b+ dendritic cells and CD103+ dendritic cells, E-cadherin-expressing dendritic cells, which express high levels of TLRs and produce colitogenic cytokines including IL-6 and IL-23, enhance intestinal inflammation by increasing T17 responses (59).

Intestinal CD11b+CD11c− macrophages produce substantial amounts of IL-10 in response to the microflora (60–62) (Fig. 1). IL-10 produced by intestinal macrophages limits intestinal inflammation through the persistence of Foxp3 expression in Treg cells (63) and inhibits IL-12 and tumor necrosis factor (TNF)-α production against commensal bacteria in intestinal myeloid cells via activation of the transcription factor Stat3. Consequently, IL-10-deficient mice and mice with Stat3 mutation specifically in myeloid cells (LysM-cre; Stat3lox/lox mice) spontaneously develop enteric inflammation (61). Interestingly, numbers of CD14+ macrophage are increased in patients with CD and produce greater amounts of colitogenic cytokines including IL-6, IL-23 and TNF-α against commensal bacteria than do healthy individuals (64). Furthermore, IL-23 derived from human intestinal CD14+ macrophages may enhance the differentiation of colitogenic IL-17- and IFN-γ-producing T cells, suggesting that abnormal innate immune responses by macrophages play a major role in the pathogenesis of IBD (64–67).

Mreg cells

We have recently identified intestinal innate-immune myeloid cells possessing a unique function. These cells are characterized by CCR1+CD11b+CD11c−, which are distributed in the intestinal lamina propria, and incapable of inducing T-cell differentiation. We found that CCR1+CD11b+CD11c− cells are further divided into two subsets based on the expression level of CCR1 (CX3CR1int and CX3CR1high). CX3CR1intCD11b+CD11c− cells have an ability to promote development of T17 cells, suggesting that CX3CR1intCD11b+CD11c− cells are identical to the above described CCR1+CD11b+CD11c− (CD70+) dendritic cells that can induce T17-cell differentiation. On the other hand, CX3CR1intCD11b+CD11c− cells suppress CD4+ T-cell proliferation in a cell–cell contact-dependent manner, and contribute to the prevention of intestinal inflammation. Mreg cells (regulatory myeloid cells) preferentially associate with CD4+ T cells via highly expressed adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1, but do not activate CD4+ T cells owing to the IL-10/Stat3-dependent suppression of CD80 and CD86 expression. LysM-cre; Stat3flox/flox mice, which spontaneously develop colitis, show defective Mreg-cell function. Administration of wild-type Mreg cells to Stat3-mutant mice ameliorates intestinal inflammation, indicating that a dysfunction of Mreg cells is involved in the pathogenesis of IBD.

Importantly, a previous study indicated that IL-10-producing Foxp3+CD4+CD25+ cells accumulate at inflamed sites in patients with IBD (68). This suggests that intestinal inflammation is not simply a result of defective Treg-cell-mediated suppression but that pathogenic T cells are regulated by multiple regulatory cells in the intestine. Accordingly, it will be important to determine whether Mreg cells are present and fully functional in patients with IBD before we can assess...
potential clinical application of Mreg cells for chronic intestinal inflammation.

Several subsets of intestinal innate-immune myeloid cells thus contribute to the maintenance of gut homeostasis, and dysregulation of activities of these cells leads to development of IBD.

Regulation of intestinal immune responses by microflora

The gastrointestinal tract in mammals harbors an estimated $10^{14}$ commensal organisms that exist in a symbiotic relationship with their host. Recent findings have elucidated that the microflora in the gut influences not only nutrient metabolism but also the development of the immune system (69–71). For instance, *Clostridium* species belonging to cluster XIV and IV induce the development of Foxp3$^+$ Treg cells in the colon (10) (Fig. 1). In addition, *Bacteroides fragilis* has been reported to protect animals from experimental colitis induced by pathogens via induction of IL-10-producing Treg cells (72) (Fig. 1). The beneficial effect of *B. fragilis* depends on the expression of polysaccharide A, which is a unique surface polysaccharide that binds to TLR2 on CD4$^+$ T cells (73). Accordingly, mice reared under germ-free conditions fail to induce oral tolerance (73).

Meanwhile, segmented filamentous bacteria (SFB) mediate T$_{h}$17-cell initiation in the small intestine (Fig. 2). Mice colonized with SFB established resistance to infection of *Citrobacter rodentium*, indicating that T$_{h}$17-cell induction by SFB was responsible for these protective immune responses (74). Mice expressing a human α-defensin gene (*DEFA5*) show loss of SFB and fewer IL-17-producing T cells (75). Moreover, SFB induces T$_{h}$1- and Treg-cell populations as well as T$_{h}$17 cells in Peyer’s patches (76), indicating that SFB may have a broad ability to coordinate the intestinal adaptive immune system.

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**Fig. 2.** Regulation of gut immune responses by innate immune cells. (A) CD103$^+$ dendritic cells activated by TLR5 ligands induce T$_{h}$1/T$_{h}$17-cell differentiation. In addition, IL-23 produced by CD103$^+$ dendritic cells induces IL-22 followed by secretion of the antimicrobial peptide RegIII$\gamma$ from intestinal epithelial cells. (B) ATP derived from commensal bacteria plays a crucial role in the induction of T$_{h}$17 cells by CX3CR1$^+$CD70$^+$ dendritic cells. (C) Segmented filamentous bacteria (SFB) are a potent inducer of T$_{h}$17-cell differentiation. (D) E-cadherin$^+$ dendritic cells enhance the accumulation of T$_{h}$17 cells by producing of a large amount of colitogenic cytokines in response to TLR signaling. In the steady state, T$_{h}$17 and T$_{h}$1 cells are responsible for protection against pathogens. However, abnormal behavior of effector T cells contributes to the onset and/or progression of intestinal inflammation. (E) In this regard, M$_{reg}$ cells tightly regulate T$_{h}$17/T$_{h}$1-cell proliferation in an IL-10/Stat3-dependent fashion.
In addition to this protective role during infection, autoimmune arthritis is triggered via T<sub>17</sub>-cell accumulation in K/BxN mice monoclonized with SFB (77). Furthermore, SFB colonization induces a high sensitivity to experimental autoimmune encephalomyelitis via induction of T<sub>17</sub> cells and autoantibody-producing B cells in the central nervous system (78, 79). T<sub>17</sub>-cell differentiation mediated by SFB colonization is thus connected to the development of autoimmune diseases while contributing to mucosal protection against pathogens. In addition to immune activation, the microflora is required to inhibit or expel pathogens by competing for nutrients, by producing biosurfactants that prevent adhesion of pathogens on mucosal surfaces, by signaling between bacteria that can lead to down-regulation of toxin production in pathogens, and by up-regulating tight-junction proteins in the intestinal epithelium.

Studies have shown that micronutrients can profoundly affect the generation and maintenance of immune responses (80). For instance, activation of the aryl hydrocarbon receptor (AhR) by 6-formylindolo[3,2-b]carbazole enhances production of IL-17 and IL-22 by T<sub>17</sub> cells (81, 82). In contrast, nuclear translocation of AhR by kynurenine promotes T<sub>reg</sub><sup>+</sup> cell induction. The activation of RAR–RXR (a heterodimer of retinoic acid receptor and retinoid X receptor) by retinoic acid, the immunologically active form of vitamin A, can also promote T<sub>reg</sub><sup>+</sup> cell generation. Furthermore, the interaction of short-chain fatty acids, such as acetate, and GPR43 (G-protein-coupled receptor 43) has been shown to promote the resolution of colitis (83). Acetate exerts anti-inflammatory effects on GPR43-expressing immune cells such as neutrophils by regulating migration and apoptosis of those cells in the intestine. In addition, acetate produced by Bifidobacterium longum has been reported to protect mice from lethal infection with Escherichia coli O157:H7 by enhancing the intestinal epithelial barrier integrity through induction of anti-inflammatory and anti-apoptotic genes (84).

Recent advances in our understanding of probiotics, which are live microorganisms that confer a health benefit, strongly suggest that these microorganisms can modulate the maintenance of gut homeostasis. In the context of intestinal inflammation, administration of probiotics can inhibit the progression of inflammation via IL-10- or TGF-β-bearing T<sub>reg</sub> cells (85, 86). In this context, one of the more common probiotics, Bifidobacterium breve, has recently been shown to induce IL-10-producing T<sub>reg</sub> cells in the colon via the TLR2-dependent activation of intestinal CD103<sup>+</sup> dendritic cells, which contributes to the prevention of intestinal inflammation (87). Probiotic administration in patients with IBD might therefore become an effective therapeutic approach during clinical remission.

**Conclusion**

Although recent advances have provided substantial insights into the gut immune homeostasis influenced by microflora, the mechanistic basis of IBD remains poorly understood. At steady state, the innate immune system plays a key role in gut homeostasis whereas during periods of altered microbiota it can be a direct cause of chronic inflammation (88). In addition, T<sub>1</sub> and T<sub>17</sub> cells contribute to the protective responses to pathogens during physiological conditions, whereas excessive activation of these cells is responsible for intestinal inflammation. Further studies to characterize the innate/adaptive immune mechanisms implicated in gut homeostasis and intestinal inflammation may thus promote advances in diagnostic and therapeutic approaches such as the development of biomarkers, biologics and reactive low-molecular-weight compounds to treat IBD.

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