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Influence of gastrointestinal commensal bacteria on the immune responses that mediate allergy and asthma

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Activity Objectives

1. To understand the concept of the microbiome.
2. To recognize the important cellular players in gut tolerance.
3. To understand the immunomodulatory effects of microbiota on the mucosal immune system.
4. To recognize the important role of vitamin D and probiotics in immunologic tolerance and protection against allergic disease.

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The human intestine contains more than 100 trillion microorganisms that maintain a symbiotic relationship with the host. Under normal conditions, these bacteria are not pathogenic and in fact confer health benefits to the host. The microbiota interacts with the innate and adaptive arms of the host's intestinal mucosal immune system and through these mechanisms drives regulatory cell differentiation in the gut that is critically involved in maintaining immune tolerance. Specifically, the microbiota can activate distinct tolerogenic dendritic cells in the gut and through this interaction can drive regulatory T-cell differentiation. In addition, the microbiota is important in driving T_H1 cell differentiation, which corrects the T_H2 immune skewing that is thought to occur at birth. If appropriate immune tolerance is not established in early life and maintained throughout life, this represents a risk factor for the development of inflammatory, autoimmune, and allergic diseases. Early-life events are instrumental in establishing the microbiota, the composition of which throughout life is influenced by various environmental and lifestyle pressures. Significant efforts are now being made to establish interventional approaches that can create a healthy microbiota that confers maximum tolerogenic immunomodulatory effects in the gut and that will protect against systemic inflammatory disease pathologies. (J Allergy Clin Immunol 2011;127:1097-107.)

Key words: *Mircobiota, immune tolerance, innate immunity, adaptive immunity, dendritic cell, effector T cell, regulatory T cell, immunosuppressive cytokines*

The collection of microorganisms that inhabit the human intestine is known as the microbiota. The genome of the microbiota is estimated to outnumber the genes in the human genome by a factor of 100.[1] It has been suggested that the human genetic environment should be considered a composite of the genes in the human genome combined with those of the intestinal microbes, collectively called the microbiome. The human microbiome project was initiated in 2007[2] in an attempt to better understand this genetic landscape. An overwhelming 100 trillion microorganisms live within the human gastrointestinal tract, details of which have been reviewed elsewhere,[3,4] and of these, 2 divisions or phyla of bacteria predominate, the Firmicutes and Bacteroidetes,[3] despite the fact that approximately 55 divisions of bacteria and 13 divisions of Archaea (single-celled organisms) have been described. The relative exclusivity of this group of microorganisms indicates that a specific type of interaction must occur between these bacteria

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Terms in boldface and italics are defined in the glossary on page 1098.

Abbreviations used
DC: Dendritic cell
FoxP3: Forkhead box protein 3
GALT: Gut-associated lymphoid tissue
GF: Germ free
IEC: Intestinal epithelial cell
PSA: Polysaccharide A
TLR: Toll-like receptor
Treg: Regulatory T
VDD: Vitamin D deficiency

and the host, which allows them to preferentially colonize the human gut.

FACTORS AFFECTING DEVELOPMENT OF THE MICROBIOTA

A fetus is sterile *in utero*, but immediately after birth and throughout life, human subjects are colonized by microorganisms that inhabit most exposed mucosal surfaces, including nasal passages and the skin, mouth, vagina, and gut. The composition of the gut microbiota is heavily influenced by host and environmental factors experienced in the first year of life, with most subjects acquiring a stable gut microflora resembling that of an adult during this period.[5]

Because of the nature of the environmental exposure that occurs in the first few days of life, the mode of delivery of a baby significantly affects the eventual composition of the intestinal microflora.[6] Newborns who are delivered by means of cesarean section have lower densities of gut bacteria and a reduced diversity of bacteria species compared with babies born by means of vaginal delivery.[7] It appears that exposure to the maternal commensal flora in the vagina, skin, and feces at birth, which is optimal during a vaginal delivery, can kick start the generation of a baby's own microbiota, favoring colonization with a more diverse range of beneficial microbes. In this issue of the *Journal of Allergy and Clinical Immunology*, Ly et al[8] review the epidemiologic evidence for the influence of mode of delivery, infant feeding patterns, probiotics, and antibiotics on intestinal flora.

EFFECT OF THE MICROBIOTA ON THE HOST

Nature has dictated that humans and microbes coexist in a multifaceted symbiotic relationship that confers benefits to both the host and the microbe. The host must gain maximum benefit from the commensal microbiota but must also protect itself against invasion by these commensal organisms that under some circumstances can become pathogenic. It has been suggested that a healthy microbiota exists when there is a balance between symbionts, commensal organisms, and pathobionts (Fig 1). Alterations in this balance can lead to dysbiosis, which has been implicated in numerous pathologies, including inflammatory bowel disease and intestinal cancer.[9]

At the most basic physiological level, the gut microbiota aids in the digestion of foods and plays an important role in the acquisition and absorption of nutrients from otherwise indigestible foods.[10] In addition, the microbiota provides resistance to colonization and prevents the overgrowth of harmful pathogenic organisms that can cause local and disseminated disease. At a structural level, the microbiota can contribute to the local architecture of the gut

GLOSSARY

ANTIMICROBIAL PEPTIDE: Antimicrobial peptides are innate immune system molecules important in host protection against bacteria and viruses and include LL-37 (a cathelicidin) and human β-defensin 2 (HBD-2). Levels of LL-37 and HBD-2 are both decreased in patients with eczema, and this might explain the increased propensity for cutaneous infections in patients with eczema.

REGULATORY T (TREG) CELLS: Treg cells are a subpopulation of T cells that suppress or regulate immune responses. They are important in maintaining homeostasis and establishing tolerance to self-antigens. There are 2 major families: naturally occurring (or thymus-derived) Treg cells ($CD4^+CD25^{bright}FoxP3^+$ cells) and adaptive (or peripheral) Treg cells, which include $FoxP3^+$ cells that develop outside the thymus from naive $CD4^+$ T-cell populations after exposure to antigen (and cytokines) and share most functional features of natural Treg cells. Adaptive Treg cells include IL-10–producing T_R1 cells and TGF-β–producing T_H3 cells. FoxP3 deficiency leads to the immune deficiency immunodysregulation, polyendocrinopathy, endocrinopathy, X-linked syndrome.

CPG: CpGs are unmethylated bacterial dinucleotides that bind TLR9, can act as immune adjuvants, and decrease allergic responses by increasing levels of regulatory and T_H1 cytokines.

IL-4, IL-13: IL-4 and IL-13 are cytokines produced predominantly by T_H2-type T cells. They bind a common α chain on their receptors and increase the production of IL-5 and IgE. IL-4 increases expression of vascular cell adhesion molecule 1 on endothelial cells, allowing trafficking of eosinophils. IL-13 is important for multiple aspects of airway remodeling, including fibrosis and angiogenesis, as well as promoting eosinophilia. Both use the signal transducer and activator of transcription 6 signaling pathway.

IL-5: IL-5 promotes the genesis, survival, activation, and chemotaxis of eosinophils. Anti–IL-5 is a biologic agent that has been used in therapeutic trials for eosinophilic asthma, hypereosinophilic syndrome, and eosinophilic esophagitis.

IL-9: IL-9 is a potent mast cell growth factor. IL-9 increases the numbers of airway mast cells in asthmatic subjects and can induce airway remodeling.

IL-17: Secretion of IL-17 is one of the defining features of T_H17 cells, which are pathogenic in many autoimmune diseases. IL-17 production is increased by IL-6 and IL-1β in combination with IL-23. IL-17 induces the production of chemokines that promote neutrophil recruitment.

RETINOIC ACID: In the intestinal tract retinoic acid production promotes the development of $FoxP3^+$ Treg cells. Retinoic acid binds to the nuclear steroid receptors peroxisome proliferator–activated receptor and retinoic acid receptor.

TOLEROGENIC DENDRITIC CELL: Tolerogenic DCs induce tolerizing rather than inflammatory T cells. Typically these DCs are immature and express low levels of costimulatory molecules that lead to T-cell tolerance. In the gut tolerogenic DCs express the surface marker CD103.

TOLL-LIKE RECEPTORS (TLRs): Pattern recognition receptors, such as TLRs, bind to PAMPs. Examples include double-stranded RNA binding to TLR3, LPS to TLR4, flagellin to TLR5, imidazoquinoline to TLR7/8, and CpG to TLR9

NUCLEOTIDE-BINDING OLIGOMERIZATION DOMAIN (NOD)-LIKE RECEPTORS (NLRS): NOD-like receptors bind PAMPs and have a conserved architecture containing a central nucleotide-binding and oligomerization domain, C-terminal leucine-rich repeats, and an N-terminal effector domain.

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BOX 1. GALT

The gastrointestinal tract is continually exposed to the environment and as such is constantly at risk from exposure to pathogens and environmental toxins. As a consequence of this, there are more lymphoid cells in the gastrointestinal mucosa than in the spleen, lymph nodes, and blood combined. Gastrointestinal lymphoid tissues are rich in cells of the innate and adaptive immune systems that are poised to defend the host from invading pathogens. GALT consists of both organized lymphoid tissues, such as mesenteric lymph nodes and Peyer patches and, more diffusely, scattered effector sites in the intestinal lamina propria and epithelium, which consist of APCs, including DCs, and $CD4^+$ T cells, which can be found in the lamina propria but also as part of the epithelial cell layer (ie, intraepithelial lymphocytes; Fig 2). $CD8^+$ T cells are also primarily found in the intraepithelial compartments. The effector sites also contain B cells and plasma cells, which produce large quantities of IgA.[12]

by degrading mucus glycoproteins that are produced by the epithelium, thus preventing mucus accumulation, in addition to promoting differentiation of the epithelium by modifying intestinal epithelial lineage morphogenesis.[11] The gut microbiota is also instrumental in promoting the development of the gut immune system (Box 1)[12] and establishing immune tolerance.

IMMUNE PRIVILEGE AND IMMUNE TOLERANCE IN THE GUT

Immune tolerance in its simplest terms refers to the ability of the host to distinguish innocuous ingested or inhaled antigens and prevent activation of an immune response to these antigens. A breakdown of this tolerance is critically involved in the pathophysiology of various diseases. The adaptive immune system plays an important role in distinguishing between self-antigens and foreign antigens, but the intestinal microbiota represents a challenge to the adaptive immune system because it contains an enormous foreign antigenic burden (dietary and commensal flora) that must be either ignored or tolerated to maintain health. The ability of the host's immune system to ignore the antigenic burden of the microbiota relies on the microbiota remaining in a distinct anatomic location where it does not induce activation of the immune system and is critically dependent on decreasing or controlling innate immune responses.[13] Commensal species have also evolved mechanisms to suppress unwanted inflammation by actively inducing immune tolerance through their ability to interact with the innate immune system or modulate the adaptive immune system.[14] There is mounting evidence that the mechanisms regulating tolerance to the commensal flora are actually critically involved in maintaining systemic immune tolerance, which is instrumental in protecting against allergic and autoimmune diseases.

HYGIENE HYPOTHESIS

In 1989, Strachan[15] proposed that a lack of exposure to microbes in early life caused by the "cleaner" environment of developed nations resulted in an impaired development of the immune system, leading to an increased risk of allergies in later life. This hygiene hypothesis was based on the observation that there was a lower risk of allergic diseases in children with larger numbers of older siblings or who were brought up on farms with exposure to livestock. However, this was an oversimplification of the situation because a direct link between infections and allergy was not clearly established.[16] Furthermore, evidence from epidemiologic and experimental studies have demonstrated that increased exposure to helminth parasites is associated with a reduced incidence of allergy and autoimmune diseases.[17] In 1998, the hygiene hypothesis was adapted to the microbiota hypothesis, which proposed that alterations in the composition of the gut microbial flora in early life caused by lifestyle influences affects the development of mucosal immune tolerance, resulting in skewed immune responses.[18]

IMMUNE MECHANISMS IN ALLERGIC DISEASES AND ASTHMA THAT MIGHT BE INFLUENCED BY GUT MICROBIAL FLORA

Allergic diseases and asthma are traditionally associated with pronounced or dysregulated T_H2 responses. However, our understanding of the mechanisms underlying the cause of this disease is continually evolving as we begin to appreciate the complexity of effector T-cell subsets. T_H2 cells are characterized by their production of ***IL-4***, ***IL-5***, ***IL-9***, and ***IL-13***, and together, these cytokines contribute to the development and maintenance of allergic inflammation. Currently, the development of mAbs that directly target T_H2 cytokine signaling pathways is showing promise in clinical trials for the treatment of asthma.[19] In apparent contrast to the predominating effects of T_H2 cells, however, T_H1 cells have also been implicated in the asthma disease process.[20] In addition, understanding the roles played by ***IL-17***–producing CD4 T cells (T_H17 cells) and cells that produce IL-9 and IL-10 but lack suppressive function (T_H9 cells)[21] in allergic asthma is now the subject of extensive research.[22,23] Patients with atopic asthma have increased levels of T_H2 cells but can simultaneously produce IL-17.[24] Therefore although allergic disease is considered a T_H2-mediated disease, it is clear that other cell types are involved and that appropriate regulatory mechanisms are in place to control all effector T-cell responses.

Cells with immunomodulatory or immunosuppressive capacity, often referred to as ***regulatory T (Treg) cells***, are essential for the maintenance of immunologic tolerance and play a critical role in controlling inflammatory responses and autoimmune diseases. Given their important role in maintaining immune tolerance, it is not surprising that defects or dysregulation in Treg cell numbers and function are associated with progression of allergic disease.[25] $CD4^+CD25^+$ forkhead box protein 3 (FoxP3)–positive Treg cells,[26] as well as the immunomodualtory cytokines IL-10[27,28] and TGF-β,[29] have been shown to play a role in controlling allergic inflammatory responses. Consistent with this, therapies that can enhance protective Treg cell responses are currently being developed for the treatment of allergic diseases. Allergen-specific immunotherapy with house dust mite antigen decreased symptoms in asthmatic children. This treatment was associated with an expansion of $CD4^+CD25^+FoxP3^+$ cells and increased IL-10 production.[30]

IMMUNOMODULATORY EFFECTS OF THE MICROBIOTA ON THE MUCOSAL IMMUNE SYSTEM

Since the establishment of the hygiene hypothesis, immunologists have strived to understand the mechanisms by which the gut microbiota directly influences specific aspects of the host's immune response that protect against atopic disease. This

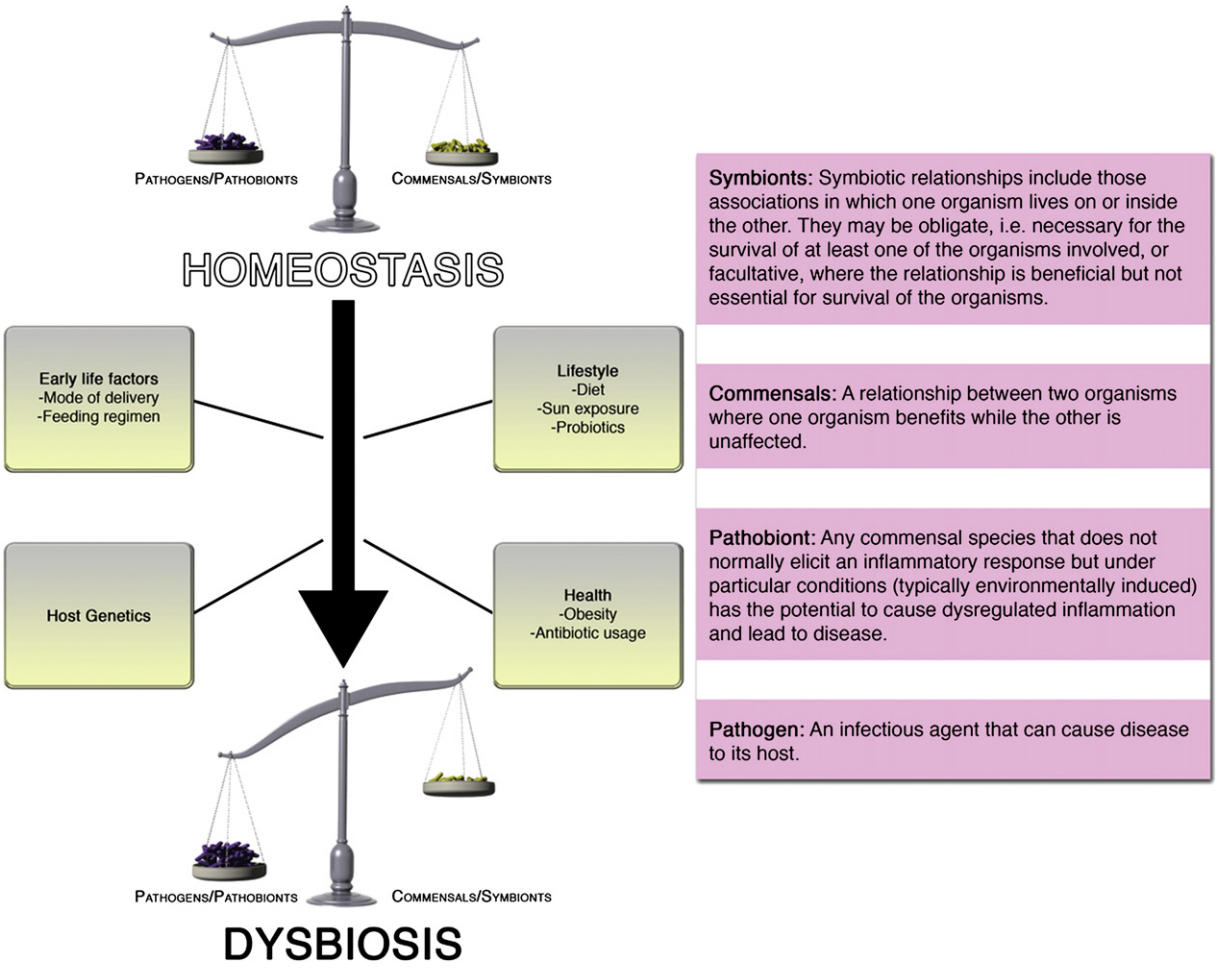

FIG 1. The healthy microbiota. A symbiotic relationship exists between the intestinal microflora and the host. The nature of this relationship depends on the type of bacterial species present. Under normal conditions, commensal bacteria live within the host and do not cause any adverse effects; instead, they provide health benefits to the host and provide colonization resistance against invasion by pathogenic bacteria. However, under certain conditions, the commensal species themselves can become pathobionts and cause pathology if they breach the intestinal barrier, resulting in the activation of proinflammatory responses. Significant alterations in the makeup of the intestinal flora can create a situation whereby the balance between beneficial commensal species and pathogenic species, pathobiont species, or both is altered to such an extent that it induces dysbiosis, which can contribute to inflammatory, autoimmune, and allergic diseases. Various external environmental factors can affect (both positively and negatively) the composition of the intestinal microbiota and therefore have the ability to alter this balance and affect human health.

protection is fundamentally dependent on the preservation of an immune-tolerant state. We will now discuss the intestinal microbiota as a master regulator of immune equilibrium that confers protection to the host against inflammatory, autoimmune, and allergic diseases. We summarize the current state-of-the-art knowledge on the mechanisms by which the intestinal microbiota modulates both regulatory and effector components of the immune system. These immunomodulatory effects should then be considered in the context of the immune mechanisms underlying the cause of allergic disease that have been outlined above.

INTESTINAL EPITHELIAL CELLS AND INNATE SIGNALING IN THE GUT

A single layer of intestinal epithelial cells (IECs) creates a barrier between the lumen of the intestine, which is an antigen-rich environment, and access to the rest of the body.[31] This barrier is maintained by IECs through physical and biochemical mechanisms. Paracellular traffic is prevented by intracellular tight junctions. Microbial attachment and invasion is impeded by a brush border, which is created by actin-rich microvillar extensions on the surface of the cells.[32] Finally, a viscous impermeable layer is formed on the surface of the epithelium through the secretion of mucus by specialized goblet cells.[33] However, despite this protective barrier, the contents of the intestinal lumen are continually sampled. This process is facilitated by the presence of specialized lymphoid structures (gut-associated lymphoid tissue [GALT]; Box 1 and Fig 2), including Peyer patches in the small intestine and lymphoid follicles that are embedded in the lamina propria throughout the intestinal tract. A layer of follicle-associated epithelium overlays the Peyer patches and lymphoid follicles and contains specialized microfold cells that specifically function to sample antigen from the intestinal lumen and deliver them through a process of transcytosis to the subepithelial dome, an

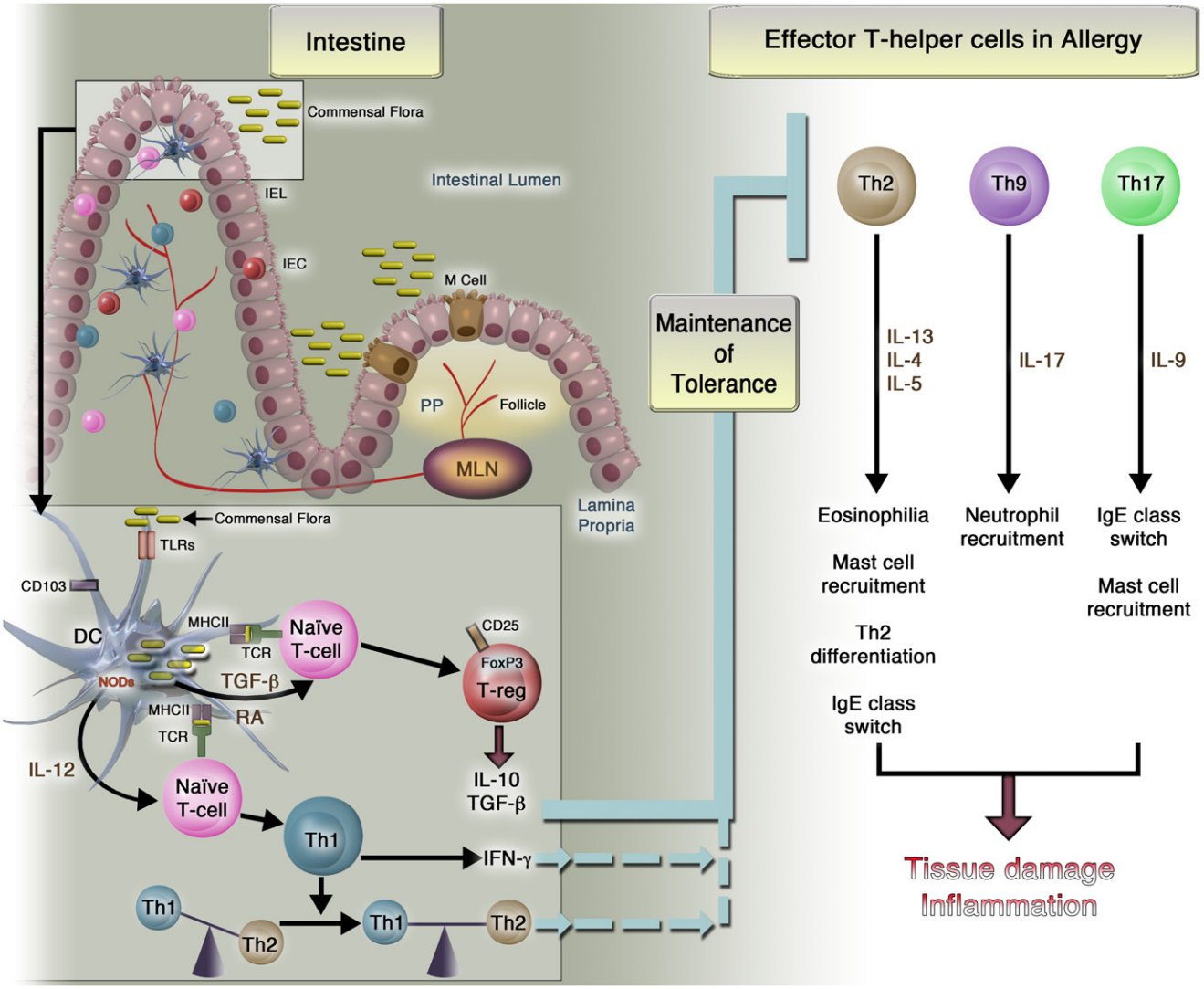

FIG 2. Immunomodulatory effects of the microbiota in the gut have the potential to decrease allergic inflammatory responses. The intestinal mucosal immune system is multifaceted, consisting of IECs, mesenteric lymph nodes *(MLN)*, Peyer patches *(PP)*, intestinal lamina propria, intraepithelial lymphocytes *(IELs)*, and microfold *(M)* cells. In the gut DCs can take up an antigen and present it to naive T cells, which then differentiate into effector or Treg cells. The nature of the T cell is dictated by cytokine signals that are produced by the DC and other cell types in the local environment in response to activation of innate signaling pathways in these cells. The intestinal microbiota is instrumental in driving differentiation of Treg cells, which produce anti-inflammatory cytokines, such as IL-10 and TGF-β. In addition, the microbiota induces T_H1 cell differentiation, which is required to establish a balance between T_H1 and T_H2 immune responses, which corrects the T_H2 skewing that is thought to occur at birth. It is unclear how the commensal flora balances T_H1 versus Treg cell responses in the gut. Expansion of Treg cell subsets in the gut is critically involved in establishing immune tolerance. Treg cells and immunomodulatory cytokines, such as IL-10 and TGF-β, are critically involved in decreasing or preventing effector T-cell responses that mediate autoimmune or allergic disease. The immunomodulatory effects of the intestinal mucosa are therefore directly relevant to protection against allergic disease pathology. *IEL*, Intraepithelial lymphocyte; *NOD*, nucleotide-bonding oligomerization domain; *RA*, retinoic acid; *TCR*, T-cell receptor.

area rich in professional antigen-presenting cells (APCs), such as dendritic cells (DCs).[34] Specialized DCs located in the lamina propria can also extend their dendrites between epithelial cell tight junctions to sample the intestinal lumen (Fig 2).

In addition to providing a physical barrier function, IECs play an integral role in distinguishing pathogenic from commensal bacteria and in regulating immune responses in the intestine. IECs are continually in contact with intestinal bacteria and therefore, not surprisingly, express various pathogen recognition receptors, including ***Toll-like receptors (TLRs)*** and ***nucleotide-binding oligomerization domain–like receptors (NLRs)***, which activate immune defenses against pathogens. Because pathogens and commensal organisms express many of the same pathogen-associated molecular patterns (PAMPs), it is important that tightly regulated control mechanisms are in place to allow the innate immune system to recognize and respond to pathogens but at the

TABLE I. Tolerogenic DC functions

Tolerogenic function	Mechanism	Reference
Increased IL-10 production controlling autocrine production of inflammatory cytokines	Direct TLR-mediated activation of DCs by bacterial PAMPS	Monteleone et al[39] and Zaph et al[43]
IgA secretion by B cells	TLR-mediated increase in iNOS expression in DCs by bacterial PAMPS	Tezuka et al[57]
Expansion of FoxP3 expression from naive CD4 cells	Activation of $CD103^+$ DCs by bacterial PAMPS induced TGF-β and retinoic acid production.	Coombes et al[58] and Niess et al[37]
Induction of IL-10–producing T_R1 cells	Activation of innate signaling pathways induces TGF-β and retinoic acid production by DCs.	Cong et al[38]
Correct T_H2 skewing	Commensal flora increased STAT6-induced production of IL-12 by DCs, which promoted T_H1 cell differentiation.	Mazmanian et al[66]

iNOS, Inducible nitric oxide synthase; *STAT6*, signal transducer and activator of transcription 6.

same time remain tolerant to commensal microbes, thus avoiding unwanted inflammatory responses in the absence of pathogens.

IECs express low levels of TLR4 and are hyporesponsive to LPS, which explains why they can tolerate exposure to large quantities of luminal bacteria. In addition, the cellular localization of the TLRs can also dictate the nature of the immune response.[35] TLR5 is expressed on the basolateral side of the epithelial cells, and therefore any bacteria encountering these receptors would have to have breached the epithelial barriers and as such be considered pathogenic.[35] In addition to their role in sensing pathogens and commensal organisms, innate signaling pathways are also involved in maintaining epithelial cell homeostasis. Activation of TLR signaling by intestinal commensal bacteria can induce growth factors and cytokines that are required to exert cytoprotective and reparative effects on the IECs.[36] These studies highlight the unique symbiotic communication that exists between the commensal flora and innate immune signaling pathways. In the gut, bacterial products expressed by commensal species are recognized by receptors, such as TLRs, that typically function to defend against pathogens. However, in this environment the interaction of the bacteria with these receptors does not induce an aggressive proinflammatory response but confers protective homeostatic benefits to the host.

Innate signaling pathways are also instrumental in regulating DC tolerogenic functions (Table I).[37,38] Lamina propria DCs can respond to TLR stimulation and produce IL-10 but little or no IL-12. This selective regulation of TLR responsiveness might play a critical role in preventing the mucosal DCs from mounting unnecessary inflammatory responses to commensal organisms.[39] In addition, innate signaling pathways are important in modulating Treg cell responses in the gut; LPS expressed by *Bacteroides* species is capable of inducing CD25 and Foxp3 expression in lamina propria lymphocytes.[40] Through these mechanisms, TLR signaling pathways have been implicated in protection against allergic disease. In animal models of food allergy, luminal bacterial signaling through TLR4 was required for protection, whereas ***CpG*** stimulation (TLR9) also rescued the allergic phenotype by modulating the T_H1/T_H2 immune balance.[41]

EPITHELIAL CELL REGULATION OF IMMUNE CELL FUNCTION

In addition to their role in providing the first line of innate defense against pathogens and commensal organisms, IECs also influence the function of APCs and lymphocytes in the intestinal microenvironment. IECs can regulate DC functions through the secretion of immunomodulatory molecules, such as thymic stromal lymphopoietin and TGF-β.[42] Thymic stromal lymphopoietin is constitutively expressed by IECs and has been shown to limit the expression of IL-12 by DCs while promoting the production of IL-10 and enhancing their ability to drive regulatory cytokine responses.[43,44] TGF-β can also limit proinflammatory cytokine production by both DCs and macrophages.[45] In addition to their effects on APCs, there is increasing evidence that IEC-derived signals can also regulate B- and T-cell functions in the intestine. Deletion of innate signaling pathways exclusively in the IECs resulted in dysregulated B- and T-cell responses in the GALT in a murine model of infection with the gut-dwelling parasite *Trichuris* species.[44] As a consequence of their anatomic location, IECs are in direct contact with intraepithelial lymphocytes and express MHC class II, allowing them to present antigen to T cells.[46]

TREG CELLS

Studies in germ-free (GF) mice have identified an important interaction between the intestinal microbiota and the development of Treg cells both locally in the gut and systemically. GF mice express lower numbers of $CD4^+CD25^+FoxP3^+$ cells both in the mesenteric lymph nodes and peripheral lymph nodes compared with conventional mice, and these Treg cells produce less IL-10.[47] Oral tolerance could not be established in GF mice because of the impaired suppressive function of their $CD4^+CD25^+$ cells and the reduced production of TGF-β and IL-10.[48] There is clear experimental evidence for the direct effects of specific bacterial species on Treg cell development; *Bifidobacterium infantis* can increase the numbers of $CD4^+CD25^+FoxP3^+$ cells in the spleen,[49] whereas strains of *Lactobacillus* species have also been shown to upregulate FoxP3 expression by $CD25^-$ cells *in vitro*.[50]

Recent studies have begun to dissect the effects of specific molecules expressed by commensal species in modulating Treg cell responses. Polysaccharide A (PSA) expressed by *Bacteroides fragilis* is sufficient to induce expansion of a specific subset of IL-10–producing $CD4^+CD25^+FoxP3^+$ Treg cells[51] that can confer protection against intestinal inflammation in mice,[52] and PSA also can provide systemic protection against an autoimmune disease of the central nervous system, experimental autoimmune encephalomyelitis.[53]

Clinical studies have also highlighted the potent effects of the intestinal microbiota on the development of Treg cells in human subjects. A positive correlation was found between the numbers of gram-positive anaerobes (lactobacilli and bifidobacteria) and

gram-negative anaerobes (*Bacteroides* and *Prevotella* species) in maternal stool and IL-10 secretion by cord blood mononuclear cells.[54] Stimulation of naive cord blood lymphocytes with gram-negative bacteria, including anaerobes, such as *Bacteroides* species, was shown to result in strong secretion of IL-10.[55] A recent phase II trial has demonstrated that daily administration of *Lactobacillus salivarius* in healthy adults for 4 weeks enhanced systemic concentrations of IL-10.[56]

APCS

Mucosal DCs continually sample luminal content in the gut and are thus constantly exposed to the gut microbiota. These DCs are actively involved in bridging the gap between the innate and adaptive immune responses in the gut. The expression of pathogen recognition receptors on the surface of the DC allows them to respond to bacterial PAMPs, such as TLRs and NLRs, which are expressed by both commensal and invading pathogenic bacteria, while at the same time presenting antigen to naive T cells. Antigen presentation in conjunction with the production of distinct immunomodulatory cytokines directs the development of specific T-cell subsets. Mucosal DCs primarily induce tolerogenic B- and T-cell responses. DCs have been shown to facilitate IgA secretion by B cells, which is important in maintaining intestinal epithelial barrier function to commensal organisms.[57] In addition, mucosal DCs are critically involved in driving Treg cell differentiation in the gut. A specific subset of $CD103^+$ DCs that are found in the mesenteric lymph nodes and lamina propria are capable of converting naive T cells to inducible $FoxP3^+$ Treg cells through a mechanism that is dependent on TGF-β and ***retinoic acid*** (a dietary metabolite).[58] In addition to DCs, a distinct population of $CD11b^{high}CD11c^-$ macrophages found in the lamina propria are also capable of directing tolerogenic responses by inducing $FoxP3^+$ Treg cell differentiation through secretion of IL-10, TGF-β, and retinoic acid.[59]

The precise mechanisms by which the intestinal microbiota can influence the development and function of APCs remain to be elucidated; however, a study has shown that DCs from GF mice are reduced in numbers and are impaired in their ability to activate T cells.[60] The ability of the microbiota to influence APC responses in the gut is further supported by studies that demonstrate that bone marrow–derived DCs incubated with *Lactobacillus* species had enhanced ability to promote Treg cell differentiation *in vitro* and prevented intestinal inflammation after adoptive transfer *in vivo*.[61] This effect was shown to be dependent on TLR2 and myeloid differentiation primary response gene 88 (MyD88) expression by the DC. Consistent with this, a recent study demonstrated that expression of TLR9 by intestinal DCs, which engages commensal bacterial DNA, might play a role in determining the balance between effector and Treg cell expansion in the gut.[62] Tolerogenic properties of mucosal DCs can also be enhanced through exposure to dietary components. A recent study has shown that engagement of a C-type lectin receptor, on lamina propria DCs, by sugar molecules promoted IL-10, but not proinflammatory cytokine, production, which increased the ability of these DCs to promote the expansion of IL-10–producing T_R1 cells.[63]

EFFECTOR T CELLS

Interaction with microbes in the gastrointestinal tract has been established as a principal environmental signal for postnatal maturation of T-cell function.[10] It is believed that neonates are skewed toward a T_H2-like response at birth[64] and that exposure to gut microbial antigens stimulates the development of T_H1 cells.[65] The shift away from T_H2 responses helps to establish immune tolerance and protects the host from the future development of atopic disease and asthma.[64] Studies in GF mice have demonstrated that in the absence of microbial colonization, T cells will naturally produce more T_H2 cytokines, such as IL-4, compared with T cells from conventionally colonized mice, which have a more balanced T_H1/T_H2 response. Colonization of GF mice with the commensal organism *B fragilis* alone was sufficient to promote a T_H1 response and corrected the T_H1/T_H2 imbalance seen in these mice; this was mediated by activation of signal transducer and activator of transcription 6 signaling in DCs that promoted IL-12–dependent expansion of T_H1 cells.[66] Clinical studies have also demonstrated that probiotic bacteria and their genomic DNA are capable of decreasing antigen-induced T_H2 responses while promoting T_H1 cytokine production.[67]

In addition to correcting T_H1/T_H2 cell immune balance, the microbiota has also been shown to have an effect on the development of T_H17 cells. T_H17 cells are abundantly expressed at steady state in the gut, primarily in the lamina propria, but in GF mice the numbers of T_H17 cells are significantly reduced. A species of commensal bacteria known as sigmentous filamentous bacteria have been recently been identified as a potent inducer of T_H17 cell differentiation in the gut and might be critically involved in providing protection against pathogenic infections.[68] Given the emerging role played by IL-17 in asthma and allergy, it is likely that the development of T_H17 cells in the gut can directly affect the underlying immunopathology of allergic disease.

CAN MANIPULATION OF THE MICROBIOTA PROTECT AGAINST ALLERGIC DISEASE?

Herein we have highlighted the mechanisms by which the microbiota can directly influence effector and regulatory immune mechanisms. These effects are critical in establishing immune tolerance, which protects against allergic disease (Fig 2). The association between the intestinal microflora and allergy is now widely accepted. Epidemiologic evidence (reviewed in this issue by Ly et al[8]) strongly suggests that modulation of immune response mechanisms in the gut can directly affect the development of allergic disease mechanisms in the lung and also other systemic sites of allergic disease, such as the skin. However, the mechanisms by which intestinal immune responses translate to systemic anti-inflammatory or immunosuppressive effects remain to be established. Furthermore, there is limited information on how the regulatory cells that develop in the gut can be directly targeted to systemic sites of allergic disease, such as the skin during atopic dermatitis or the lung, for example, to decrease allergic inflammatory responses during asthma. In addition, it is unclear whether the phenotypes of these cells alter once they are outside the intestinal immune system. A recent review highlighted the mechanisms by which the development of immune tolerance in the lung is directly related to the pathogenesis of asthma; however, as yet, we have little appreciation of the extent to which immunologic processes in the gut, which lead to the development of tolerance, are relevant to systemic allergic diseases and moreover whether these effects might be site specific, for example, are the mechanisms of immune tolerance required to protect against

asthma distinct from those required to protect against food allergies?

Studies to address these types of questions have been facilitated by advances in the development of animal models of allergic airway disease that results from intestinal microbial disruption. Mice treated with antibiotics followed by a single oral gavage of *Candida albicans* exhibit significant alterations in the composition of the intestinal commensal flora. These animals were then exposed to an aerosol challenge of a common allergen. Introduction of allergen to the lungs of mice that had a disrupted microbiota resulted in a local increase in CD4 cell–mediated inflammation.[69] This model is the first to directly demonstrate that allergy can develop as a consequence of an altered intestinal microbiota and will provide a platform to dissect the immune-mediated mechanisms of microbiota-induced protection against allergy.

It is clear that the microbiota can have profound immunomodulatory effects that are instrumental in maintaining immune tolerance. Environmental or dietary influences that can manipulate the intestinal microbial ecosystem and have the capacity to influence immune responses at a distant site therefore have the potential to treat or, more importantly, prevent asthma and other allergic diseases.

PROBIOTICS

The World Health Organization has defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host."[70] Probiotics are typically of the *Lactobacillus* or *Bifidobacterium* species that, when ingested, increase the levels of these beneficial colonizers in the gut.[71] Prebiotics are nondigestible food ingredients, in particular nondigestible carbohydrates and oligosaccharides, that selectively stimulate the growth of the host's own specific microflora; for example, inulin and oligofructose promote the growth of bifidobacteria.[72]

The rationale for the use of probiotics in the treatment of allergic diseases is supported by the hygiene hypothesis, and there are encouraging data emerging on the use of probiotics in the treatment of atopic eczema in neonates.[73] However, to date, the overall clinical evidence on the benefits of probiotics in the prevention of allergic disease is inconclusive.[74] It is likely that quantity and time of administration, as well as the interaction with specific components of the host's microflora, will heavily influence the immunomodulatory effects of probiotics (and prebiotics). The situation is complicated by the fact that that not all probiotic strains have similar immunomodualtory capabilities.[50] Individual species within the genus *Lactobacillus* can differentially affect allergic status in infants,[75] most likely because of their contrasting capacities to induce regulatory cytokine production.[76] The differential effects of probiotic species is unsurprising based on the fact that distinct immunomodulatory effects in the gut can be attributed not only to individual species of commensal bacteria but also to distinct molecules expressed by these organisms.[66] It remains to be established whether a global increase in a specific bacterial species over a specific length of time will be sufficient to induce long-term sustainable immunologic effects that could decrease or control allergic inflammation in all subjects. In this regard perhaps the use of prebiotics that can enhance or promote the specific growth of our own intrinsic bacterial communities might actually represent a more useful approach but will most likely depend on the dose and timing of the prebiotic administration, as well as underlying host genetics factors.

OBESITY

Clinical studies have established a link between obesity and atopic disease.[77] The underlying immunologic mechanisms behind this link can in part be explained by a decrease in immune tolerance as a consequence of immunologic signals produced by the adipose tissue.[78,79] Notwithstanding the direct contributions made by the obese state to the disruption of immune tolerance, it is also plausible that obesity could alter tolerogenic responses as a result of indirect effects on the gut microbiota. Studies have shown that obesity is associated with substantial changes in the composition and metabolic functions of the gut microflora.[80] This altered "obese flora" is capable of extracting increased levels of energy from the diet compared with the normal nonobese flora.

Although specific microbiota might predispose to or enhance weight gain, it is also possible that obesity-associated alterations in intestinal microflora might have a wider-reaching effect on the immune system. If obesity results in a reduction or imbalance in specific commensal organisms that are required to maintain immune tolerance, this might directly affect the development of allergic diseases. Consistent with this, 16S RNA sequencing has demonstrated a 50% reduction in the abundance of Bacteroidetes in genetically obese mice compared with that seen in nonobese mice fed the same diet.[81] It has been well documented that *Bacteroides fragilis* have the ability to promote Treg cell responses and protect against inflammatory disease.[51-53]

VITAMIN D

There is increasing evidence that Vitamin D might play a role in protecting against asthma and allergic disease. As a consequence of lack of exposure to sunlight, vitamin D deficiency (VDD) is now common in populations worldwide.[82] Studies have established a clear association between VDD and the increased incidence of asthma and other atopic diseases.[83] Polymorphisms in the vitamin D receptor gene[84] and other genes in the vitamin D signaling pathway are associated with increased susceptibility to allergy and asthma.[85] Vitamin D has been shown to have direct effects on lung function that might in part explain its protective role in asthma[86]; furthermore, vitamin D can also exert potent immunomodulatory effects that help to establish tolerance. The vitamin D metabolite 1,25-dihydroxyvitamin D3 has been shown to induce ***tolerogenic dendritic cells***, promote $CD4^+CD25^+FoxP3^+$ Treg cell activation, and induce IL-10 production.[87] In addition to its immunomodulatory effects on the adaptive immune system, vitamin D can also influence innate immunity by promoting ***antimicrobial peptide*** production, particularly at the epithelial surfaces,[88] and in line with this, VDD has been associated with increased susceptibility to pathogenic infections.[89] There is increasing interest in the role of vitamin D at the intestinal mucosal surface, and it is thought that VDD, which results in decreased production of antimicrobial peptides by IECs, might result in perturbations in the intestinal microbial ecology and create a situation of dysbiosis. In support of this hypothesis, diet-induced VDD in mice was shown to predispose the mice to colitis as a result of alterations in the enteric bacteria.[90] To date, no study has demonstrated a direct effect of VDD or vitamin D supplementation on the composition of the intestinal flora, and

it is likely that the levels of exposure to vitamin D, as well as the timing of exposure, would influence its effects. However, it seems likely that the indirect effects of vitamin D on the intestinal microbiota could contribute to its protective role in allergy and asthma, and this remains an active area of research.

FUTURE PERSPECTIVES

As we have discussed herein, the dialogue between the microbiota and the host is multifaceted and involves specific interactions between unique molecules expressed by the bacteria, with receptors on specific cells of the host's immune system. A critical goal for researchers is to identify what constitutes a healthy microbiota. The field has been significantly advanced in recent years through the introduction of culture-independent techniques, such as 16s RNA sequencing, for identifying intestinal microbial species.[91] The advent of metagenomics means that scientists can now sequence an entire collection of DNA in a microbial sample and identify previously unculturable component species. Segmented filamentous bacteria is an example of a nonculturable commensal species that is now a major focus of attention because of its role in coordinating T-cell responses in the intestinal mucosa.[68]

Can we really manipulate our microbiota directly to confer health benefits? There is clinical evidence to suggest that we can. Bacteriatherapy or fecal transplantation has been used to treat antibiotic-associated *Clostridium difficile* infection. Systemic broad-spectrum antimicrobial agents can disrupt the ecologic bacterial balance in the colon and facilitate overgrowth of *C difficile*, which can cause severe diarrhea and clinical symptoms of colitis. A recent study has demonstrated that the introduction of fecal flora from one subject, who was genetically profiled before transplantation, resulted in the establishment of a stable microbial community in the recipient that was genetically identical to the donor flora. This change in bacterial composition was accompanied by resolution of the disease.[92]

An alternative therapeutic strategy might be to harness the immunomodulatory properties of specific bacterial components and administer these molecules to adjust the immune imbalances occurring in allergic subjects. A carbohydrate molecule, PSA, isolated from the gram-negative commensal organism *B fragilis* has been shown to promote Treg cell activation, and as a result of this mechanism, treatment with PSA was protective in animal models of inflammatory[52] and autoimmune[53] diseases. In addition, administration of purified PSA was sufficient to readdress the T_H2 immune skewing that occurs in GF mice by driving T_H1 cell expansion in the gut.[66] These data from animal models highlight the potential for the development of bacteria-derived molecules as immunotherapeutics in the treatment of allergic disease.

The fact that microbiota are different in every subject poses critical challenges in the development of therapeutic or prophylactic approaches to promote a healthy microbiota. At the species level, it is thought that a subject's intestinal microbiota is as unique as his or her fingerprint. The human microbiome project was established with the specific goal of leveraging new, high-throughput technologies to characterize the human microbiome of at least 250 healthy volunteers in addition to determining specific associations between alterations in the microbiome and health and disease. This and related initiatives might in the future enable us to selectively enhance or suppress specific target organisms in a subject's microbiota in a way that could provide the precise immune signals required to protect against allergy and also other diseases, such as cancer and autoimmune diseases.

What we know

- **The intestinal mucosa is an immune-privileged site, which under normal conditions can tolerate exposure to a huge antigen load without resulting in an inflammatory response.**
- **The intestinal microbiota plays a key role in promoting maturation of the immune system, particularly during the neonatal period, and is influenced throughout life by environmental factors.**
- **The intestinal microbiota can affect both the innate and adaptive immune pathways.**
- **The immunomodulatory effects of the intestinal microbiota are integral to establishing immune tolerance through expansion of Treg cells and induction of immunosuppressive cytokines.**
- **A breakdown or dysregulation of immune tolerance in the host will result in unwanted inflammatory immune responses against innocuous antigens, resulting in allergic disease.**
- **Maintenance of immune tolerance is critical in protection against allergic and autoimmune diseases.**
- **Microbiota play a role in protection against allergic and autoimmune diseases by helping to maintain immune tolerance.**
- **Harnessing the immunomodulatory effects of commensal species, the molecules they express, or both represents an attractive approach for developing novel therapeutics for the treatment of allergic diseases.**

What we still do not know

- **The mechanisms by which immunomodulatory effects in the gut actually translate to protective systemic anti-inflammatory effects**
- **Whether the establishment of intestinal immune tolerance will influence the development of distinct allergic disease in a similar manner depending on the site of disease**
- **The precise composition of a healthy microbiota, which is required to confer maximum immune-mediated protection**
- **The best strategy for establishing a healthy microbiota during fetal life, immediately after birth, and throughout life**
- **How to manipulate an unhealthy microbiota to re-establish its positive effects on health**

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