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Coordination of tolerogenic immune responses by the commensal microbiota

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Abstract

All mammals are born ignorant to the existence of microorganisms. Soon after birth, however, every mammal begins a lifelong association with a multitude of microbes that lay residence on the skin, mouth, vaginal mucosa and gastrointestinal (GI) tract. Approximately 500-1000 different species of microbes have highly evolved to occupy these bodily niches, with the highest density and diversity occurring within the intestine [1]. These organisms play a vital role in mammalian nutrient breakdown and provide resistance to colonization by pathogenic microorganisms. More recently, however, studies have demonstrated that the microbiota can have a profound and long-lasting effect on the development of our immune system both inside and outside the intestine [2]. While our immune system has evolved to recognize and eradicate foreign entities, it tolerates the symbiotic microorganisms of the intestine. How and why this tolerance occurs has remained unclear. Here we present evidence that the commensal microbes of the intestine actively induce tolerant responses from the host that coordinate healthy immune responses. Potentially, disruption of this dialogue between the host and microbe can lead to the development of autoimmune diseases such as inflammatory bowel disease (IBD), rheumatoid arthritis (RA), or Type I diabetes (TID). As a wealth of publications have focused on the impact of the microbiota on intestinal immune responses and IBD, this chapter will focus on the extra-intestinal impacts of the microbiota from development to disease and integrate the known mechanisms by which the microbiota is able to actively communicate with its host to promote health.

Keywords

commensal microbiota; autoimmunity; tolerance

Acquisition of the microbiota

Mammalian mucosal surfaces represent a rich microbial habitat. An astounding 100 trillion of these microorganisms live within our gastrointestinal tract, with densities and diversity of microbes increasing from the stomach to the colon. Because these organisms outnumber host cells by an order of magnitude, many have proposed that mammals should be considered ‘super-organisms’ composed of both mammalian and microbial cells where the two are intimately linked for health [3].

From birth mammals begin to acquire a consortium of microorganisms from their environment, many of which will maintain lifelong relationships with their hosts. The identity of these microorganisms is only beginning to be understood by sequencing 16S ribosomal DNA from human fecal samples. A study performed by Relman and colleagues

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sampled 14 human infants, including a pair of di-zygotic twins, over the first year of life to lend insight into the temporal patterns and composition for the assembly of the microbiota [4]. The overall density of bacteria was relatively unstable throughout the first week of life and then persisted to about 10^9- 10^{10}/g of stool. The one infant delivered by caesarean section had lower densities of bacteria, suggesting that mode of delivery is important in acquisition of microbial organisms. The infants' microbial community varied significantly between individuals with the exception of the fraternal twins, who had a higher similarity in their temporal patterns of microbial acquisition. The earliest colonizers were aerobes such as *Staphylococcus* and *Streptococcus* species whereas anaerobic bacteria, such as *Eubacteria* and *Clostridia* appeared later. Additionally, the appearance and disappearance of several other taxa, including *Prevotella* and *Veillonella* was noted. Despite the extreme variation early in life, a profile characteristic to an adult microbiota type was seen by the end of the first year. Studies performed on three human adults identified 395 phylotypes at the strain level and a single Archaeal phylotype. The major species found include the genera *Bacteroides, Eubacterium, Clostridum* and *Ruminococcus* [5]. Approximately 80% of the sequences from these three individuals, represented species that have not yet been cultivated, demonstrating how little is known regarding the composition of the microbiota. As was reported with infants, analysis indicated that microbial composition was extremely variable between individual. Microbial diversity on the planet as a whole is quite diverse, with 55 divisions of Bacteria and 13 divisions of Archaea described and potentially many more to discover. Despite the astounding bacterial diversity of the outside world, the human intestine is dominated by only 2 divisions of bacteria, the Bacteroidetes and the Firmicutes and one member of the Archaea [6]. That such an exclusive group of microorganisms are given residence, suggests that the relationship between these bacteria and their host is a specific one, with both the bacteria and host shaping the evolution of their respective partners. Questions of how bacteria are ‘selected’ (or chose) to live within a given host and whether each species has a specialized niche remain unanswered, but will certainly be important endeavors of future investigations.

Can the microbiota shape the development of the extra-intestinal immune environment?

The development of animals that lack colonization by any microorganism, referred to as germ-free animals, have allowed for the understanding of how profoundly the microbiota impacts the development of the host. Commensal organisms have significant affects on the development of intestinal tissues and cells types and these developmental defects have been extensively reviewed elsewhere [2, 7]. Interestingly, germ-free mice have several extra-intestinal defects as well, suggesting that the microbes harbored within the GI tract are capable of shaping systemic immunity [8]. The spleen serves as one of the primary immunologic organs that surveys the blood for the presence of foreign entities. B and T cells collect within this organ to form an organized structure, with dendritic cells, macrophages and natural killer (NK) cells also present. In the absence of microbial colonization mice have fewer and smaller germinal centers within the spleen and fewer gamma-globulin containing cells [9]. Additionally, a study published over 30 years ago found that immunoglobulin G (IgG) and IgM antibody response of germ-free mice in the spleen is deficient when challenged with DNP-BSA [10]. In this same study, lymphocytes isolated from the spleen had an attenuated response to the T cell mitogen, ConA, demonstrating that both T and B cell reactions are impaired in the absence of microbial colonization. Later it was reported that the number of CD4+ T cells residing within the spleen was two-fold lower in germ-free mice and produced more interleukin-4 (IL-4) when compared to conventionally colonized animals, indicating that germ-free mice have a Th2 bias [11]. The influence of the microbiota extends beyond development and guides functional aspects of systemic immunity as well.

Germ-free mice are more susceptible to infectious pathogens such as *Shigella flexneri*, *Bacillus anthracis*, and *Leishmania* [2]. In a study using a systemic challenge of *Listeria monocytogenes*, it was demonstrated that germ-free mice had increased bacterial burden in the liver, spleen and peritoneal cavity [12]. Moreover, trafficking of T lymphocytes to the peritoneal cavity in response to *Listeria* infection was impaired in germ-free mice, suggesting that the microbiota is important for the proper function of the adaptive response upon infection.

The intestinal immune system goes to great lengths to ensure that the microbes harbored within the GI tract do not escape to the systemic compartment. In addition to barrier defenses such as a thick mucus layer and the production of anti-microbial peptides by intestinal epithelial cells, dendritic cells that take up commensal organisms are unable to penetrate past the mesenteric lymph nodes and elicit an IgA response to further prevent commensals from breaking through the intestinal barrier [13]. Given the strict restriction of commensal bacteria to the intestinal lumen, it remains unclear how these organisms could affect systemic responses and immunity. Additionally, commensal microorganisms are found at other sites in the body and could therefore be responsible for the systemic effects seen in germ-free mice. A direct demonstration that a commensal organism residing within the intestine is capable of coordinating T cells responses in the spleen was shown when germ-free mice were mono-associated with the prominent human commensal, *Bacteroides fragilis* [14]. Mono-association with *B. fragilis* restores the reduced CD4+ T cell numbers and induces production of the Th1 cytokine interferon-γ (IFN-γ), thereby correcting the Th2 bias seen in germ-free mice. More importantly, this activity is associated with a single molecule made by *B. fragilis*, polysaccharide A (PSA). Indeed, mono-association of mice with a mutant of *B. fragilis* that lacks expression of PSA (*B. fragilis*ΔPSA) does not correct the systemic CD4+ T cell defect. These data demonstrate that individual species of the intestinal microbiota can influence extra-intestinal immune responses and present the possibility that these organisms can influence systemic diseases.

Communicating with the microbiota

Pathogen manipulation of host immunity has been a major scientific focus over the last couple of decades. However, mammals are host to a diverse consortium of bacteria to which we remain immunologically tolerant. Since pathogens and commensals have many of the same motifs that elicit immunity within the host, how the host tolerates this extraordinary bacterial burden while maintaining the ability to mount productive immunity towards pathogens has remained enigmatic. Initially, studies suggested that the host was largely unaware of the presence of their microbial guests. However, emerging evidence suggests that rather than being ignorant, the host is actively tolerating the commensal consortium of intestinal organisms. The mechanisms that govern this tolerance remain largely unknown. We propose that the host is not the only participant in directing immune responses, but rather that an active dialogue occurs between host and microbe that results in a relationship that has benefits to both parties. The words of this communication come in the form of specific bacterial and host molecules that can coordinate the biology of both participants. Here we will review the mechanisms by which the host senses the microbiota and in turn the signals utilized by commensal organisms to coordinate tolerogenic host responses (Table 1).

The mammalian immune system can be divided into two major arms; innate and adaptive immunity. The innate immune system is built upon the sensing of conserved microbial associated molecular patterns (MAMPs) such as cell wall components of bacteria by Toll-like receptors (TLRs) and Nod-like receptors (NLRs) found on numerous cell types including macrophages, dendritic cells and even T cells. Following ligand binding, TLRs initiate signal transduction cascades that begin with adaptor proteins of the MyD88 family,

and culminate in widespread changes in gene expression including induction of NF-κB. TLRs regulate a variety of different genes, including those that function as transcriptional activators, immunomodulatory cytokines, regulators of phagocytosis, mediators of antigen presentation and co-stimulation, and managers of cell proliferation. This represents the first wave of an immune response. Activated innate immune cells, such as DCs, will then elicit induction of the adaptive arm of the immune response which includes the activation of antigen specific T and B cells. The T helper cell response is fine-tuned for the type of pathogen encountered, and can consist of 4 lineages characterized by the expression of specific proteins. T helper 1 (Th1) cells produce large amounts of IFN-γ, while Th2 cells are marked by IL-4 secretion. Th17 cells make copious amounts of IL-17 and have recently been implicated in multiple autoimmune diseases. Finally, regulatory T cells (Tregs) represent a lineage of helper T cells that suppress immune responses, either by directly down-regulating T cell activity or suppressing cells of the innate immune system. These cell types are characterized by the expression of the transcription factor Foxp3 and secrete suppressive cytokines such as IL-10 and transforming growth factor- β (TGF-β) [15]. While the innate immune system utilizes TLRs and NLRs to directly recognize the presence of bacteria, the adaptive immune system recognizes bacterial specific antigens via T and B cell receptors. Thus, each lineage is capable of maintaining a dialogue with the microorganisms itself.

The intestine represents one of the largest mucosal surfaces on the human body. As such, defending this area against microbial penetration is a complicated task and involves multiple cell types. Intestinal epithelial cells (IECs) form a single cell barrier between the lumen of the intestine preventing access to the rest of the body. Thus, IECs represent the frontline of defense against the invasion of microorganisms as well as the first-line of communication. As an initial defense to the entry of microorganisms, specialized cells types within the epithelial layer secrete mucins that help form a thick protective layer over the IECs [16]. Indeed, this mucus layer has been shown to be devoid of bacteria and thus functions to limit penetration of microbes within the intestinal wall [17]. Despite this barrier, IECs still come into contact with luminal bacteria and must respond accordingly. Therefore, it is no surprise that IECs express both TLRs and NLRs. Some of these receptors, such as TLR4, are expressed at low levels of IECs while others, such as TLR5 are expressed normally [18]. Accordingly, IECs have been reported to be hyporesponsive to LPS, potentially explaining why these cells can tolerate the large bacterial burden within the intestine. However, feeding mice purified LPS was sufficient to induce the expression of the anti-microbial peptide RegIIIγ, demonstrating that IECs are capable of responding to LPS within the lumen of the intestine [19]. If IECs are capable of mounting inflammatory responses to Toll ligands, how is tolerance achieved? A seminal study from Medzhitov and colleagues reported that MyD88 deficient animals are severely susceptible to intestinal injury [20]. Indeed, this study demonstrated that TLR signaling elicits the production of tissue protective factors that are imperative for intestinal homeostasis and suggested that TLR signals in the intestine may not always elicit inflammation. More importantly, they demonstrated for the first time that commensal organisms within the intestine may actively guide host protective responses. Supporting this, IEC specific inhibition of NF-κB (a pathway induced by TLR signaling) through conditional ablation of NEMO results in spontaneous and chronic intestinal inflammation [21]. Long before these studies had been performed, germ-free mice had been reported to have a slower rate of epithelial cell turnover, further validating the idea that commensally derived signals are important for intestinal integrity. Not only have commensal bacteria been implicated in IEC homeostasis, but these organisms can also control IEC cell signaling. Pathogenic *Salmonella enterica* was co-incubated with the commensal organism *Bacteroides thetaiotaomicron* on intestinal epithelial cells and a cDNA array was used to monitor inflammatory responses [22]. While *S. enterica* alone initiated strong expression of inflammatory mediators such as IL-8 and tumor-necrosis factor (TNF), co-incubation with

the commensal organism *B. thetaiotaomicron* down-regulated this inflammatory activity. Interestingly, the ability of *B. thetaiotaomicron* to suppress *S. enterica*-induced inflammation was specific, as a related strain, *Bacteroides vulgates* did not produce the same attenuating effect. The mechanism by which *B. thetaiotaomicron* suppresses inflammation was through enhancing the nuclear export of the transcription factor NF-κB. Inhibition of NF-κB signaling in IECs was also demonstrated for a non-pathogenic *Salmonella* strain [23]. The anti-inflammatory effect could be abolished by non-denaturing heat killing of the organisms and was not present in bacterial lysates or conditioned media suggesting that the anti-inflammatory effect of this strain was mediated through direct interactions of the epithelium with viable bacteria. In contrast, media supernatants from a mix of several *Lactobacillus* and *Bifidobacteria* strains have been shown to enhance epithelial cell integrity[24]. Therefore, while IECs are equipped with the ability to mount inflammatory responses toward bacteria, it seems that the enteric flora express specialized molecules to coordinate a tolerant host response. The identity of these molecules produced by the bacteria and the receptors to which these molecules associate remain completely unknown.

Mucosal dendritic cells (DCs) are known to sample luminal contents and thus represent a second cell type that is in direct contact with the commensal microbiota. Dendritic cells bridge innate and adaptive immunity by directly responding to bacterial presence through the expression of TLRs and NLRs, and subsequently presenting bacterial antigens to T cells. Within the intestine, DCs have been shown to induce largely tolerogenic T and B cell responses. Indeed, TNF-α and iNOS expressing DCs induce T cell independent IgA secretion from B cells, which limits penetration of commensal organisms past the epithelial barrier [25]. The second mechanism by which these cell types elicit tolerance is through the induction of regulatory T cells (Treg). CD103+ DCs have been identified to reside within the mesenteric lymph nodes (MLNs) and convert a naïve T lymphocyte into a Treg with functionally suppressive capacity [26, 27]. These data indicate that specialized DCs exist exclusively within the intestine and function to maintain tolerance. While it is unclear to what extent the microbiota influences the development of CD103+ DCs, studies looking at CD11c+ cells in the MLN suggest the DCs derived from germ-free mice are fewer in number and are less able to stimulate T cell responses, indicating that the microbiota can influence DC function [28]. Supporting this, bone marrow derived dendritic cells (BMDCs) incubated with commensal *Lactobacillus* species are able to induce Treg responses [29]. Additionally, transfer of *Lactobacillus*-treated DCs prevented intestinal inflammation in a MyD88 and TLR2 dependent manner, suggesting that bacterial molecules are capable of being recognized by DCs and coordinating their tolerogenic potential.

Tregs are considered one of the dominant mechanisms of tolerance induction within mammals, and have indeed been shown to be paramount for the maintenance of intestinal homeostasis. Indeed, transfer of effector T cells in the absence of Tregs elicits robust and chronic intestinal inflammation while co-transfer of effector T cells with Tregs ameliorates disease [30]. Additionally, Treg specific ablation of IL-10 results in spontaneous intestinal inflammation, but not systemic autoimmunity, indicating that specific subsets of Tregs function to maintain homeostasis within the intestine [31]. A few reports have indicated that the microbiota can influence the development of T regulatory subsets, though others have reported contradictory results [7]. Demonstrating that microbial recognition can influence the development of Tregs, a recent study demonstrated that TLR9 deficient animals have an increased frequency of Tregs at intestinal sites [32]. Specific commensal bacteria residing within the intestine have been shown to influence the development of Tregs in systemic compartments. *Bifidobacteria infantis* can increase the numbers of CD4+Foxp3+ Tregs in the spleen. Further, transfer of *B. infantis*-induced Tregs can inhibit pro-inflammatory NF-κB activity [33]. Additionally, *Lactobacillus* strains have been demonstrated to induce IL-10 independent tolerogenic responses, suggesting that multiple commensal bacteria have

evolved mechanisms to induce T regulatory cells within the intestine. Molecules from these organisms that direct the development of Treg cells have not yet been identified. It has recently been demonstrated that a single capsular polysaccharide, PSA, from the prominent human commensal, *Bacteroides fragilis*, is able to protect from experimental colitis through the induction of IL-10 producing tolerogenic T cells [34]. The ability of PSA to influence host T cell biology has been demonstrated to act through presentation of PSA on MHCII by an antigen presenting cell [35], demonstrating that microbial antigens can indeed direct host immune responses and represents another level of communication that can be utilized by the microbiota. It seems likely that the mechanisms by which Treg biology is influenced by the commensal bacteria is through the direction of DCs. Therefore, it is likely that commensal microbial antigens are being presented to either naïve T cells for conversion into Treg cells or directly being presented to the Tregs themselves. An alternative mechanism by which the microbial consortium could influence Treg biology is through the engagement of TLRs. While this is a fairly unexplored area of research, one report demonstrating that Myd88 deficient Tregs are unable to protect from experimental intestinal inflammation offers the possibility that TLR ligands can directly govern Treg biology [36]. Recent data indicate that proper Treg homeostasis requires production of small single stranded RNAs called miRNAs. Although several miRNAs are likely involved in Treg biology, mice genetically deficient in a single miRNA, miR-155, have reduced Treg numbers due to a lack of overall fitness [37]. Interestingly, miR-155 expression levels are known to be modulated following immune stimulation by microbial ligands. This suggests that commensal flora might influence miRNA expression in host immune cells, such as Tregs, that are important for regulating overall immune homeostasis.

The identification of PSA as a molecule able to induce tolerogenic responses, suggests the possibility that commensal bacteria have evolved specialized molecules in order to coordinate their own tolerance within the intestine. Pathogens have evolved specialized proteins to subvert or control host immunity to enhance infectivity. Perhaps commensals have also evolved mechanisms to coordinate their ability to colonize a given host. Understanding what the microbial tools of communication are and in turn how the host can understand this message will be imperative to our understanding of host-microbial mutalism.

Autoimmunity: when the dialogue becomes interrupted

The hygiene hypothesis was first proposed 20 years ago to explain the dramatic rise in atopic diseases seen over the preceding decade [38]. This hypothesis posits that the aberrant immune responses such as allergy are a result of smaller family sizes and improvements in personal cleanliness which have reduced the exposure to microbial stimulation. An extension of this hypothesis is the ‘microflora hypothesis’ or altered microbiota hypothesis proposed by Noverr and Huffnagle [39]. This hypothesis proposes that changes in the gastrointestinal microbiota because of antibiotic use and dietary differences in developed countries have disrupted the normal microbial mediated mechanisms of immunological tolerance. There is a significant amount of epidemiologic and clinical data to support the altered microflora hypothesis [39]. Both of these hypotheses make two critical points: 1) that the environmental changes can impact the microbiota and 2) that this perturbation in the composition of the microbiota can lead to disease. Several studies have explored the idea that antibiotics can disrupt the composition of the microbiota. Relman and colleagues used pyrosequencing technology to generate large numbers of 16S rDNA tags from fecal samples taken from three individuals before and after ciprofloxacin treatment [40]. They found that treatment with antibiotics reduced the diversity of the microbiota with significant impacts on over one-third of the community. The microbiota closely resembled that of the pretreatment state within 4 weeks, however, several taxa failed to recover within 6 months. These data demonstrate that antibiotics can have devastating consequences to the composition of the

microbial community, many of which might be permanent. One consequence of broad spectrum antibiotic therapy is the colonization of the host by antibiotic resistant pathogens, an example of which is vancomycin resistant *Enterococcus* (VRE). It has been unclear how antibiotic mediated depletion of commensal organisms allows for colonization by VRE. A recent study demonstrated that mice given a course of antibiotics have reduced expression of the C-type lectin, RegIIIγ [19]. RegIIIγ is known to kill gram-positive bacteria, such as VRE, and is induced by intestinal gram-negative symbionts such as *B. thetaiotaomicron* [41]. Thus, antibiotic treatment can compromise the microbial induced mechanisms of host bacterial killing and thereby allow pathogenic bacteria an opportunity to colonize. Together, these data support the notion that antibiotics can have severe impacts on the composition of the microbial community.

The second supposition of the altered microbiota hypothesis is that a change in the composition of the microbial community can lead to disease. Several reviews have discussed the extensive evidence for the role of the microbiota in intestinal inflammatory autoimmunity [7]. Here we discuss the evidence for the involvement of commensal bacteria in systemic immune aberrations including allergy, rheumatoid arthritis (RA) and type I diabetes (T1D).

The hygiene hypothesis was originally formulated in response to the dramatic increase in the incidence of allergies. Since its inception, at least 14 reports containing over 2,000 individuals have been published that demonstrate the correlation between allergy and an altered microbiota [8]. In most of the studies, a decrease in *Bifidobacteria* preceded the development of allergy. Additionally, higher counts of Clostridial species, such as *Clostridium difficile*, were found to be associated with development of allergies. While many of these correlations are compelling, it is difficult to assess whether these changes in microbial composition are causative. To do this, a defined animal model to study allergy is required. The majority of murine models of allergic airway disease use systemic sensitization prior to intranasal challenge, however allergies in humans do not arise from immunization with an allergen. Therefore, there are currently no suitable animals models to explain how allergen sensitization can occur in the absence of systemic challenge or to test a role for the microbiota in allergy development. In response to this Huffnagle and colleagues have developed a mouse model of allergic airway disease that results from antibiotic therapy and microbial disruption [42]. Immunocompetent animals are treated for 5 days with cefoperazone in the drinking water followed by a single oral gavage of *Candida albicans*. This leads to a stable increase in the fungal microbiota and alteration of the commensal intestinal bacteria. These mice are then exposed to an intranasal challenge of an allergen derived from *Aspergillus fumigatus* spores. Interestingly, in an environment where the natural intestinal microbiota is disrupted, introduction of a common allergen to the lungs results in T cell mediated airway autoimmunity. The development of this model has several important implications. It directly demonstrates that allergy can develop when the microbiota becomes disrupted and provides a platform for manipulation of particular commensal species to determine their individual impact on the development of allergy. Future studies should identify specific commensal species that can prevent allergy induction and the mechanisms by which these organisms accomplish this.

Rheumatoid arthritis is a disabling musculoskeletal autoimmune disease whose fundamental causative factors remain unknown. It is widely accepted that both genetic and environmental predisposing factors are involved; however, disease heritability is estimated to be between only 50-60%. This indicates that environmental factors play an important role in RA development [43]. Several observations have lead to the idea that the intestinal microbiota might be involved in disease induction. In patients with inflammatory arthritis, degradation products of bacterial cell walls and nucleic acids are detected in inflamed joints. Moreover,

injection of bacterial cell wall fragments from several intestinal commensals have been shown to induce arthritis [44]. This has led investigators to look at the composition of the intestinal microbiota of patients with early RA. The results of this study indicate that patients with RA had significantly less *Bifidobacteria* species, bacteria of the *Bacteroides-Porphyromonas-Prevotella* group and *Bacteroides fragilis* subgroup, supporting the notion that the intestinal microbiota is altered during disease [45]. Supporting the notion that the microbiota plays a role in RA development an animal model of Collagen induced arthritis was used. Disease was induced in germ-free and conventional animals were induced and progression of the RA development monitored. Interestingly, germ-free animals had markedly worse disease than animals with a complex microbiota, demonstrating that the microbiota has a suppressive effect on the development of RA [46]. This indicates that there are organisms within our intestine that are mediating beneficial immune responses that potentially actively suppress the development of joint inflammation.

Type I diabetes is an autoimmune disease that results from the T cell mediated destruction of insulin-producing β cells. The incidence of TID has steadily increased over the past several decades in developed countries suggesting an environmental component to disease induction [47]. Supporting this, incidence of disease in experimental animal model systems varies with the microbial status of the housing facility [48]. Moreover, diabetes development in the non-obese diabetic (NOD) mouse model is increased when mice are re-derived in a germ-free environment. Because TLRs are receptors that are known to recognize bacteria, a recent study set out to understand how microbial sensing impacted the progression of T1D. To do this, NOD mice were crossed with Myd88 deficient animals and disease monitored [49]. While wild-type NOD mice developed diabetes as early as 10 weeks, MyD88 deficient animals were completely devoid of autoimmunity, suggesting that microbial signals initiate disease. However, when these animals are rederived in a germ-free environment the incidence of disease dramatically increases, suggesting that the presence of the microbiota is protective even in the absence of signaling by TLRs. Therefore in this scenario, bacterial sensing by TLRs enhances the development of TID, while mechanisms that do not require TLRs induce protective responses. Although there has not been a reported connection between commensal microbiota and the autoimmune conditions multiple sclerosis (MS) or lupus, a few TLR pathways have been shown to reduce disease severity in specific mouse models. For instance, engagement of TLR9 reduces disease in lupus, and a TLR-dependent tolerance mechanism in B cells blunts T cell mediated tissue damage in MS. While TLR engagement on cells generally leads to production of pro-inflammatory cytokines, it also induces certain anti-inflammatory molecules, like IL-10, which may be at the heart of TLR dependent mechanisms of tolerance in autoimmune settings. Because TLRs can recognize motifs that are found on commensal bacteria, it is plausible that TLRs can link commensal microbes to both lupus and MS as well. To truly understand the mechanisms of how the microbiota influences immune health and development of these various autoimmune diseases, it will be critical to have good animal model systems where the composition of the microbiota can be manipulated. While most of what is known about microbial mediated tolerance comes from studies performed in IBD, these examples lend evidence that the communication between the host and microbe is imperative to host health and suggest that the microbiota could have impacts on a multitude of other human diseases.

Conclusion

The complex consortium of microbes that we harbor within our gastrointestinal tract are not just passive bystanders, rather these organisms seem to actively shape our immune system responses both inside and outside the intestine. The multiple diseases that have been suggested to be associated with a disruption in the commensal microbiota highlight the importance of understanding the individual species that make up a ‘healthy’ microbiota.

While it seems clear that the microbiota influences disease progression and/or prevention, the mechanism by which it can accomplish this task remain completely unknown. We have presented evidence that there is an intimate communication between host and microbe that involves bacterial molecules and host derived sensors. While much of the data to date has explored TLRs and NLR receptors as the bacterial communication devices, there is evidence that alternative means, such as presentation of enteric bacterial derived molecules by DCs, can influence immune responses that affect autoimmunity. The focus of future endeavors will be to identify these molecules as well as the host receptors that recognize them, as beneficial microbial products could offer new therapeutics to enhance human health.

References

1. Hooper LV, Gordon JI. Commensal host-bacterial relationships in the gut. Science. 2001; 292:1115–8. [PubMed: 11352068]
2. Smith K, McCoy KD, Macpherson AJ. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. Semin Immunol. 2007; 19:59–69. [PubMed: 17118672]
3. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell. 2006; 124:837–48. [PubMed: 16497592]
4. Palmer C, Bik EM, Digiulio DB, Relman DA, Brown PO. Development of the Human Infant Intestinal Microbiota. PLoS Biol. 2007; 5:e177. [PubMed: 17594176]
5. Gill SR, Pop M, Deboy RT, et al. Metagenomic analysis of the human distal gut microbiome. Science. 2006; 312:1355–9. [PubMed: 16741115]
6. Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. Science. 2005; 308:1635–8. [PubMed: 15831718]
7. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol. 2009
8. Noverr MC, Huffnagle GB. Does the microbiota regulate immune responses outside the gut? Trends Microbiol. 2004; 12:562–8. [PubMed: 15539116]
9. Bauer H, Horowitz RE, Levenson SM, Popper H. The response of the lymphatic tissue to the microbial flora. Studies on germfree mice. Am J Pathol. 1963; 42:471–83. [PubMed: 13966929]
10. Ohwaki M, Yasutake N, Yasui H, Ogura R. A comparative study on the humoral immune responses in germ-free and conventional mice. Immunology. 1977; 32:43–8. [PubMed: 321340]
11. Dobber R, Hertogh-Huijbregts A, Rozing J, Bottomly K, Nagelkerken L. The involvement of the intestinal microflora in the expansion of CD4+ T cells with a naive phenotype in the periphery. Dev Immunol. 1992; 2:141–50. [PubMed: 1386544]
12. Inagaki H, Suzuki T, Nomoto K, Yoshikai Y. Increased susceptibility to primary infection with Listeria monocytogenes in germfree mice may be due to lack of accumulation of L-selectin+ CD44+ T cells in sites of inflammation. Infect Immun. 1996; 64:3280–7. [PubMed: 8757865]
13. Macpherson AJ, Uhr T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science. 2004; 303:1662–5. [PubMed: 15016999]
14. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell. 2005; 122:107–18. [PubMed: 16009137]
15. Fontenot JD, Rudensky AY. A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. Nat Immunol. 2005; 6:331–7. [PubMed: 15785758]
16. Jass JR, Roberton AM. Colorectal mucin histochemistry in health and disease: a critical review. Pathol Int. 1994; 44:487–504. [PubMed: 7921193]
17. Johansson ME, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc Natl Acad Sci U S A. 2008; 105:15064–9. [PubMed: 18806221]

18. Abreu MT, Fukata M, Arditi M. TLR signaling in the gut in health and disease. J Immunol. 2005; 174:4453–60. [PubMed: 15814663]
19. Brandl K, Plitas G, Mihu CN, et al. Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. Nature. 2008; 455:804–7. [PubMed: 18724361]
20. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell. 2004; 118:229–41. [PubMed: 15260992]
21. Nenci A, Becker C, Wullaert A, et al. Epithelial NEMO links innate immunity to chronic intestinal inflammation. Nature. 2007; 446:557–61. [PubMed: 17361131]
22. Kelly D, Campbell JI, King TP, et al. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol. 2004; 5:104–12. [PubMed: 14691478]
23. Neish AS, Gewirtz AT, Zeng H, et al. Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. Science. 2000; 289:1560–3. [PubMed: 10968793]
24. Madsen K, Cornish A, Soper P, et al. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology. 2001; 121:580–91. [PubMed: 11522742]
25. Tezuka H, Abe Y, Iwata M, et al. Regulation of IgA production by naturally occurring TNF/iNOS-producing dendritic cells. Nature. 2007; 448:929–33. [PubMed: 17713535]
26. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med. 2007; 204:1757–64. [PubMed: 17620361]
27. Izcue A, Coombes JL, Powrie F. Regulatory T cells suppress systemic and mucosal immune activation to control intestinal inflammation. Immunol Rev. 2006; 212:256–71. [PubMed: 16903919]
28. Walton KL, He J, Kelsall BL, Sartor RB, Fisher NC. Dendritic cells in germ-free and specific pathogen-free mice have similar phenotypes and in vitro antigen presenting function. Immunol Lett. 2006; 102:16–24. [PubMed: 16105690]
29. Foligne B, Zoumpopoulou G, Dewulf J, et al. A key role of dendritic cells in probiotic functionality. PLoS One. 2007; 2:e313. [PubMed: 17375199]
30. Mottet C, Uhlig HH, Powrie F. Cutting edge: cure of colitis by CD4+CD25+ regulatory T cells. J Immunol. 2003; 170:3939–43. [PubMed: 12682220]
31. Rubtsov YP, Rasmussen JP, Chi EY, et al. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity. 2008; 28:546–58. [PubMed: 18387831]
32. Hall JA, Bouladoux N, Sun CM, et al. Commensal DNA limits regulatory T cell conversion and is a natural adjuvant of intestinal immune responses. Immunity. 2008; 29:637–49. [PubMed: 18835196]
33. O'Mahony C, Scully P, O'Mahony D, et al. Commensal-induced regulatory T cells mediate protection against pathogen-stimulated NF-kappaB activation. PLoS Pathog. 2008; 4:e1000112. [PubMed: 18670628]
34. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature. 2008; 453:620–5. [PubMed: 18509436]
35. Cobb BA, Wang Q, Tzianabos AO, Kasper DL. Polysaccharide processing and presentation by the MHCII pathway. Cell. 2004; 117:677–87. [PubMed: 15163414]
36. Fukata M, Breglio K, Chen A, et al. The myeloid differentiation factor 88 (MyD88) is required for CD4+ T cell effector function in a murine model of inflammatory bowel disease. J Immunol. 2008; 180:1886–94. [PubMed: 18209086]
37. Lu LF, Thai TH, Calado DP, et al. Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. Immunity. 2009; 30:80–91. [PubMed: 19144316]
38. Strachan DP. Hay fever, hygiene, and household size. Bmj. 1989; 299:1259–60. [PubMed: 2513902]
39. Noverr MC, Huffnagle GB. The 'microflora hypothesis' of allergic diseases. Clin Exp Allergy. 2005; 35:1511–20. [PubMed: 16393316]

40. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol. 2008; 6:e280. [PubMed: 19018661]
41. Cash HL, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. Science. 2006; 313:1126–30. [PubMed: 16931762]
42. Noverr MC, Noggle RM, Toews GB, Huffnagle GB. Role of antibiotics and fungal microbiota in driving pulmonary allergic responses. Infect Immun. 2004; 72:4996–5003. [PubMed: 15321991]
43. Edwards CJ, Cooper C. Early environmental factors and rheumatoid arthritis. Clin Exp Immunol. 2006; 143:1–5. [PubMed: 16367927]
44. Toivanen P. Normal intestinal microbiota in the aetiopathogenesis of rheumatoid arthritis. Ann Rheum Dis. 2003; 62:807–11. [PubMed: 12922950]
45. Vaahtovuo J, Munukka E, Korkeamaki M, Luukkainen R, Toivanen P. Fecal microbiota in early rheumatoid arthritis. J Rheumatol. 2008; 35:1500–5. [PubMed: 18528968]
46. Breban MA, Moreau MC, Fournier C, Ducluzeau R, Kahn MF. Influence of the bacterial flora on collagen-induced arthritis in susceptible and resistant strains of rats. Clin Exp Rheumatol. 1993; 11:61–4. [PubMed: 8453801]
47. Karvonen M, Tuomilehto J, Libman I, LaPorte R. A review of the recent epidemiological data on the worldwide incidence of type 1 (insulin-dependent) diabetes mellitus. World Health Organization DIAMOND Project Group. Diabetologia. 1993; 36:883–92. [PubMed: 8243865]
48. Patterson CC, Dahlquist G, Soltesz G, Green A. Is childhood-onset type I diabetes a wealth-related disease? An ecological analysis of European incidence rates. Diabetologia. 2001; 44 3:B9–16. [PubMed: 11724424]
49. Wen L, Ley RE, Volchkov PY, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature. 2008; 455:1109–13. [PubMed: 18806780]

Table 1

Tolerogenic responses utilized by the host that are either genetically encoded by the host or induced by the bacteria themselves

Cell Type	Host response	Bacteria	Mechanism
Epithelial cells	RegIIIγ	*B. thetaiotaomicron*	unknown
	hyporesponsive to LPS		↓ expression of TLR4
	barrier integrity	Bacteria	Myd88 signaling
	↓IL-8 and TNF-a	*B. thetaiotaomicron*	↓ NF-κB
	↓ inflammation	nonpathogenic *Salmonella*	inhibit IkBa degradation
	barrier integrity	*Lactobacillus*/*Bifidobacteria*	secreted protein factor
	barrier integrity	*B. breve*	secreted factor
Dendritic cells	IgA from B cells		TNF-α, iNOS, April, BAFF
	CD103+ Tregs		Induction of Tregs
	↓ inflammatioin	Lactobacillus	DC induced Tregs
Tregs		*B. infantis*	induction of Tregs
		Lactiobacillus rhamnosus GG	Induction of Tregs
		B. fragilis	IL-10 expressing T cells
		VSL#3	TGF-β + Tregs
B cells	intestinal homeostasis	Bacteria	IgA
	↓ sytemic abs to commensals	unknown	MyD88 signaling