

Published in final edited form as:

*Cell Host Microbe*. 2012 October 18; 12(4): 496–508. doi:10.1016/j.chom.2012.09.009.

## Intestinal commensal microbes as immune modulators

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### Abstract

Commensal bacteria are necessary for the development and maintenance of a healthy immune system. Harnessing the ability of microbiota to affect host immunity is considered an important therapeutic strategy for many mucosal and non-mucosal immune-related conditions, such as inflammatory bowel diseases (IBD), celiac disease, metabolic syndrome, diabetes and microbial infections. In addition to well-established immunostimulatory effects of the microbiota, the presence of individual mutualistic commensal bacteria with immunomodulatory effects has been described. These organisms are permanent members of the commensal microbiota and affect host immune homeostasis in specific ways. Identification of individual examples of such immunomodulatory commensals and understanding their mechanisms of interaction with the host will be invaluable in designing therapeutic strategies to reverse intestinal dysbiosis and recover immunological homeostasis.

### Introduction

Mucosal surfaces are colonized by a complex and dynamic microbial ecosystem termed “microbiota”. An astounding number and diversity of microbes, including fungi, bacteria, archaea, and viruses, are present in any given moment throughout our bodies. The vast majority of these organisms are not disease-causing invaders, but have made the host their one-and-only home. Through co-evolution, these “commensals” have established one of the most impressive examples of mutualistic relationship in the natural world, in which both microbes and their animal host depend on each other for optimal survival. Indeed, we rely on our microbiota for many basic physiologic and metabolic functions, as well as proper immune functions. Commensals provide immune protection in several ways. They can defend their mucosal home by directly combating invading pathogens or by mobilizing host anti-microbial immune defenses. They can also affect host immunity in a more inconspicuous, but equally important, way by directing the development of host immune cell subsets at steady state and therefore affecting mucosal and systemic innate or adaptive immune responses. Recent studies have identified examples of commensal bacterial species with such immunomodulatory roles. The existence of multiple members of the microbiota

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that affect host immune homeostasis in different ways means that differences in the composition of this community may contribute to individual differences in immune responses during infection, autoimmunity, cancer or other immunological conditions. Unveiling the underlying cellular and molecular mechanisms of each of these examples holds the promise to lead to exciting new ways for regulating mucosal immunity.

## Microbial-host interactions in the intestine

Decades of studies in germ-free (GF) animals have established the importance of microbiota for proper host immune function (Macpherson and Harris, 2004). GF animals were first created more than a century ago (Nuttal and Thierfeledr, 1895–1896) and long-term husbandry of GF rats has been possible since the 1940's (Reyniers et al., 1946). However, until recently, the composition of gut microbial communities remained largely unknown. Advances in high-throughput sequencing in the last few years have led to extensive cataloguing of the human microbiota (HMP, 2012). In addition to correlating changes in microbiota composition with disease, this has allowed for the identification of commensal species with specific immune effects. The vast majority of these studies have focused on the bacterial component of the microbiota, however characterization of the fungal and viral components and their function is under way (Iliev et al., 2012; Reyes et al., 2012).

The individual's microbiota composition is dynamic. It changes with age and fluctuates with environmental changes, such as geographical location, diet, antibiotic use, or influx and efflux of external microbes (Clemente et al., 2012). In addition, vastly different microbial communities reside in different parts of the body (Costello et al., 2009). Based on their colonization ability, bacteria in the gut can be transient or permanent. Transient bacteria represent microbes that are introduced during adult life from the external environment and do not permanently colonize the intestinal tract for different reasons, such as lack of appropriate adaptations for colonization or inability to compete with the resident microbiota. Many food-associated microbes, including pathogens and conventional commercial probiotics, are part of this category. Transient organisms can affect the immune system in different ways and be innocuous, pathogenic, or even beneficial, e.g. ingestion of probiotic-containing foods. These organisms have not co-evolved and therefore do not establish a mutualistic relationship with the host, but rather try to survive in the gut environment despite established host defenses. Indeed, many acute intestinal pathogens have developed strategies to forcefully colonize the intestine, which in most cases induces a strong immune response aimed at clearing the pathogen. The pathologic immunological consequences of this inflammatory response may persist for years after the clearance of the transient organisms as in post-infectious irritable bowel syndrome (Spiller and Garsed, 2009).

In contrast to transient bacteria, permanent bacteria are long-term members of the microbial community. Their colonization occurs in successive waves during ontogeny and they have developed evolutionary adaptations to establish a permanent relationship with the host. In most cases they have co-evolved with the host and are not normally found as free-living organisms. These are the true commensal bacteria. Commensals have multiple effects on the immune system of the host. On one hand the presence of a large number of "innocuous" bacteria has **immunostimulatory** effects. Commensals stimulate general recruitment of immune cells to the mucosa, as well as generation and maturation of organized gut-associated lymphoid tissues (Macpherson and Harris, 2004). They also stimulate protective epithelial cell functions, such as mucus and anti-microbial peptide secretion (Hooper and Macpherson, 2010). On the other hand, recent studies have identified the presence of commensal species with **immunomodulatory** effects. These effects are specific for individual bacteria or groups of bacteria, i.e. for specific components of the microbiota. They involve reversible changes in differentiation or effector function of host immune cell

subsets. In this way, microbiota composition can influence the type and robustness of host immune responses. Here, we refer to such permanent microbiota members with immunomodulatory effects as **autobionts**. In contrast to transient pathogens or pathobionts (see below), the immune effects of autobionts are more subtle because they do not cause any overt change in the health state of the host. Rather, they help maintain and regulate the host's healthy immune steady state. Permanent microbiota members that can demonstrate detrimental effects under special conditions are called **pathobionts**. Pathobionts colonize the host, but do not cause disease with a full complement of normal microbiota. However, they can expand and cause disease if microbiota or host immune homeostasis are perturbed (for example after antibiotic treatment or under conditions of intestinal inflammation). The immune effects and characteristics of different intestinal bacteria are summarized in Table 1.

In contrast to pathogens, pathobionts, and even probiotics, very little is known about the mechanisms by which autobionts exert their immunomodulatory effects. Until recently, one reason for this was the lack of specific examples of immunomodulatory commensals. Another reason is the difficulty in culturing these organisms *ex vivo* and the relative lack of genetic tools to study their genome function. In this review we focus on recently described examples of immunomodulatory commensals and speculate on potential cellular and molecular mechanisms involved in their interaction with the host and the establishment of a healthy steady immune state. Knowledge of these mechanisms can benefit the development of future therapies for intestinal diseases (Clemente et al., 2012).

## The intestinal microbial community influences immunity

During steady state conditions, the microbiota affects the development and function of various immune cell populations, including IgA-secreting plasma cells, Th17 cells, regulatory T (Treg) cells, invariant natural killer T (iNKT) cells,  $\gamma\delta$ T cells, NK cells, macrophages, dendritic cells (DCs), and innate lymphoid cells (ILCs) (Honda and Littman, 2012). For example, IgA<sup>+</sup> plasma cells in gut lymphoid tissues and lamina propria (LP) are greatly reduced in GF or antibiotics-treated conventional animals (Macpherson and Harris, 2004). In another example, the abundance and function of CD4<sup>+</sup> T cells expressing the FoxP3 transcription factor (Tregs) or interleukin (IL)-17 (Th17 cells) in the intestinal mucosa at steady state are affected by the microbiota. In GF or antibiotic-treated mice, the percentages of both Tregs and Th17 cells are markedly reduced, and expression of the immune suppressive cytokine IL-10 in Treg cells is severely reduced (Atarashi et al., 2008; Atarashi et al., 2011; Ivanov et al., 2008). These reductions are quickly restored by transplantation of intestinal or fecal microbiota from conventionally raised/specific pathogen free (SPF) mice. As described below, the development of these CD4<sup>+</sup> T cell subsets is differentially regulated by certain components of the intestinal microbiota including *Bacteroides fragilis*, Clostridia species, and segmented filamentous bacteria (SFB) (Atarashi et al., 2011; Ivanov et al., 2009; Round and Mazmanian, 2010). Th17 cells contribute to host defense against infection with pathogenic microbes but can also augment harmful autoinflammatory functions, whereas Tregs play critical roles in immune suppression. Therefore, under healthy conditions Th17 cells and Tregs should co-exist in a well-regulated balance. The particular combinations and relative abundances of the corresponding commensal species would generate distinct immune environments and immune responses in the host.

In addition to inducing development or recruitment of host immune cell subsets, microbiota may also affect the function of these subsets. For example, gut microbiota provides an environment not only for the accumulation of IgA<sup>+</sup> cells but also for the functional maturation of IgA<sup>+</sup> plasma cells by inducing the generation of iNOS<sup>+</sup> IgA<sup>+</sup> plasma cells (Fritz et al., 2012). iNOS<sup>+</sup> IgA<sup>+</sup> plasma cells are absent in GF mice, but present in

conventionally-raised mice. This subset plays critical roles in the enhancement of IgA<sup>+</sup> plasma cell development and the host defense against enteric pathogens, such as *Citrobacter rodentium* (Fritz et al., 2012). Commensal bacteria also affect NK cell function. Even though NK cell numbers are normal in GF mice, NK cell priming and anti-viral activity is deficient in the absence of microbiota (Ganal et al., 2012). This effect is due to microbiota-directed introduction of epigenetic changes and induction of type I Interferons from monocyctic macrophages, which are required for proper NK cell priming (Ganal et al., 2012).

Microbiota-controlled immune effects may also play role in regulation of the microbiota itself. Commensal-induced IgA<sup>+</sup> plasma cells contribute to controlling microbiota abundance and composition. For instance, mice carrying a knock-in mutation of activation-induced cytidine deaminase (AID) (AID<sup>G23S</sup>), which can mediate normal IgA class switching but cannot induce somatic hypermutation and high-affinity IgA responses, exhibit excessive proliferation of anaerobic bacteria in the small intestine (Wei et al., 2011). Similar overgrowth of microbiota was also reported in programmed cell death-1 (PD-1) deficient mice, which have increased follicular helper T (T<sub>FH</sub>) cells development, and therefore low-affinity IgA-producing plasma cell are aberrantly selected in germinal centers (Kawamoto et al., 2012). Thus, microbiota is required for the development of fully functional IgA<sup>+</sup> cells, which in turn function to maintain microbial homeostasis in the gut.

The microbiota also plays suppressive roles in immune cell function and accumulation in the gut. For example, it represses constitutive production of IL-22 in lymphoid tissue inducer (LTi) cells and NKp46<sup>+</sup> cells (both of which are ROR $\gamma$ <sup>+</sup> ILCs, see detailed review by Tait Wonjo and David Artis in this issue) through epithelial expression of IL-25 (Sawa et al., 2011). Another example is that early exposure to gut microbiota provides an epigenetic suppressive marking in the regulatory element of CXCL16 gene in the host, resulting in life long suppression of CXCL16 expression in gut and lung (Olszak et al., 2012). This suppression is accompanied by reduced abundance of iNKT cells in the colon and lung at steady state and affects host resistance to colitis and asthma (Olszak et al., 2012). Taken together, intestinal microbiota provides diverse signals for activation and suppression of the immune system, thereby having the ability to skew host immune status towards either effector or regulator dominance.

Commensals have immunomodulatory functions not only in the intestine. For example, resident commensals in the skin induce local Th17 and Th1 responses that are crucial in protection from bacterial infections (Naik et al., 2012). More importantly, these responses were compartmentalized to the skin and were independent of gut commensals (Naik et al., 2012).

Commensals influence pathogenesis of many diseases. Because this community is evolutionarily established to sustain healthy immune steady state, any major perturbations in its composition may have negative effects and perpetuate the cycle of chronic inflammation, allergy, or metabolic syndrome. Such, “dysbiosis” can be induced by diet, pharmacological agents, infection, inflammation, and host genetics (Honda and Littman, 2012). Once established, the dysbiotic microbiota may become stable and transplantable to GF or even conventionally raised animals. Indeed, cohousing of wild-type mice with disease-prone mutant mice, such as *Tbet*<sup>-/-</sup>*Rag*<sup>-/-</sup>, *Nrlp6*<sup>-/-</sup>, or *Asc*<sup>-/-</sup> mice, results in transfer of dysbiotic microbiota and predisposition of the wild-type mice to disease, including colitis and metabolic syndrome (Elinav et al., 2011; Garrett et al., 2007; Henao-Mejia et al., 2012).

Dysbiosis may lead to elimination of beneficial bacteria or outgrowth of pathobionts. Pathobionts are permanent members of the microbiota, present at low levels and innocuous under normal conditions. They can become pathogenic if allowed sufficient expansion due

to loss of microbiota or immune homeostasis. For instance, overgrowth of members of Prevotellaceae and TM7 has been implicated in host susceptibility to DSS colitis in *Nrlp6*<sup>-/-</sup> mice (Elinav et al., 2011). In *Tbet*<sup>-/-</sup>*Rag*<sup>-/-</sup> mice, *Proteus mirabilis* and *Klebsiella pneumoniae* were identified to, at least in part, be responsible for the phenotype of spontaneous colitis (Garrett et al., 2010). Most importantly, disease susceptibility was transferable to wild-type mice.

The composition of the gut microbiota is also altered by diet. Milk fat- and taurocholic acid-rich diets induced marked increase in *Bilophila wadsworthia* colonization, which is associated with enhancement of Th1 responses and acceleration of colitis development in *Il10*<sup>-/-</sup> mice (Devkota et al., 2012). It should be noted that depending on the host genotype, otherwise innocuous symbionts may become pathogenic. Indeed, in an inflammatory bowel disease (IBD) mouse model with deficiencies in IL-10 and TGF- $\beta$  signaling, *Bacteroides thetaiotaomicron*, a well-characterized symbiotic species, potently induces colitis (Bloom et al., 2011).

## Autobionts – mutualistic immunomodulatory microbes

As discussed above, there is abundant evidence that microbiota directs host immunity. Regulation of immune responses by influencing development, differentiation, or effector function of different cells of the immune system is especially interesting, because it results not simply from the presence of innocuous bacteria, but from the biological activity of commensals. In many cases these effects are functionally distinct, e.g. induction of Th17 cells vs Tregs, and depend on the activity of different members of the commensal community. The relative abundance of these “autobionts” can direct the general type of immunity in the host mucosa of an individual at a given time. Currently there are relatively few specific examples of commensals with immunomodulatory effects as discussed below. More are surely going to be identified in the future.

Despite considerable species diversity, the intestinal microbiota in most mammals consists of bacteria belonging to two major phyla – Gram-negative Bacteroidetes and Gram-positive Firmicutes. This probably reflects evolutionary adaptations of these phyla to survive in the gut environment and both of them contain important immunomodulatory commensals.

### **Bacteroides fragilis promotes Treg function**

*B. fragilis* was the first commensal to be implicated in affecting T helper cell balance by promoting Th1 development systemically (Mazmanian et al., 2005). However, further studies showed that *B. fragilis* also affects mucosal T cell homeostasis by promoting regulatory T cell function (Round and Mazmanian, 2010). *B. fragilis* is a Gram-negative member of the phylum Bacteroidetes. It is not a very abundant member of the gut microbiota, however the genus *Bacteroides* is well represented in human gut and has a superior ability to utilize the nutrients in the gut microenvironment (Flint et al., 2008). For example, the genome of the prototypical dominant commensal member of the class, *B. thetaiotaomicron*, contains several hundred proteins involved in harvesting and metabolizing of dietary polysaccharides (Sonnenburg et al., 2005). These are probably adaptations that have allowed *Bacteroides* to establish mutualistic relationship with the host, by 1) being able to flourish in the plant polysaccharide-enriched gut environment and 2) being able to provide biological “by-products” necessary for the well-being of the host. Different Bacteroidetes species produce different beneficial biological “by-products”, which may have helped establish them as permanent symbionts. For example, *B. thetaiotaomicron* is one of the major producers of short-chain fatty acids (SCFAs), which are necessary for proper host metabolic and immune functions. *B. fragilis* seems to have pronounced immunomodulatory functions. *B. fragilis* is not normally present in conventionally raised specific pathogen-free

(SPF) mice and colonization with *B. fragilis* protects mice from colitis in the T cell transfer and 2,4,6-trinitrobenzene sulfonic acid (TNBS) models (Mazmanian et al., 2008). This protection is due to the expansion of immune suppressive IL-10 producing Tregs by the bacteria (Round and Mazmanian, 2010). The introduction of *B. fragilis* as a permanent member of the microbiota also affects T cell homeostasis in the absence of inflammation. Colonization of SPF or GF mice with *B. fragilis* leads to an induction of IL-10 production by Foxp3<sup>+</sup> Tregs even at steady state (Round and Mazmanian, 2010). Thus, *B. fragilis* colonization modulates intestinal T cell homeostasis by boosting Treg function. This anti-inflammatory effect of the bacteria likely represents an evolutionary adaptation for establishing mutualism. Indeed, when Tregs were depleted, *B. fragilis* could not efficiently colonize host tissues (Round et al., 2011). Most importantly, the identification of *B. fragilis* as a modulator of Treg function allowed for investigation of the bacterial and host mechanisms involved. It was found that the anti-inflammatory effects of *B. fragilis* require the expression of bacterial capsular polysaccharide A (PSA). PSA-deficient *B. fragilis* mutants were incapable of inducing IL-10 production by Tregs and did not provide protection from colitis (Mazmanian et al., 2008; Round and Mazmanian, 2010). Instead, lack of PSA expression led to expansion of Th17 cells and loss of the mutualistic ability of *B. fragilis* to colonize host tissues (Round et al., 2011). Moreover, treatment of mice with purified PSA is sufficient to replicate the effects of the bacteria, including induction of IL-10 production by Tregs, suppression of Th17 cell production, protection in colitis models, and colonization of the host (Mazmanian et al., 2008; Round et al., 2011; Round and Mazmanian, 2010). These studies represent an elegant example of how investigating an immunomodulatory commensal and its effect on the host immune system can lead to elucidation of underlying molecular mechanisms and identification of clinically relevant immunomodulatory molecules.

#### Cluster IV and XIVa Clostridia induce Treg differentiation

Intestinal Clostridia are a heterogeneous group that forms the core of Firmicutes of the normal commensal microbiota. Clostridia are Gram-positive, rod-shaped, endospore-forming bacteria. They are a highly heterogeneous class that is composed of at least 19 clusters based on genomic similarity (Collins et al., 1994). The prototypical Clostridia from cluster I contain frequent environmental toxin-producing members, such as *Clostridium perfringens*, *C. difficile*, and *C. tetani*. These Clostridia may be present in the intestine, but usually as transient pathogens or, at best, pathobionts (e.g., *C. difficile*). In contrast, most commensal intestinal Clostridia are non-toxinogenic members of clusters XIVa and IV. They are typically described as fusiform-shaped bacteria and constitute 10–40% of the total microbiota (Frank et al., 2007). Cluster XIVa includes the genera *Clostridium*, *Eubacterium*, *Ruminococcus*, *Coprococcus*, and *Roseburia*. The cluster IV group includes species belonging to the *Clostridium*, *Faecalibacterium*, and *Ruminococcus* genera. Clostridia colonize the mucus layers in vicinity of the epithelium, in contrast to Bacteroidaceae, Enterococcaceae, and Lactobacillaceae, which colonize in regions of the central lumen, suggesting unique influences of Clostridia on host physiology (Nava and Stappenbeck, 2011). Indeed, the cluster XIVa Lachnospiraceae family is significantly less abundant in IBD patients compared to healthy subjects (Frank et al., 2007). Loss of mucosa-associated Clostridia and cluster IV Clostridia, particularly *Faecalibacterium prausnitzii*, is observed in IBD patients (Sokol et al., 2008). Although it remains unclear whether the decrease in Clostridia is a cause or effect of chronic inflammation, it is likely that maintenance of the Clostridia community is necessary to prevent IBD. In addition to the role in the intestinal (local) immune homeostasis, Clostridia also affect systemic immunity. Indeed, it has been shown that reduction of Clostridia clusters XIVa and IV by neonatal vancomycin treatment, promotes airway hypersensitivity in a mouse model (Russell et al., 2012). Furthermore,

decreased abundances of clusters IV and XIVa Clostridia have been associated with atopy during childhood (Candela et al., 2012).

The levels of Clostridia clusters IV and XIVa in adult mouse colon microbiota were found to correlate with the numbers of Tregs (Atarashi et al., 2011; Russell et al., 2012). Consistent with a role in colonic Treg induction, GF mice colonized with 46 strains of Clostridia clusters XIVa and IV showed accumulation of Treg cells in the colon (Atarashi et al., 2011). The 46 strains of Clostridia were originally isolated from the sporulating microbiota fraction of conventional mice based on their capacity to normalize the enlarged cecum in GF mice (Itoh and Mitsuoka, 1985). These Clostridia were found to induce near steady state numbers of colonic Tregs in GF mice, in contrast to other intestinal bacteria including Treg-associated autoantigens such as *B. fragilis* (Atarashi et al., 2011). Whereas most Treg cells in the colon of GF mice were Helios<sup>hi</sup>, which has been proposed as a marker for thymically derived Treg cells (Thornton et al., 2010), Treg cells in mice colonized with 46 strains of Clostridia clusters XIVa and IV were mostly Helios<sup>lo</sup> (Atarashi et al., 2011), suggesting that Clostridia robustly trigger peripheral differentiation of induced Treg (iTreg) cells. Consistent with these findings, 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 (Geuking et al., 2011). Treg cells in the intestine exhibit different characteristics from those in secondary lymphoid organs, and they express CD103, killer cell lectin-like receptor G1 (KLRG1), granzyme B (Gzmb), IL-10 and IL-35 (Feuerer et al., 2010). In particular, IL-10 plays an indispensable role in the suppression of aberrant activation of Th17 cells, myeloid cells, and  $\gamma\delta$ T cells in the intestine. Indeed, Treg-specific disruption of IL-10 results in severe colitis (Rubtsov et al., 2008), as does a Treg-specific deficiency of STAT3, which regulates many of the above-mentioned genes (Chaudhry et al., 2009). STAT3-deficient Tregs lack IL-10 expression and the mice develop spontaneous, Th17-mediated, commensal microbiota-dependent fetal colitis (Chaudhry et al., 2009). Colonization of GF mice with the 46 strains of Clostridia clusters XIVa and IV, induced not only increase in Treg numbers, but also high levels of IL-10 production (Atarashi et al., 2011). In humans, *F. prausnitzii*, which belongs to Clostridium cluster IV, increases IL-10 expression in peripheral blood mononuclear cells *in vitro* (Sokol et al., 2008). Therefore, autochthonous Clostridia constitutively induce accumulation and functional activation of Tregs in the colon and the relative abundance of Clostridia in the microbiota may strongly affect the immune status of the host.

### SFB induce Th17 cell differentiation

T cell homeostasis in the intestine can be defined as the balance between T cell subsets that promote immune responses and T cell subsets that subdue immune responses. IL-17-producing Th17 cells are pro-inflammatory CD4 T cells that contribute to disease pathogenesis in a number of chronic autoimmune inflammatory conditions, including IBD, multiple sclerosis, rheumatoid arthritis, psoriasis, and certain cancers. At the same time Th17 cells are crucial for efficient immune responses against mucosal pathogens, including viruses, bacteria, and fungi. Th17 cells differentiate from naïve T cells under the combined effects of TGF- $\beta$  and pro-inflammatory cytokines, such as IL-6, IL-23, and IL-1 $\beta$ . The latter group of cytokines is upregulated in secondary lymphoid tissues during certain infections, which instructs Th17 cell differentiation in this context. However, at steady state, in the absence of infection or overt inflammation, Th17 cells are highly enriched in the intestinal LP and are not present in secondary lymphoid tissues (Ivanov et al., 2006). In the gut, Th17 cells co-exist with Foxp3<sup>+</sup> Tregs. The two subsets share common developmental pathway and alternative differentiation fates. This includes shared dependence on TGF- $\beta$  and direct interaction and mutual functional inhibition of the two master transcriptional regulators, ROR $\gamma$ t (Th17 cells) and FoxP3 (Tregs) (Zhou et al., 2008). This overlap of cytokine and

transcriptional networks results in an elegant balance between two functionally opposing T cell subsets. Importantly, this balance is flexible and can be quickly reversed depending on the required immune response. One of the most important factors controlling the homeostasis between these two T cell subsets in the gut is the composition of intestinal microbiota. Th17 cells are not present in GF mice, but are induced upon colonization with the full complement of gut bacteria from SPF mice (Atarashi et al., 2008; Ivanov et al., 2008). In contrast, colonization with culturable intestinal isolates, including Treg-inducing commensals, such as *B. fragilis* or the mix of 46 Clostridia described above, does not lead to Th17 cell induction (Ivanov et al., 2009; Round et al., 2011), arguing that commensal microbiota contains unknown Th17 cell-inducing bacteria. This was further supported by the discovery that C57BL/6 mice from a colony at the Jackson Laboratory did not contain gut Th17 cells due to the lack of Th17 cell-inducing bacteria and total microbiota from these mice could not induce Th17 cells in GF animals, in contrast to microbiota from C57BL/6 mice from a colony at Taconic Farms (Ivanov et al., 2008). Comparison of the microbiota between these two colonies revealed that Taconic B6 mice are highly enriched in segmented filamentous bacteria (SFB), which are absent from Jackson B6 mice (Ivanov et al., 2009). Interestingly, mono-colonization of GF animals with SFB or introduction of SFB into Jackson B6 mice induces Th17 cell differentiation in the LP (Gaboriau-Routhiau et al., 2009; Ivanov et al., 2009), identifying SFB as a Th17 cell-inducing autobiont.

SFB are Gram-positive anaerobic bacteria that are known to permanently colonize the intestinal tract of many animal species. SFB or SFB-like bacteria have been described in invertebrates, such as termites and cockroaches, and in vertebrate animals such as fish, chickens, rabbits, mice, rats, cats, dogs, sheep, cows, pigs, zebras and monkeys (Klaasen et al., 1992). However, SFB have not yet been detected in humans (Sczesnak et al., 2011). SFB are members of the Firmicutes phylum and based on 16S rRNA sequence, were assigned to Clostridia (Snel et al., 1995). The similarity to Clostridia was later confirmed by the full SFB genomic sequence (Sczesnak et al., 2011). In fact, more than 60% of SFB ORFs are derived from Clostridial ORFs (Sczesnak et al., 2011). Despite this close similarity, SFB do not cluster with any of the sequenced Clostridial genomes. Moreover, the SFB genome is almost three times smaller than the average Clostridial genome. Such genome reduction is typical for obligate symbionts and therefore may be a mutualistic evolutionary adaptation to establish SFB as a permanent member of the gut microbiota.

Even though SFB induce Th17 cells, they do not cause any overt pathology, such as intestinal inflammation. It is unclear why SFB-induced Th17 cells do not induce inflammation. It could be due to control by Tregs and the opposing effects of Treg-inducing autobionts. However, SFB-monocolonized mice do not develop spontaneous colitis, which argues against suppressive effects of other bacteria. Another possibility is that either the levels or the effector function of SFB-induced Th17 cells are not sufficient for induction of inflammation. Indeed, naturally occurring intestinal Th17 cells do not cause colitis in the T cell transfer model of colitis in contrast to transfer of Th17 cells isolated from colitic mice (Ono et al., 2012). Small intestinal Th17 cells, presumably induced by microbiota, even had some regulatory activity in this model (Ono et al., 2012). Thus, Th17 cells induced by SFB may be qualitatively different from pathogenic Th17 cells induced during colitis. The lack of pathological changes in the face of induction of a “pro-inflammatory” T cell subset may reflect an evolutionary adaptation that has helped preserve SFB as part of the microbiota. This also suggests that SFB, SFB-induced Th17 cells, and other SFB immunomodulatory effects are beneficial for the host. Indeed, SFB colonization leads to improved protection against infections with intestinal pathogens, as shown for *Citrobacter rodentium* in mice and enteropathogenic *E. coli* in rabbits (Heczko et al., 2000; Ivanov et al., 2009). Th17 cells are important mediators of protection against intestinal infections, which could be the underlying mechanism of SFB-mediated protection. However, SFB have other more general



immunostimulatory effects, such as induction of IgA production, general CD4 T cell and IEL accumulation (Umesaki et al., 1995), and stimulation of anti-microbial peptide production from epithelial cells (Ivanov et al., 2009). Therefore, whether the protective effects of SFB are mediated by Th17 cells remains to be established. SFB are currently the only known commensal species, and hence the only autobiont, that can induce Th17 cells, even though indirect evidence suggests that other Th17 cell-inducing commensals may exist (Atarashi et al., 2008; Ivanov et al., 2009).

## Mechanisms of commensal immunomodulatory effects

As defined and discussed here, autobionts are commensal bacteria that modulate immune homeostasis by affecting the development, differentiation or effector function of different immune cell subsets. Whether these effects represent a natural immune response to the presence of the bacteria or a mutualistic adaptation has not been clearly established in all cases. However, the immune changes induced by autobionts possess several characteristics that distinguish them from conventional immune responses against pathogens or other commensals. Firstly, the response is unique for the bacteria. For example SFB is the only known commensal to induce Th17 cells. Secondly, the response itself is specialized and usually restricted to a certain immune cell subset. For example, the presence of commensal bacteria induces a general recruitment of all lymphoid subsets to the gut and maturation of secondary lymphoid structures. A pathogen invasion will induce a full cascade of pro-inflammatory immune responses, such as release of a battery of pro-inflammatory cytokines and infiltration of multiple immune cell subsets. In contrast, *B. fragilis* predominantly induces IL-10 production from Tregs, Clostridia from clusters IV and XIVa induce Foxp3<sup>+</sup> Tregs, and SFB induce specifically Th17 cells. Most importantly, the effects of autobionts are “subtle” and do not present as overt immune changes, such as immune deficiency or inflammation. Invasive intestinal pathogens also induce Th17 cells, but in contrast to SFB, this induction is part of the general tissue damaging inflammatory immune response with induction and infiltration of multiple inflammatory subsets. Thus, the effects of autobionts are fundamentally different from those of pathogens or innocuous commensals. Therefore, characterization of the molecular and cellular mechanisms underlying these effects is of particular interest and may be extremely relevant for the development of immunomodulatory therapies with low side effects.

## Modification of Pattern-Recognition Receptor (PRR) signaling

Toll-like receptors (TLRs) and Nod-like receptors (NLRs) are crucial innate immune receptors for the general detection of bacteria. They are also crucial for the establishment of intestinal homeostasis by the microbiota. Loss of TLR signaling negatively affects intestinal epithelial cell (IEC) regeneration after injury and aggravates intestinal inflammation (Rakoff-Nahoum et al., 2004). Ligation of TLRs by commensals may also inhibit immune activation by IECs through various mechanisms (Cerf-Bensussan and Gaboriau-Routhiau, 2010). Deficiencies in PRR signaling also control homeostasis at the level of microbiota composition. Indeed, genetic deficiencies of *Nod1*, *Nod2*, *Nlrp3*, and *Nlrp6* have all been shown to affect microbiota homeostasis, which leads to increased disease susceptibility (Bouskra et al., 2008; Elinav et al., 2011; Henao-Mejia et al., 2012; Petnicki-Ocwieja et al., 2009). Interestingly, all these studies involved perturbations of NLR signaling. In contrast, a recent comprehensive study demonstrated that loss of TLR signaling does not affect microbiota composition and that the distinct microbiota composition of TLR-deficient mice is due to housing isolation rather than defective innate immunity (Ubeda et al., 2012). Regardless of its effects on microbiota composition, TLR activation is likely to be involved in mediating immunomodulatory effects of commensals in general. The effects of *B. fragilis* and PSA on Th1 and Treg function require TLR2 signaling (Round et al., 2011). TLR2 expression seems to be required on Tregs themselves *in vitro*, although whether this is the

case *in vivo* remains to be investigated (Round et al., 2011). In contrast, induction of Tregs by the 46 strains of Clostridia occurred normally after colonization of GF *Myd88*<sup>-/-</sup> mice, which lack the signaling adaptor for several TLRs (Atarashi et al., 2011), although whether it is controlled by other PRRs has not been investigated. Whether TLR-signaling is involved in the induction of Th17 cells by SFB, is not completely clear. Th17 cells are still present in normal numbers in the LP of SPF *Myd88*<sup>-/-</sup> mice, as well as in *Myd88*<sup>-/-</sup> *Trif*<sup>-/-</sup> mice, which lack both TLR signaling adaptors and therefore all TLR signaling. or *Rip2*<sup>-/-</sup> mice which lack downstream Nod1/2-signaling (Atarashi et al., 2008; Ivanov et al., 2009; Ivanov et al., 2008). However, full genome sequencing of SFB has revealed that SFB harbor flagellar assembly proteins with flagellins capable of binding to TLR5 (Kuwahara et al., 2011). Moreover, there are reports that TLR signaling is important for intestinal Th17 cell generation. The numbers of Th17 cell-bound ROR $\gamma$ <sup>+</sup> CD4 T cells, as identified by GFP reporter expression, were decreased in the absence of MyD88 (Shaw et al., 2012). In support of this, *Tlr9*<sup>-/-</sup> mice have decreased numbers of LP Th17 cells (Hall et al., 2008). An additional complication is potential heterogeneity of responses through different TLRs mediated by different commensals. For example, it is possible that activation of some TLRs inhibits, while activation of other TLRs promotes Th17 cell differentiation. In this scenario, ablation of signaling through both activating and inhibitory TLRs in *Myd88*<sup>-/-</sup> mice will not lead to considerable changes in Th17 cell number. The precise role of PRRs in the Th17 cell inductive capacity of total microbiota and specific commensals, such as SFB, awaits controlled colonization experiments.

### Modification of LP immune cell function and antigen specificity

Autobionts are non-invasive members of the luminal microbiota, however they affect immune cell function in the LP and even systemically. In most cases, the affected host cells are not in direct contact with the bacteria. Rather, they interact with bacterial products or are affected by the activity of bacteria-detecting cells. The nature of the immune cells involved in inducing Th17 or Treg cells by commensals is an area of active investigation. Naïve T cells differentiate into effector cells upon appropriate activation by a cognate antigen in the context of an appropriate cytokine environment. Therefore, T cell modulating commensals, such as SFB and Clostridia, must specifically affect one, or both, of these processes. The specificity can be provided by induction of a unique cytokine environment, appropriate level of TCR stimulation by commensal-deriver antigens, or both. These signals can be transmitted to T cells by the activation of unique subsets of immune cells in the LP. Dendritic cells (DCs) are major modulators of T cell responses, because they can serve as potent antigen-presenting cells (APCs) and at the same time contribute to the local cytokine environment through detection of microbial substances by their PRRs. The intestinal LP harbors distinct subsets of intestinal DCs (iDCs) and several of them have been implicated in regulating intestinal T cell homeostasis and in particular the Th17/Treg balance (Bogunovic et al., 2012; Varol et al., 2010). iDCs can produce the major cytokines involved in Th17 and Treg differentiation, including IL-6, IL-23, and TGF- $\beta$ , and have been directly implicated in regulating T cell homeostasis (Swiatczak and Rescigno, 2012). Indeed iDCs have been shown to induce Treg and Th17 cell differentiation *in vitro*. CD103<sup>+</sup> iDCs induce Treg differentiation *in vitro* through the production of the vitamin A-derived nuclear hormone receptor agonist retinoic acid (RA) (Coombes et al., 2007; Sun et al., 2007). A number of different iDC subsets have been shown to induce Th17 cell differentiation *in vitro*, including CD70<sup>hi</sup>, TLR5<sup>+</sup>, and CD11b<sup>+</sup>CD103<sup>+</sup> DCs (Atarashi et al., 2008; Denning et al., 2011; Uematsu et al., 2008). However, the exact contribution of any of these iDC subsets, or even iDCs in general for Th17 or Treg induction *in vivo* is not completely clear. Probably the most direct data come from studies of DC-specific deletion of various molecules. DC-specific deletion of Notch2 leads to a specific loss of the CD103<sup>+</sup>CD11b<sup>+</sup> iDC subset with corresponding decrease in Th17, but not Treg, cell differentiation, which

seems to implicate this subset in Th17 rather than Treg induction (Lewis et al., 2011). iDCs express the integrin  $\alpha\text{v}\beta\text{8}$ , which is an important activator of intestinal TGF- $\beta$ , a cytokine crucial for both Th17 and Treg differentiation. DC-specific deletion of integrin  $\alpha\text{v}$  or  $\beta\text{8}$  leads to significant disruption in Treg and Th17 cell differentiation and development of colitis (Acharya et al., 2010; Lacy-Hulbert et al., 2007; Paidassi et al., 2011; Travis et al., 2007). In addition, DC-specific loss of the transcription factors T-bet and Stat3, the cytokine IL-10, and the Wnt signaling mediator  $\beta$ -catenin, all lead to disrupted Th17/Treg balance and chronic colitis (Garrett et al., 2007; Manicassamy et al., 2010; Melillo et al., 2010). Therefore, although a role for iDCs in regulating gut T cell homeostasis seems obvious, the exact contribution of different DC subsets *in vivo* will need to be examined further. In contrast, the role of iDCs in microbiota-mediated immune effects is not known. Even though CD103<sup>+</sup> iDCs express RA-producing enzymes and are capable of promoting Treg induction *in vitro*, whether the *in vivo* effects of autoantigens, such as Clostridia or *B. fragilis* are mediated through iDCs remains to be investigated. In the case of Th17 cells, SFB colonization or colonization with other Th17 cell-inducing bacteria stimulates production of Th17 cell-regulating cytokines, e.g. IL-23, IL-6, and TGF- $\beta$  from LP DCs (Atarashi et al., 2008; Ivanov et al., 2009). CD103<sup>+</sup>CD11b<sup>+</sup> DCs isolated from mice with Th17 cell-inducing microbiota, were the only gut DC subset capable of inducing Th17 cells *in vitro* (Denning et al., 2011) and this was dependent on microbiota effects, because the same cells isolated from Jackson B6 mice (that lack SFB and Th17 cells) were deficient in Th17 cell induction. The exact role of DCs in SFB-mediated induction of Th17 cells *in vivo* remains to be established.

LP DCs may mediate T cell induction by commensals in several ways. They may sample commensal-derived antigens, either by directly contacting the bacteria through extension of dendrites into the gut lumen (Rescigno et al., 2001), or by detecting bacterial products that gain access to the LP. They may then present these antigens to intestinal T cells to induce commensal-specific T cells. Alternatively, LP DCs may be conditioned by commensals indirectly, e.g. by cytokines produced by other cells in response to the bacteria. For example, SFB attachment may induce cytokine production by IECs that may modulate DC function. Indeed, SFB induce serum amyloid A (SAA) production from IECs, which in turn may stimulate DC production of IL-23 (He et al., 2006; Ivanov et al., 2009), a cytokine required for Th17 cell maintenance.

Another important question that will help shed light into autoantigen-specific mechanisms is: what are the antigen specificities of autoantigen-induced Th17 and Treg cells? If autoantigens provide antigens to induce autoantigen-specific T cells, identification of the APCs involved can help identify cells that receive signals from the commensal as well as how commensals or their antigens are detected by the immune system. At the same time, characterization of Treg or Th17 cell-inducing autoantigen-derived antigens will identify potential commensal genes involved in the process, which may help pave the path for design and development of microbial products as immunomodulatory therapeutics. On the other hand, if most autoantigen-induced T cells are not specific for the bacteria, autoantigens are mostly affecting cytokine environment in the gut. A recent study using high-throughput sequencing demonstrated that a large proportion of inducible colonic Tregs recognize commensal-derived antigens (Lathrop et al., 2011). It has also been shown that Foxp3<sup>+</sup> Tregs with TCRs that are specific for flagellins related to those of Clostridia cluster XIVa contribute to the induction of IgA<sup>+</sup> B cells in the intestine (Cong et al., 2009). Still, it is currently not known whether Tregs induced by commensal Clostridia are mostly specific for Clostridial antigens. In the case of Th17 cells, one study found that Th17 cells still develop in the gut of non-commensal TCR-transgenic (Tg) mice, which argues that presentation of commensal antigens is not required for Th17 cell induction (Lochner et al., 2011). Surprisingly, T cell activation and Th17 cell differentiation still occurred in the absence of cognate antigen

(Lochner et al., 2011), which may reflect potential cross-reactivity of the Tg TCRs to unknown endogenous or commensal-derived antigens. In contrast, in another study, transferred activated TCR Tg T cells did not differentiate into Th17 cells in the LP (Hand et al., 2012). More importantly, these studies were performed in mice with undefined commensal microbiota, which may contain multiple Th17 cell-inducing species, including pathobionts such as *Helicobacter spp* (Muller and Solnick, 2011). Therefore, the role of individual Th17 cell-inducing commensals, such as SFB, has not been investigated and whether they preferentially induce commensal-specific Th17 cells is not known.

### Modification of intestinal epithelial cell (IEC) function

IECs form a single cell barrier that separates the microbiota from host tissues and immune cells. IECs also represent a cell type to which both microbiota and immune cells have direct access and may, therefore, play a major role in transmitting microbiota's immunomodulatory signals. There are multiple examples of how commensal bacteria affect IEC function. Signals from microbiota are constantly being processed by IECs to maintain proper barrier function (Hooper and Macpherson, 2010; Rakoff-Nahoum et al., 2004). At the same time IEC produced cytokines are crucial in modulating LP immune responses (He et al., 2007; Saenz et al., 2008). Exactly how these two types of events are connected is not completely clear. However, it is logical to imagine a scenario where immunomodulatory commensals modify IEC function either by directly binding to IECs or by secreting substances that ligate IEC receptors on the apical side. Following IEC activation, IEC-derived cytokines will be secreted on the basolateral side to influence the activity of LP immune cells, such as DCs, that in turn will affect T cell homeostasis. Such sequence of events is highly likely especially for mucosa-associated auto-bionts. SFB are a prototypical example. SFB colonize the intestine of many animal species in the post-weaning period. The majority of microbiota are kept away from direct contact with the epithelium by a layer of mucus enriched in anti-microbial substances (Johansson et al., 2008; Vaishnava et al., 2011). SFB differ from most commensal in that they can penetrate the mucosal layer and adhere tightly to IECs in the terminal ileum as well as to follicle-associated epithelium (FAE) of Peyer's Patches (Klaasen et al., 1992). The host and bacterial molecules involved in this adherence are unknown. However the interaction resembles ligation of a surface receptor on the IEC apical site by a bacterial adhesin. Actin cytoskeletal reorganization is seen in the IEC at the point of contact (Jepson et al., 1993), which implies initiation of downstream signaling events and modification of IEC function by the adhering commensal. Indeed, SFB are known to affect gene-expression in IECs, including MHC class II expression, surface fucosylation, and anti-microbial peptide production (Ivanov et al., 2009; Umesaki et al., 1995). They also seem to induce cytokine-like molecules such as SAA, which can modify the function of LP cells (Ivanov et al., 2009). Whether adherence to IECs or any of these effects on IEC function are required for the Th17 cell-inductive capacity of SFB is not yet known. SFB interaction with IECs may also bring the bacteria in close proximity to sampling cells, such as M cells in PPs and intestinal villi or interdigitating DCs (Rescigno et al., 2001). In any case, the strong association of SFB with IECs is a unique feature of this commensal and is likely to be involved in its immunomodulatory effects. Whether other mucosa-associated commensals also have strong immunomodulatory effects remains to be investigated.

### Modification of host metabolic functions

Auto-bionts may influence the balance of intestinal immune cells by generating immunomodulatory metabolites. Microbiota is integral for the generation of dietary metabolites (Holmes et al., 2012) and plays major role in metabolic conditions, such as obesity (Turnbaugh et al., 2006), metabolic syndrome (Vijay-Kumar et al., 2010), and nonalcoholic fatty liver disease (Hena-Mejia et al., 2012). In addition to energy harvest,

microbial metabolites have profound effects on immune responses. A comparison of sera from GF and conventional mice by mass spectrometry revealed that the microbiota play critical roles in metabolizing tryptophan, phenylalanine and tyrosine, and producing tryptophan metabolites such as indoxyl sulfate and the antioxidant indole-3-propionic acid (IPA), and other organic acids containing phenyl groups, such as phenyl sulfate (Wikoff et al., 2009). Importantly, these aromatic hydrocarbons are known to influence the differentiation of T cells and ILCs (Qiu et al., 2012; Veldhoen et al., 2008). The aryl hydrocarbon receptor (AhR) is a receptor for tryptophan derivatives and has been implicated in immune regulation by tryptophan metabolites. AhR is expressed in differentiating Th17 cells, and AhR ligands upregulate IL-22 expression and boost Th17 cell differentiation (Veldhoen et al., 2008). Other reports have shown that AhR activation can result in control of pro-inflammatory responses and expansion of the Treg compartment (Gandhi et al., 2010; Quintana et al., 2008). Thus, the control of Th17 and Treg differentiation by AhR seems to be complex. AhR is also expressed by IL-22-producing ILCs and IELs, and *AhR*<sup>-/-</sup> mice have fewer IL-22-producing ILCs and IELs in the intestine (Li et al., 2011; Qiu et al., 2012). Tryptophan metabolism in general, may be important for T cell homeostasis. In this context, it is noteworthy that IECs from mice colonized with 46 strains of Clostridia express high levels of indoleamine 2,3-dioxygenase (IDO) (Atarashi et al., 2011), an enzyme that degrades tryptophan and has been implicated in Treg induction (Matteoli et al., 2010). Furthermore, it has been shown that uptake of dietary amino acids, including tryptophan, by IECs via the amino acid transporter B<sup>0</sup>AT1 complexed with angiotensin-converting enzyme 2 (ACE2) is essential for the production of antimicrobial peptides and control of gut microbial ecology (Hashimoto et al., 2012). Therefore, it will be interesting to address the involvement of amino acid metabolites in commensal-mediated immune modulation.

Bacteroidetes and Clostridia employ a wide variety of enzymes capable of degrading polysaccharides and oligosaccharides into short chain fatty acids (SCFAs), such as acetic acid, propionic acid, and butyric acid (Flint et al., 2008). SCFAs influence host physiology, including gut motility and colonocyte development. In addition, SCFAs contribute to maintenance of intestinal immune homeostasis. Indeed, butyrate metabolism is impaired in patients with ulcerative colitis, and topical sodium butyrate treatments or butyrate enemas are effective therapies (Scheppach et al., 1992; Vernia et al., 2003). GF mice are highly susceptible to dextran sulfate sodium (DSS)-mediated colitis, but treatment of GF mice with acetate in drinking water markedly improves colitis disease indices (Maslowski et al., 2009). SCFAs are known to act as extracellular signaling molecules that activate G-protein coupled receptors, such as GPR41 and GPR43. GPR41 is expressed by a subset of IECs and controls microbiota effects on energy harvest and obesity (Samuel et al., 2008). GPR43 is expressed by neutrophils and eosinophils, and transmits microbial-derived immunomodulatory signals. *Gpr43*<sup>-/-</sup> neutrophils have an intrinsic hyper-reactive phenotype, including hyper-production of reactive oxygen species (ROS), and high chemotactic activity (Maslowski et al., 2009). It is not known whether SCFA-signaling is involved in the induction of Th17 cells and Tregs by autoantigens. However, considering that Bacteroidetes and Firmicutes are high producers of SCFAs (Flint et al., 2008), SCFA-GPR signaling may represent an important molecular pathway activated by commensals to regulate immune and inflammatory responses and maintain the mucosal barrier.

## Therapeutical use of intestinal microbes or their products/effects

Alteration of the gut microbiota in humans via fecal transplants of stool from healthy donors has been effective in a large number of case studies of refractory *C. difficile* infection (Khoruts and Sadowsky, 2011). While fecal transplantation has established a proof of principle for the feasibility of manipulating human microbiota as a therapeutic strategy (Khoruts and Sadowsky, 2011), treatment with specific microorganisms is more desirable

for medical purposes. Many of the conventional probiotic microorganisms were traditionally used to preserve food products by fermentation and are not necessarily part of the normal microbiota (O'Toole and Cooney, 2008). In other words, no probiotic has been rationally isolated from the microbiota with the purpose of boosting specific arms of the host immune system or to fix a particular commensal dysbiosis. As described earlier, several lines of evidence suggest that dysbiotic microbiota can be a highly stable complex and therefore refractory to treatment with individual transient probiotic strains. Indeed, despite promising data from animal models, most probiotics tested to date have demonstrated, at best, mediocre effects in the clinic, particularly for treatment of IBD. Thus, there is compelling need to identify more robust therapeutic organisms that are compatible to the host and can affect the host immune system in a well-controlled, physiological fashion. Ideally, these organisms will be able to induce broader changes to the microbial ecosystem and have the ability to ameliorate dysbiosis (Figure 1). Such therapeutic organisms may include some traditional probiotics, but immunomodulatory autoantibiotics represent the most promising candidates. Autoantibiotics are capable of becoming permanent members of the microbiota and therefore may be able to restore dysbiosis and inhibit the growth of pathogens or pathobionts. Indeed, SFB colonization can reduce replication of enteropathogenic *E. coli* in rabbits (Heczko et al., 2000), *Salmonella enteritidis* in rats (Garland et al., 1982), and *Citrobacter rodentium* in mice (Ivanov et al., 2009). Colonization with indigenous Clostridia and Lactobacilli isolated from conventional mice was shown to effectively control *E. coli* expansion in the large intestine of mice (Itoh and Freter, 1989). At the same time, autoantibiotics have focused and independent effects on the immune system that are relatively more subtle and aimed at sustaining intestinal immune homeostasis. SFB and Clostridia induce controlled expansion of Th17 cells or Tregs independently of each other and even in GF mice, and the induced Th17 or Treg cells do not exceed the levels in SPF mice and do not cause overt inflammatory disease or immune deficiency. Therefore, the mechanisms controlling autoantibiotic-induced immune effects are more "physiological". Characterization of the cellular and molecular mechanisms responsible for the effects in each example of autoantibiotic-host interaction is crucial in designing therapies that will not rely on colonization with live bacteria, but instead use specific small molecules which mimic their beneficial effects. For example, even though SFB have not yet been found in human metagenomic databases (Sczesnak et al., 2011) the molecular and cellular mechanisms of SFB immune modulation may still be preserved and taken on by other commensals in humans. Characterization of such molecular mechanisms will allow for development of rationally designed combinations of targeting agents to boost the immune system in a desired way or correct effects of intestinal dysbiosis to treat various immune diseases.

## Acknowledgments

This work was supported by grants to K.H. from Japan Science and Technology Agency for CREST, the Japan Society for the Promotion of Science NEXT program, and the Waksman Foundation of Japan Inc., and by grants to I.I.I. from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the Crohn's and Colitis Foundation of America (CCFA).

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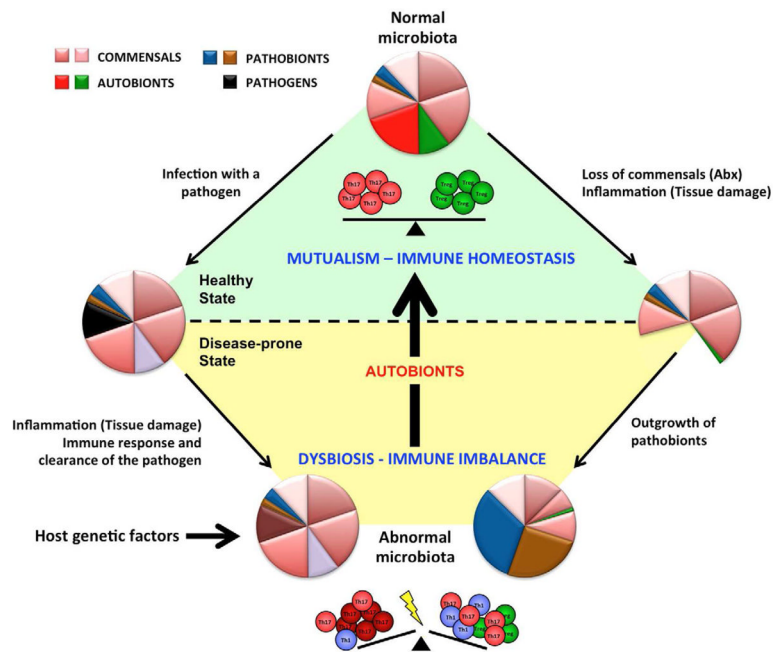


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**Figure 1. Mutualistic commensals with immunomodulatory effects sustain healthy immune homeostasis**

Autoions are permanent members of the normal commensal microbiota. They control immune homeostasis in the lamina propria by, for example, inducing different subsets of effector T cells (control of T cell homeostasis). The relative proportions of Th17 and Treg cells depend on the relative presence of different autoions, e.g. in different individuals, different intestinal locations, or at different stages in ontogeny. These mutualistic interactions sustain the healthy steady state. Loss of autoions and general dysbiosis perturbs also the immune balance of the host. Dysbiosis may occur in multiple ways. Invasive intestinal pathogens cause transient infections, but may lead to longterm perturbations of the microbiota due to strong inflammatory responses against the pathogen. Antibiotic (Abx) treatments or inflammation caused by physical damage to the mucosa may also lead to dysbiosis and the outgrowth of pathobionts, which are permanent members of the microbiota, but do not cause disease in the presence of autoions. Host genetic factors may also initiate or perpetuate dysbiosis. Dysbiosis leads to loss of the immunomodulatory effects of autoions and results in a perturbed immune balance, which under appropriate conditions may manifest itself in disease. The disease state augments dysbiosis in a vicious circle. Autoions and conventional probiotics are both microorganisms with beneficial effects. Probiotics have transient effects and can boost host immunity. Autoions are part of the normal microbiota and have developed evolutionary adaptations to colonize the host, regulate host immunity and establish a healthy immune state. Autoions can therefore reverse dysbiosis as well as immune homeostasis.

**Table 1**  
Non-pathogenic bacterial members of the intestinal microbiota with immune effects

Concept	Examples*	Association with Host	Immune Effects	Mechanisms*
Probiotics	<ul style="list-style-type: none"> <li>confer health benefit to the host when administered in adequate amount</li> <li>not necessarily part of the "normal microbiota"</li> <li>may affect beneficial microbiota (indirect effects)</li> </ul>	Transient	Innocuous Immunostimulatory	Cytokine induction, TLR activation, Pathobiont and pathogen suppression, Lactic acid Short-chain fatty acids
Autobionts	<ul style="list-style-type: none"> <li>direct influence on host immune cell homeostasis or function</li> <li>part of the "normal microbiota."</li> </ul>	Permanent Host-dependent Symbiotic	Immunomodulatory	Largely Unknown (TLR2, metabolites (?), antigens (?), effects on IEC function (?))
Pathobionts	<ul style="list-style-type: none"> <li>do not cause disease in the presence of normal microbiota in healthy host</li> <li>cause disease when microbiota or host immunity is perturbed</li> </ul>	Permanent Parasitic/Infectious	Innocuous Detrimental	Invasive mechanisms, Spore formation, Toxins

\* Examples and mechanistic studies have been performed in mice (with the exception of *F. prauznitsii* and *C. difficile*). See text for details and references. (?) Speculative mechanisms