Published in final edited form as:

Curr Opin Immunol. 2010 February ; 22(1): 63–72. doi:10.1016/j.coi.2010.01.008.

Microbial control of regulatory and effector T cell responses in the gut

Timothy Hand and Yasmine Belkaid

NIAID, National Institutes of Health, Laboratory of Parasitic Diseases, 4 Center Drive, Bethesda MD 20892

Keywords

Commensal; symbiont; pathogen; Foxp3; Th17; Th1

The human intestine harbors and is in constant contact with 1000 trillion microbes, composed of an estimated 15,000 strains¹. Recent studies have changed our perspective of commensal microbes from benign but inert passengers, to active participants in the processing of food into useful metabolic components, the post-natal development of mucosal and systemic immunity, and in its long-term steady-state function. Although mucosal surfaces have to constitutively integrate a multitude of microbial derived signals, new evidence suggests that defined bacteria or microbial products can play a dominant role in the induction of distinct class of immune responses. In this review we will focus on recent findings associating microbes that colonize or invade the gut, specialized mucosal DCs, and induction of effector or regulatory response in the GI tract.

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

As the intestine is constantly exposed to antigens derived from food and commensal bacteria, a commonly held preconception is that tolerance and the induction of regulatory responses constitute the default response to antigens presented in this environment. Nevertheless, this view has to be adjusted as it is becoming clear that a certain degree of constitutive effector response and inflammation is beneficial for the host, not only to maintain integrity of the tissue but also to allow the host to develop protective responses when required. This implies that the steady state regulation of this environment relies on the maintenance of a balance of antagonistic signals allowing the induction and maintenance of various classes of effector lymphocytes. Indeed, at steady state, the gut is home to a large number of lymphocytes that have the capacity to produce regulatory (e.g. IL-10 or TGF- β) and/ or effector cytokines (e.g. IL-17 and IFN- γ ^{2,3}). This constitutive production of cytokines is tightly controlled by the flora as germ-free mice show extensive deficiencies in intestinal immune system development and basal cytokine production^{2,4}. In the absence of flora, the CD4⁺ T cell population is diminished, disproportionately affecting Th1 and Th17 cells, while T_{reg} frequencies are maintained or increased². Colonization of germ free mice with complex microbiota

Correspondence: Y Belkaid: ybelkaid@niaid.nih.gov.

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orchestrates a broad spectrum of T helper (Th1, Th17) and regulatory T cell responses⁵. Some of the factors that govern the induction of constitutive effector responses in the GI tract and how such conditioning affect subsequent effector responses against pathogens will be discussed in this review

Steady state and induction of regulatory responses

The intestinal mucosa contains a large number of antigen presenting cells whose complexity has only begun to be addressed⁶. Recent studies demonstrated that in the lamina propria two main subsets of DCs can be distinguished on the basis of the expression of CD103 or CX3CR1^{7–9}. CD103⁺DCs are shorter-lived and capable of efficiently presenting antigen to naive T cells, in part due to their increased ability to traffic to draining lymph nodes, while CX3CR1⁺ DCs are longer-lived, tissue resident and poor antigen presenting cells⁹. This apparent functional dichotomy can be also traced to their distinct origin. Indeed, DCs expressing CD103 arise from macrophage–DC precursors through a Flt-3 ligand growth factor mediated pathway, while CX3CR1⁺ Lp DCs derived from grafted Ly6C^{hi} monocytes under the control of GM-CSF^{7, 8}. Using infectious agents and colitis models, several groups reported that in the gut, inflammation and IL-17 induction is usually associated with DCs that do not express CD103^{7, 8, 10}. For instance, colitis can be induced by TNF- α secreting CX3CR1⁺ Lp DCs and an increased frequency of these cells can lead to intestinal inflammation⁷. On the other hand, under steady state conditions, the gut-associated lymphoid tissue is a preferential site for the peripheral induction of regulatory T cells^{11–14} suggesting that the pro-inflammatory nature of CX3CR1⁺DCs is counterbalanced by other subsets of DCs. Recent evidence suggests that CD103⁺DCs are playing a major role in the maintenance of this equilibrium. One of the specific features of CD103⁺DCs is their capacity to metabolize the vitamin A metabolite RA, previously shown to imprint gut homing receptors on lymphocytes^{15–18} and promote IgA secretion by gut activated B cells¹⁷. Another effect of RA on the immune regulation of the GI tract is associated with its capacity to enhance the TGF- β mediated generation of Foxp3⁺ T_{reg} cells^{11–13, 19–23}. Of note, other cells, including epithelial cells and MLN stromal cells can express enzymes associated with vitamin A metabolism^{24,25} suggesting that CD103- APCs may acquire RA from alternative sources and store it²³. A recent report demonstrated that stimulation of DCs via TLR2 induced vitamin A metabolizing enzymes, suggesting that these enzymes may be tightly controlled by interaction with microbes²⁶. In addition to inducing Foxp3 expression by T cells, RA can conversely inhibit the generation of Th-17^{13, 19, 21, 22} suggesting that RA may play an important role in maintaining the balance between effector and regulatory populations in the GI tract. The actions of RA are mediated by nuclear receptors which are ligand-inducible transcriptional regulators belonging to two distinct families: RAR and RXR. Each family consists of the three genetic isotypes (RAR- or RXR α , β and γ). Foxp3 induction was abolished in RAR α deficient T cells²⁷ and conversely, these cells produced elevated levels of both IL-17 and IFN- γ . At least two mechanisms have been identified by which RA can enhance the capacity of TGF- β to induce T_{reg} cells. One of these mechanisms is the capacity of RA to suppress the production of effector cytokines that interfere with Foxp3 expression^{27–29}. Additionally, RA can also act directly on naïve T cells to favor TGF- β mediated Foxp3⁺ T_{reg} cell induction³⁰. Despite the recent identification of these LpDC subsets, the current characterization of mucosal APC-T cell interaction remains an oversimplification. That there is no clear consensus on the nomenclature or gating strategies used by individual groups precludes major synthesis. In addition, the composition and function of gut APCs is likely to be affected by inflammation as well as their location along the length of the intestine, as will be discussed in more detail below.

Negative control of T_{reg} induction by microbes

It is becoming clear that activation status as well as inflammatory mediators modulate the capacity of DCs to induce T_{reg} cells *de novo*^{85–90}. For instance IL-6 and TGF- β in tandem can direct the production of IL-17 secreting T cells over T_{reg} cells^{85, 86} and Th1 and Th2 effector cytokines have an antagonistic effect on T_{reg} cell conversion²⁹. In addition, activated DCs or strong costimulation limit the induction of Foxp3⁺ T_{reg} cell in favor of the induction of effector responses^{20, 91, 92}. When activated with commensal derived DNA, LpDCs produce large amount of IL-6 and consequently are impaired in their capacity to induce T_{reg}³. Notably, other representative bacterial TLR ligands including 2 and 4 did not markedly affect LpDC induced T_{reg} cell conversion *in vitro* consistent with the regulatory properties of TLR4 and 5 in this environment^{65, 67, 70}. In absence of commensal DNA-derived signal such as in mice deficient in TLR9, T_{reg} cell frequencies are dramatically increased within intestinal effector sites⁷⁷. This observation suggests that via TLR9, commensals can constrain the constitutive generation of T_{reg} cells in the GI tract and contribute to the maintenance of the tonicity of this environment

Because inflammatory signals can interfere with T_{reg} cell induction, acute infections in the GI tract are expected to prevent expression of Foxp3. Indeed, the constitutive induction of T_{reg} following exposure to oral antigens is inhibited after oral infection with the protozoan *T. gondii*⁹³. Upon infectious challenge, the gut-resident APCs are replaced by inflammatory cells that have not been conditioned by the gut environment⁹⁴. Indeed, LpDCs from infected mice are less efficient at inducing T_{reg} cells *in vitro* via their capacity to induce IFN- γ producing T_{eff}⁹³. Similarly, gut DCs exposed to Toxoplasma antigen *in vitro* are impaired in their capacity to induce T_{reg} cells demonstrating that tolerogenic DCs can lose their capacity to induce T_{reg} cells when activated by defined commensal products (Bacterial DNA) or pathogens, in favor of the induction of effector cells⁹³. Previous reports examining both gut and lung inflammation support the idea that restricted or defective T_{reg} cell conversion can enhance immunopathology^{95, 96} supporting the idea that interference with constitutive T_{reg} induction during infection may directly contribute to the pathological process.

Contextual roles of commensals on host homeostasis

There is clear evidence that some dietary microorganisms, termed probiotics, can play a beneficial role in the health of their host³¹. Promising results have been obtained with probiotics in the treatment of human inflammatory diseases of the intestine and in the prevention and treatment of atopic eczema in neonates and infants³². There can be exquisite specificity in the effects of individual commensal species; for example, within the genus *Lactobacillus* different species are associated with differing *in vivo* allergic status in infants³³ and contrasting capacity to induce regulatory cytokines *in vitro*³⁴. Moreover, different strains or mutants of particular *Lactobacillus* species stimulated very different immunological outcomes in mice^{35, 36}.

Some of the effects of probiotic treatment appear to be associated with an increase in the frequency of Foxp3⁺T_{reg} cells as well as induction of lymphocytes (expressing Foxp3 or not) producing regulatory cytokines. For instance, in mice, probiotic *Lactobacillus* and/or *Bifidobacterium* treatment suppressed TNBS-induced colitis^{37, 38}, as well as allergic responses via induction of TGF- β ³⁹ and activation of T_{reg} cells⁴⁰. Similarly, treatment of colitic mice with the probiotic cocktail VSL3 increased TGF and IL-10 producing T cells⁴¹. In a model of pathogen-induced inflammation, treatment of mice with *Bifidobacteria infantis* activated T_{reg} cells that could inhibit inflammation and induced activation of NF- κ B in recipient mice⁴². Some of the regulatory effects of probiotics are likely to occur via modulation of DCs^{34, 43} but could also arise as a consequence of their interaction with

epithelial cells. Indeed, some commensal-derived products can decrease tight junction permeability⁴⁴ and control the induction of antimicrobial peptides^{45, 46}.

On the other hand, the flora itself can become a liability as the breakdown of tolerance to commensal bacteria can contribute to the progression of IBD. Inflammatory responses in human and experimental IBD are directed toward certain subsets of commensal organisms that have pathogenic potential, such as *Helicobacter*, *Clostridium* and *Enterococcus* species, with some microorganisms, such as *E. coli*, reported to be strongly associated with Crohn's disease⁴⁷. Furthermore, lines of evidence suggest that perturbations in gut flora alone, can lead to gastrointestinal dysfunction⁵⁰. However these bacteria often can peacefully coexist in their host as part of the constitutive flora and their capacity to trigger or contribute to these disorders is partly controlled by host genetic factors. Indeed, genome wide association studies identified disease-associated mutation in genes that are involved in sensing of bacteria (*NOD2*)⁴⁸ and control of innate responses (*IL23R*)⁴⁹.

That gut inflammation is triggered by a combination of dysregulated microbiota and host genetic factors is best illustrated by a recent work looking at the role of the transcription factor Tbet in innate immune responses. In the absence of T-bet, *RAG2*^{-/-} mice (TRUC) spontaneously develop a juvenile ulcerative colitis resulting from pro-inflammatory response to commensal microbiota⁵¹. The mechanism underlying this pathological process appears to be two fold. First, TRUC mice display enhanced TNF- α production. Secondly, these mice harbor a colitogenic flora that can be transmitted to T-bet competent mice⁵¹. T-bet deficiency by DCs can also lead to the spontaneous development of colorectal cancer dependent on inflammation responses to commensal bacteria in a MyD88 independent fashion⁵². The association between chronic *Helicobacter pylori* and gastric cancer constitute the best documented example of microbe-associated inflammation as trigger of carcinogenesis⁵³. More recently, the human commensal *Enterotoxigenic Bacteroides fragillis* (ETBF) has also been shown to trigger colonic tumors in the multiple intestinal neoplasia (Min) strain of mice via induction of IL-17⁵⁴.

Furthermore, there can be significant interactions between commensals and pathogens. For example, a shift in bacterial composition can aggravate the immunopathology caused by toxoplasmosis⁵⁵. During infection with *T. gondii*, there is reduced floral complexity, either because of relative loss of more "regulatory" strains, or simply as a broad reflection of an altered homeostasis accompanying pathogenesis. Disruption of the microbiota composition has been also documented in the context of other enteric infections such as *Citrobacter rodentium* or *Salmonella typhimurium*^{56, 57}. Understanding how shifts in the microbiota induced by pathogens contributes to both pathology and protective responses in the GI tract need to be further explored.

Role of *Bacteroides fragillis* and Polysaccharide A in gut homeostasis

Whereas most individual bacteria failed to efficiently stimulate intestinal T cell responses, a restricted number of individual bacteria can control the tonicity of the gut immune system⁵. The first demonstration that a single commensal molecule could promote beneficial immune responses was provided by the identification of the polysaccharide A (PSA). PSA is produced in large quantities by a prominent human symbiont *Bacteroides fragillis*⁵⁸. Colonization of germ free mice with *B. fragillis* or treatment with purified PSA is sufficient to restore most of the defects observed in the development the immune system⁵⁸. Furthermore, via PSA expression, *Bacteroides fragillis* can protect mice from experimental colitis induced by *Helicobacter hepaticus*, a commensal bacterium with pathogenic potential⁵⁹. This protective activity is associated with the capacity of PSA to induce or expand IL-10 producing CD4⁺T cells⁵⁹. Hence, immunoregulation may revolve around highly specific host-microbial

molecular interactions, presumably reflecting a long and intimate co-evolution of their symbiotic relationship.

Control of IL-17 production by Segmented Filamentous Bacteria

A further demonstration of the impact of specific commensal organisms on immune system development and skewing was illustrated by the observation that mice lacking a key group of microbes (since identified as Segmented Filamentous Bacteria (SFB)) were unable to mount a Th17 response in the small intestine. In addition, the T_{reg} cell compartment of these mice was expanded^{25, 60}. When administered to mice in a defined bacterial cocktail, SFB was sufficient to induce intestinal inflammation in SCID mice reconstituted with CD45RB^{high}CD4⁺ T cells⁶¹. As shown by scanning electron microscopy, SFB adhere tightly to Peyer's Patches of the small intestine and concomitantly induce local expression of IFN- γ , IL-10 and IL-17. Compared to germ free mice, SFB colonization increased IFN- γ producing CD4 cells, IL-17 producing cells and Foxp3⁺ T_{regs} in the small intestine and colon. However, absolute numbers of these cells were not restored to the level those of mice with a complete microbiota suggesting that as expected, SFB may be sufficient for such changes but need to synergize with other microorganisms to coordinate the full maturation of the intestinal immune system.

Role of TLRs in the control of GALT T_{reg}/T_{eff} responses

Paradoxically, commensal and pathogenic microbes interact with the host immune system through conserved ligands that are cardinal features of microorganisms⁶². Many of these ligands signal through the Toll-like family of receptors (TLR)⁶². TLRs are widely expressed by cells of hematopoietic origin, as well as non-hematopoietic cells, including the epithelial cells lining the intestinal tract⁶³. The initial identification of TLRs in the splenic and peripheral blood leukocyte compartments paved our understanding of how engagement of these molecules by invasive pathogens can activate inflammatory cascades that initiate adaptive immunity⁶⁴. However, sites colonized by commensals differ in that they are in constant contact with TLR ligands. Yet, the response to TLR signaling by the commensal flora has only recently begun to be elucidated. For example, it is now clear that TLR signaling in the intestinal epithelial compartment is crucially involved in the maintenance of intestinal homeostasis and tissue repair⁶⁵. Commensal floral interactions with TLRs have also been shown to mediate tolerance to food antigens⁶⁷. Furthermore, these signals also positively regulate the sampling of luminal contents by DCs from the underlying lamina propria compartment⁶⁶.

Role of Flagellin in the control of gut immunity

Bacterial flagellin is a structural protein that forms the main portion of flagella and promotes bacterial chemotaxis, adhesion and invasion of host tissue. TLR5 recognizes the conserved domain of flagellin monomers. Unlike other TLR family members, TLR5 is not expressed on macrophages or conventional DCs in mice and poorly expressed by intestinal epithelial cells. Instead TLR5 is highly expressed in lamina propria DCs from the small intestine and in particular on CD11c^{hi}CD11b^{hi} dendritic cells (containing both CX3CR1 and CD103⁺DCs)^{68, 69}. Flagellin stimulated LpDCs do not produce IL-10 and TNF α but instead express chemokines, prostaglandin and antimicrobial peptides⁶⁸. In addition, LpDCs stimulated with flagellin produce both IL-6 and IL-12⁶⁸. Whereas DCs from non-GALT tissues induce Th1 cells in response to TLR ligands, CD11c^{hi}CD11b^{hi} LpDCs induce ROR γ T functional Th17 cells as well as Th1 cells from naïve CD4⁺T cells in response to flagellin *in vitro* and *in vivo*⁶⁹. Recent studies have shown that RA negatively regulates Th17 cell differentiation induced by IL-6 plus TGF- β in a dose dependent manner¹³. However, LpDCs and in particular the CD11c^{hi}CD11b^{hi} subset of LpDCs, can specifically induce Th17 in the context of TLR5

stimulation, despite their expression of enzymes associated with the generation of RA, ⁶⁹. Interestingly the effect of RA on DC mediated Th17 differs according to its concentration. At high concentration, RA inhibits both Th1 and Th17 while at low concentration RA clearly favor Th17 induction while limiting Th1 responses ⁶⁹. Such a role for RA in promoting Th17 is highly compatible with the observation that the GALT that is naturally exposed to RA, contains a high number of resident IL-17 producing cells. Thus, such interaction may be critical to trigger effector responses to pathogenic flagellated bacteria. Intriguingly, some commensal bacteria also have flagella, suggesting that they may have evolved mechanisms to escape TLR5-mediated host detection in the intestine. On the other hand, even in absence of pathogens, TLR5 ligands are clearly sensed as evidenced by the observation that mice lacking TLR5 spontaneously develop colitis ⁷⁰. Furthermore, mice lacking TLR5 over-express genes associated with innate and adaptive immunity contributing not only to colitis development but also to their enhanced protection against infection to enteric pathogens such as Salmonella ⁷¹. Recent studies identified that in the context of Crohn's Disease, flagellins are immunodominant antigens of the microbiota ⁷². An explanation of how the mucosal immune system prevents harmful responses against flagellin was provided by a study demonstrating that intestinal IgA can regulate the activation of peripheral flagellin-specific CD4⁺T cells⁷³. Importantly, T_{reg} cells control such antigen IgA B cell responses in an antigen specific manner via production of TGF- β . This study uncovered a new role for T_{reg} as a major helper T cell for the induction and maintenance of intestinal IgA B cell responses and prevention of responses against major flora antigen under steady state condition ⁷³.

Role of commensal derived DNA

TLR9 recognizes unmethylated cytosine phosphate guanosine (CpG) dinucleotides, which are abundant in prokaryotic DNA found in intestinal flora. Using synthesized sequences containing CpG, it has been shown that engagement of TLR9 expressed on DCs, T_{reg} and conventional T cells can limit T_{reg} cell suppressive function ^{74, 75}. Previous work identified an association between Crohn's disease and a promoter polymorphism in the TLR9 gene in humans ⁷⁶. Such an association supports the idea of a role for gut floral DNA (gfDNA) sensing in the pathophysiology of inflammatory bowel diseases (IBD). In mice, gut flora derived DNA (gfDNA) plays a major role in intestinal homeostasis through toll like receptor 9 (TLR9) engagement ⁷⁷ and constitutive interaction between gfDNA and TLR9 in the gut can act as an immunological adjuvant and critically controls the balance between T_{reg} and T_{eff} ⁷⁷. Naïve TLR9 deficient mice displayed a striking increase in the frequency of Foxp3⁺ T_{reg} within intestinal effector sites, accompanied by a significant reduction in constitutive IL-17 and IFN- γ production by T_{eff} cells. Complementing this, gfDNA, strongly constrained the capacity of lamina propria DCs to induce T_{reg} conversion *in vitro*. Further, T_{reg}/T_{eff} disequilibrium in TLR9 deficient mice led to impaired immune responses to oral infection with microsporidia and to oral vaccination, which could be rescued through neutralization of T_{reg} ⁷⁷. Other studies have shown that *in vitro*, gut flora bacteria are not all equal in their capacity to stimulate TLR9 and do so with varying levels of efficiency that correlate with the frequency of [CG] dinucleotides ^{77, 78}. Thus, it is tempting to speculate that alteration of T_{reg} cell homeostasis mediated by TLR9 signaling may be differentially regulated by specific gut flora species.

Role of commensal derived ATP

Adenosine 5'-triphosphate (ATP), can modulate immune cell function by means of activation of the ATP sensor P2X and P2Y receptors. Commensal bacteria have been shown to generate large amounts of ATP ⁷⁹. Consistent with this observation, germ free animals have reduced ATP in their feces compared to conventional mice. ATP derived from commensal bacteria can activate a subset of lamina propria cells, CD70_{high}CD11c_{low} cells, leading to the differentiation of Th17 cells⁷⁹. Systemic or rectal administration of ATP into these germ-free mice results in

a marked increase in the number of lamina propria Th-17 cells. A CD70^{high}CD11c^{low} subset of the lamina propria cells expresses IL-6, IL-23p19 and TGF- β -activating integrin- α V and - β 8, in response to ATP stimulation, and preferentially induces Th17 differentiation. The critical role of ATP is further underscored by the observation that administration of ATP exacerbates a T-cell-mediated colitis model with enhanced Th17 differentiation⁷⁹. This data provide the first evidence that a commensal-derived metabolite could direct a specific immune response.

Role of short-chain fatty acid (SCFAs)

Short chain fatty acids (SCFA) are produced by colonic bacteria after fermentation of dietary fibers. The type of SCFA produced depends on the metabolizing bacteria, with Bacteroides producing high level of acetate and propionate while firmicutes produce high amount of butyrate⁸⁰. During the onset of colitis the amount of SCFA is significantly reduced⁸¹ and increased intake of either dietary fibers or SCFA is clinically beneficial in the treatment of various forms of colitis^{82, 83}. A recent study demonstrated that interaction between SCFA and its receptor the G-protein coupled receptor 43 (GPR43) was required to control inflammatory responses during experimental colitis or asthma⁸⁴. Evidence also suggests that expression of GPR43 is associated with the expression of other innate receptors such as TLRs or C5aR in the GI tract⁸⁴. Thus SCFA provides another example of a link between dietary elements and control of gut homeostasis.

Systemic effect of mucosal microbial populations

One consequence of the immune system's reliance on microflora for optimal immunoregulation, is that alteration of microbial communities may result in unintended activation of immune effector mechanisms. Such alteration in microbial communities can arise as a consequence of exogenous factors including urbanization, global travel, dietary changes⁹⁷ and perhaps most importantly, in response to antibiotic treatment. Recent evidence suggests that acute antibiotic treatment has dramatic consequences on the taxonomic richness and diversity of the commensal microbial community that can last for an extended period of time^{98, 99}. In experimental models, antibiotic associated disturbance of the microbiota disrupts not only local responses to food antigen or infection^{67, 100, 77} but has also consequences on systemic responses. Indeed, treatment of mice with antibiotics led to a reduction in the incidence of experiment autoimmune encephalitis (EAE)¹⁰¹. As well, diabetes in the Non-Obese Diabetes (NOD) mouse model has been related to their housing conditions and presumably their commensal flora. When NOD mice were made deficient for MyD88, induction of disease was delayed and connected with a divergent commensal flora compared to MyD88 intact controls¹⁰². The long-term consequences of such perturbations for the human-microbial symbiosis are more difficult to discern, but chronic conditions such as asthma and atopic disease have been associated with childhood antibiotic use and an altered intestinal microbiota^{104–106}. Therefore the role of microbes or microbe metabolites in shaping regulation of innate and adaptive response extends beyond mucosal sites to provide systemic control of host homeostasis.

If changes in the commensal population impact upon systemic immune responses then it is not surprising to find that infection occurring in the same milieu, directly or via shift in microbial community, can also exert substantial systemic effects. The influence of infection on “bystander” or unrelated immune responses, provides a mechanistic explanation of the more general “hygiene hypothesis”. This hypothesis posits that increasing rates of allergy and asthma in Western countries could be the consequence of reduced infectious stresses during early childhood¹⁰⁷. Experimental work has lent strong support for this hypothesis. For example, during gastrointestinal infection, helminth-driven Treg cell suppression of effector functions protects against subsequent airway inflammation¹⁰⁸. Similar infections change responses to

blood-stage malaria¹⁰⁹ and interfere with vaccinations^{110, 111}. Evidence for bystander suppression in human GI helminth infection is also accumulating, with lower allergy rates in infected children^{112, 113}, and lower inflammatory responses to autoantigen during MS¹¹⁴. Indeed, helminth therapy is being tested as a potential strategy to ameliorate intestinal inflammation in Crohn's Disease and Ulcerative Colitis¹¹⁵. Notably, various suppressive cell types are observed in these infections, including "regulatory B cells" and alternatively activated macrophages, although the interdependence and sequence of activation of these regulatory components have yet to be discerned¹¹⁶. The presence of symbiotic and chronic infections in the GI tract could lead to the maintenance of a pool of cells with regulatory activities that would maintain host immune homeostasis and enhance the threshold required for immune activation. Over time, established GI infections may create a new homeostatic set point, in which reactivity to the chronic pathogen is minimized, with wider implications for responsiveness to self-antigens and allergens. At this point, it remains unclear to what extent any recalibration of host immunity is purely induced by the pathogen, or by perturbation of the commensal population, or is a result of endogenous controls within the immune system itself. On the basis of both human and experimental studies discussed above, it seems likely that all three components play an essential role in reaching a stable and nonpathogenic steady state for the longer term.

Acknowledgments

This work was supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

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Figure 1. Conditioning of T cell by commensals

The gut is colonized by a complex microbiota composed of microbes with potential beneficial (symbionts) or pathogenic effects for their host. This site is constantly conditioned by dietary elements such as vitamin A or fiber that can regulate the homeostasis of this site via their metabolites (respectively retinoic acid (RA) or short chain fatty acid (SCFA)). In the lamina propria or Peyer's patches, various subsets of DCs (e.g. for the lamina propria CD11c⁺CD103⁺ or CD11b⁺CD14⁺CXCR1⁺DC) can be found. These DCs can induce Foxp3⁺T_{reg} via their capacity to metabolizing and release RA (CD103⁺DCs) or induce various classes of effector responses following exposure to microbial derived products (e.g. Flagellin, Bacterial DNA) or metabolites (ATP).