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Brief Reviews

The Journal of Immunology

The Influence of the Microbiome on Allergic Sensitization to Food

Catherine H. Plunkett* and Cathryn R. Nagler*,†

The alarming increase in the incidence and severity of food allergies has coincided with lifestyle changes in Western societies, such as dietary modifications and increased antibiotic use. These demographic shifts have profoundly altered the coevolved relationship between host and microbiota, depleting bacterial populations critical for the maintenance of mucosal homeostasis. There is increasing evidence that the dysbiosis associated with sensitization to food fails to stimulate protective tolerogenic pathways, leading to the development of the type 2 immune responses that characterize allergic disease. Defining the role of beneficial allergy-protective members of the microbiota in the regulation of tolerance to food has exciting potential for new interventions to treat dietary allergies by modulation of the microbiota. *The Journal of Immunology*, **2017, 198: 581–589.**

The incidence of allergic diseases has increased dramatically over the last 50 y, particularly in developed countries. The rise in prevalence occurred in such a short time frame that genetics alone cannot explain it. This same time period was marked by improvements in sanitation, stark dietary changes, and increased vaccination and antibiotic use in Western societies, all of which were linked to increasing susceptibility to allergic and autoimmune diseases (1–4). What these lifestyle changes have in common is their ability to alter the populations of commensal microbes (the microbiota) that live in and on our bodies (1). The hygiene hypothesis was the first to imply a link between microbes and allergy by suggesting that the lower incidence of allergic diseases in children with older siblings resulted from increased exposure to infectious disease in early childhood (5). This idea was expanded in subsequent epidemiological studies that found that children raised in rural environments had a lower incidence of allergic disease than those in urban settings and had greater environmental exposure to microbial products, such as LPS (6–10). More recent work revised the original hygiene hypothesis concept to include increased antibiotic use and vaccination as other lifestyle changes that have reduced childhood infections and altered the microbiota (11). Cesarean birth and formula feeding have also disturbed nature's coevolved strategy, altering founder microbial taxa and increasing susceptibility to diseases associated with this "Western" lifestyle (12–14). Consumption of a highly processed modern diet, high in fat, low in fiber, and quite different from that of our ancestors, has had profound consequences for the composition of the intestinal microbiota (15–17). Collectively, these studies suggest that environmental and lifestyle changes have affected the relationship between the commensal microbiota and its human host and contributed to the increasing incidence of allergic disease.

The skin and all mucosal surfaces are populated by a site-specific microbiota (18). The microbes present can include bacteria, viruses, bacteriophages, Archaea, fungi, parasitic worms, and protists (19, 20). Commensal bacteria are the best characterized, particularly in the gastrointestinal tract, where their density increases from an estimated 10^4 to 10^8 per milliliter of luminal contents in the small intestine to $\sim 10^{11}$ organisms per milliliter of luminal content in the colon, the highest bacterial density of any environment analyzed (21). In addition to this large community of bacteria, the gastrointestinal tract contains more immune cells than any other organ. The two are in intimate communication; maintenance of homeostasis between these microbes and the immune system is essential to health. Exciting new research is beginning to identify the mechanisms by which beneficial functions of the microbiota regulate tolerance to dietary Ags (22, 23).

In this review, we discuss the role of the microbiota in maintaining tolerance to food and examine how commensal dysbiosis promotes the development of food allergy. Finally, we examine the clinical evidence for a role for the microbiota in regulating food allergen sensitization and explore strategies for the development of microbiome-modulating therapeutics to prevent or treat food allergy.

Extending the hygiene hypothesis to the microbiota

The pathogenesis of food allergy involves an aberrant type 2 immune response to dietary Ags. The most common allergenic foods are tree nuts, peanuts, milk, eggs, shellfish, fish,

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Abbreviations used in this article: DC, dendritic cell; fTh2, food Ag–specific Th2 cell; GAP, goblet cell–associated Ag passage; ILC, innate lymphoid cell; LN, lymph node; mLN, mesenteric lymph node; RA, retinoic acid; SCFA, short-chain fatty acid; Treg, regulatory T cell.

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wheat, and soy, although a great number of other foods can elicit an allergic response (24). A healthy immune response to food Ags is a state of nonresponsiveness, referred to as oral tolerance (25). When oral tolerance is not induced, food Ags can instead evoke a response that is characterized by differentiation of naive T cells into food Ag–specific Th2 cells (fTh2s), which produce large amounts of IL-4 and IL-13 that drive B cells to produce Ag-specific IgE (26). IgE binds to the surface of mast cells and, upon re-exposure, Ag cross-linking results in mast cell degranulation. The release of mast cell mediators, such as histamine, leads to the symptoms associated with food allergy, including anaphylaxis (27). In support of the epidemiological observations attributing a protective role to the microbiota in constraining allergic responses, germ-free mice, which are born and raised in a sterile environment, have an exaggerated systemic type 2 immune response, which is characterized by high levels of IgE, and are more susceptible to oral Ag–induced anaphylaxis than are mice colonized with a diverse microbiota (28, 29). Early work demonstrated that administration of LPS to germ-free mice is sufficient to restore oral tolerance (30). In keeping with this observation, mice unable to signal via TLR4, the receptor for LPS, exhibit increased allergen-specific IgE and exacerbated anaphylactic symptoms in response to repeated intragastric administration of peanut extract plus cholera toxin compared with TLR4-sufficient mice (31). TLR4 is one of a group of pattern recognition receptors that the immune system uses to detect microbe-derived products, including LPS and DNA. Microbial sensing through TLRs is critical for maintaining intestinal homeostasis and limiting inflammation (32). Oral administration of a broad-spectrum antibiotic mixture evoked food allergen sensitization in TLR4-sufficient mice, suggesting that intestinal bacteria were the source of the TLR4 ligand (31). Recent work has begun to reveal an even more complex role for LPS in allergic disease (33). Fecal samples were collected during the first 3 y of life from genetically related, but geographically separated, children at high (Finnish), low (Russian), and transitional (Estonian) risk for the development of autoimmune and allergic disease, including food allergy. The investigators found that the low-risk Russian children had higher proportions of *Bifidobacterium*, whereas Finnish and Estonian children had increased abundance of *Bacteroides*. Surprisingly, metagenomic analysis revealed striking differences in LPS synthesis between the Finnish and Russian cohorts. Russian children had LPS mostly originating from *Escherichia coli*, whereas the bulk of the LPS in Finnish children originated from *Bacteroides*. Importantly, the LPS variant produced by *Bacteroides* was structurally and functionally distinct from *E. coli* LPS. *E. coli* LPS is strongly immunostimulatory, and chronic exposure results in a refractory state known as endotoxin tolerance (34), which is thought to contribute to the protective effects of the microbiota suggested by the hygiene hypothesis (35). *Bacteroides*-derived LPS was less immunostimulatory to primary human PBMCs than *E. coli* LPS, and when PBMCs were treated with the two LPS variants mixed together, the high cytokine production elicited by *E. coli* LPS was abrogated. These findings raise the possibility that colonization early in life with a low-immunostimulatory microbiota can impair aspects of immune education and predispose to inflammatory diseases, such as food allergy (35, 36). A better understanding of how various components of the microbiota influence immune system development will inform therapeutic strategies aimed at restoring the benefits conferred by particular microbial communities.

Oral tolerance to food Ags

The gastrointestinal tract is under constant bombardment by microbial and food Ags. Therefore, of necessity, the healthy intestinal immune system is geared toward a tolerogenic response characterized by the presence of large numbers of regulatory T cells (Tregs). Originally, it was believed that oral tolerance was primarily mediated by the generation of food Ag–specific Tregs (37). Ag encountered in the lamina propria is taken up by a population of $CD103^+$ intestinal dendritic cells (DCs) that migrate to the draining mesenteric lymph nodes (mLNs). Within the mLN, large amounts of retinoic acid (RA), a vitamin A derivative, and TGF-β produced by $CD103^+$ DCs and LN stromal cells instruct Ag-specific naive T cells to differentiate into Tregs (38–40). Additionally, RA and TGF-β induce upregulation of the gut-homing receptors CCR9 and α4β7 on these newly differentiated Tregs to recruit them back to the intestinal lamina propria (38–40), where these Tregs are expanded by the production of IL-10 from resident CX3CR1 macrophages (41). Some of these newly expanded Tregs may also enter into the circulation to mediate systemic tolerance to orally available Ags (42). In support of this model, oral tolerance is abrogated in the absence of gut homing or in animals lacking CX3CR1 macrophages (41). Other work showed that oral Ag exposure induces an allergic phenotype in mice with vitamin A deficiency (43). RA-deficient mLN DCs drive naive T cells toward a pathogenic Th2 phenotype instead of Tregs (43). Collectively, these data support the concept that food Ag–specific Tregs are critical for protection from dietary allergies (41, 42). However, these studies only showed that Ag-specific Tregs, induced by oral administration of model food Ags, such as OVA, induce nonresponsiveness to subsequent peripheral immunization (42). As such, it has not been clear whether Treg development is a feature common to all food Ags found in a complex diet or whether these food Ag–specific Tregs also contribute to intestinal homeostasis. Recent work using germ-free mice weaned onto an elemental diet void of Ags demonstrated that the majority of Tregs in the small intestine are indeed induced in response to food Ags in a complex diet (44). The induction of small intestinal Tregs occurs rapidly following the introduction of solid food and decreases over 4–6 wk following removal of food Ags (44). Mice weaned onto Ag-free diets had a greater proportion of Ag-specific T cells differentiating into inflammatory T cells rather than Tregs following oral Ag administration, suggesting that Tregs raised against dietary Ags limit proinflammatory responses to model Ags, such as OVA. Therefore, food Ag–specific Tregs contribute to protection against allergic sensitization to dietary Ags, possibly by reinforcing a tolerogenic environment in the small intestine.

Microbiota-mediated tolerance to food Ags

In addition to dietary Ags, the intestinal immune system must maintain a tolerogenic response to the microbiota resident within the gut lumen. Components of the microbiota strongly induce colonic Tregs, as demonstrated by a deficit in Tregs in

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the colonic lamina propria of germ-free mice, which increases in frequency following colonization (45, 46). Colonic Treg induction was attributed to Clostridia, a class of mucosa-associated Firmicutes (45, 47). Spore-forming Clostridia isolated from mouse and human feces strongly induce colonic Tregs (45, 47). However, it was suggested more recently that colonic Tregs can also be induced by other members of the microbiota (48). It is unclear whether there is a common mode of action of Treg induction between these diverse bacterial groups or whether they stimulate specific TLRs. It is also not known whether all (or most) Tregs induced by commensal bacteria bear bacteria-specific TCRs (49). Moreover, there is evidence that bacteria-induced Tregs also contribute to tolerance toward other Ags, including those from food. Indeed, Clostridia-induced Treg expansion was associated with protection from food allergen sensitization (22, 45, 47). Because there is heterogeneity within the intestinal Treg population, it is possible that particular bacteria may induce specific populations of Tregs that have different functions for maintaining homeostasis (50). Kim et al. (44) observed that antibiotic treatment markedly reduces RORγt$^+$ Tregs in the colonic lamina propria, suggesting that these Tregs are bacteria dependent, whereas mice fed an Ag-free diet had a selective reduction in RORγt$^-$ Tregs in the small intestine, indicating these are food Ag dependent. This finding supports previous literature that commensal bacteria induce a population of RORγt$^+$ Tregs in the colon (48, 51). DCs from the small intestine and colon migrate to anatomically different mLNs and induce immunologically distinct T cell responses (52, 53). Colonic and small intestinal DCs differ phenotypically and functionally, which may reflect the different antigenic burdens encountered by DCs at these physiologically distinct sites along the GI tract (52, 53). This finding may help to explain why bacteria and food Ags induce distinct populations of Tregs that differ in location and phenotype. Evidence that bacteria-mediated Treg expansion can protect from food allergen sensitization (22, 45, 47) suggests that food- and bacteria-derived Tregs work cooperatively to mediate oral tolerance and protect from food allergen sensitization (Fig. 1). It is possible that bacteria-specific Tregs migrate to the small intestinal lamina propria (perhaps through recirculation via the mLN) and secrete IL-10 to reinforce the tolerogenic environment (54). Indeed, a population of RORγt$^+$ Tregs is present within the small intestine, albeit at a lower frequency than in the colon, and is reduced following antibiotic treatment, supporting the idea that microbe-driven Tregs can also localize at this site (44). In light of this, the tolerogenic environment maintained by bacteria-derived Tregs likely contributes to the production of food Ag–specific Tregs, rather than proallergic Th2 cells, upon subsequent exposure

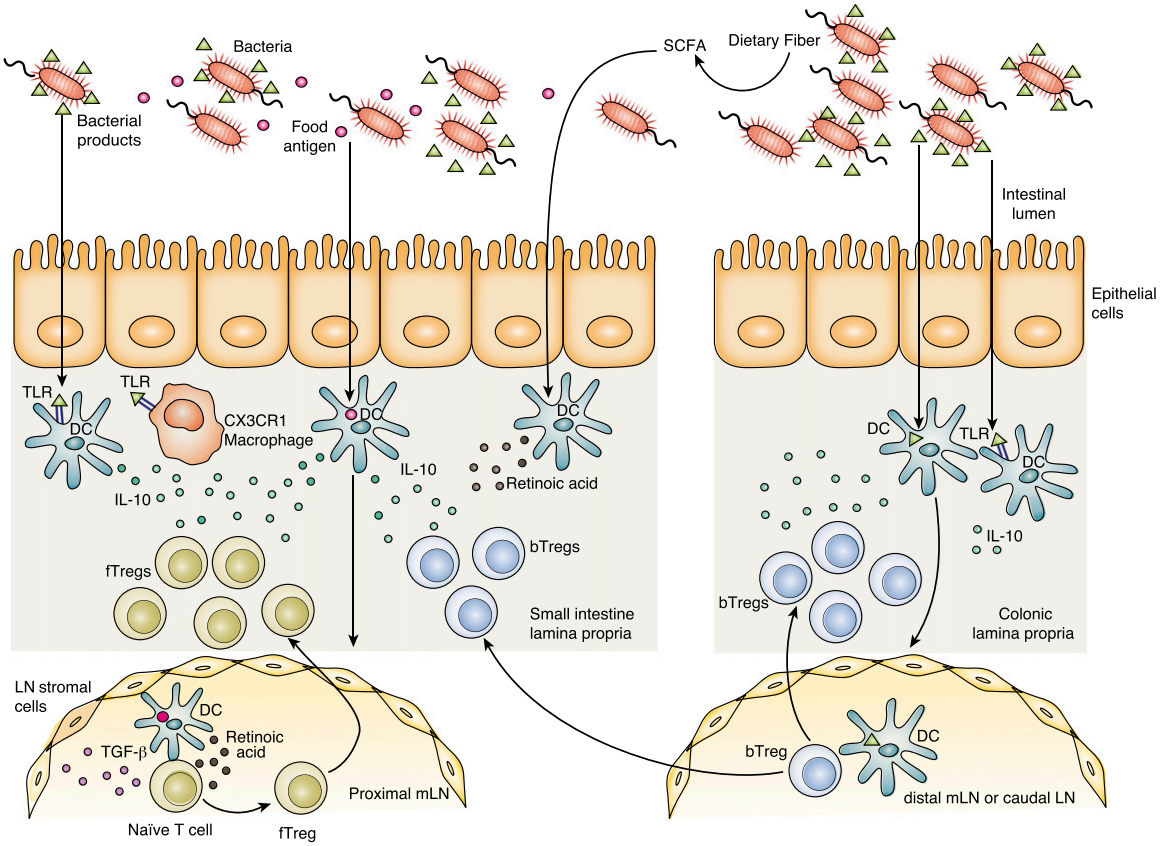

FIGURE 1. Induction of tolerance to food and bacterial Ags in the intestine: food Ag is taken up by DCs in the small intestine that migrate to the proximal mLN. TGF-β and RA, produced by LN stromal cells and DCs respectively, induce the differentiation of naive T cells to food Ag–specific Tregs (fTregs). RA and TGF-β also induce upregulation of gut-homing receptors on these newly differentiated Tregs to recruit them back to the lamina propria. TLR signaling by bacterial products induces the production of IL-10 by CX3CR1 macrophages resident in the lamina propria, supporting Treg expansion in this site. Bacterial products are also taken up by colonic DCs that migrate to the distal mLN and caudal LN to induce differentiation of bacterial-specific Tregs (bTregs). Although predominant in the colon, bTregs also migrate to the small intestine where they release IL-10 to maintain the tolerogenic immune environment. Fermentation of dietary fiber to SCFAs may enhance RA production by DCs and promote Treg differentiation. TLR signaling by bacterial products, such as LPS, induces a tolerogenic phenotype in colonic and small intestinal DCs that promotes differentiation of Tregs.

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to food Ags. In addition, RORγt$^+$ Tregs are found in small numbers in other body sites, including the lung, spleen, and skin (55), suggesting that these cells disseminate throughout the body. This may help to explain the role of the intestinal microbiota in protection from other allergic diseases, including asthma.

Other studies are beginning to identify the signaling pathways for bacteria-mediated Treg induction that are important for preventing allergic sensitization to food. Several reports implicated microbial sensing by host TLRs in the induction of colonic Tregs that maintain tolerogenic responses (46, 56). Mice lacking MyD88, an adaptor molecule for downstream TLR signaling, develop more severe intestinal inflammation in response to epithelial damage induced by dextran sodium sulfate, suggesting an important role for microbial sensing in limiting inflammatory responses (32). TRAF6 is another important adaptor molecule for TLR signaling that acts downstream of MyD88 to activate transcription factors, such as NF-κB, to induce cytokine production (57). Mice with a specific deletion of TRAF6 in CD11c$^+$ APCs (Traf6ΔCD11c) have reduced numbers of Tregs in their small intestine and present with a spontaneous type 2 inflammation at this site (58). Exacerbation of the inflammatory phenotype in germ-free Traf6ΔCD11c mice further supports a role for microbe-induced Tregs in preventing pathogenic type 2 intestinal inflammation (58, 59). Interestingly, this phenotype was specific for the small intestine, because no overt inflammation was observed in the colon. This may be due to the different physiological functions of the small and large intestine, because the large intestine functions primarily to reabsorb water, whereas the small intestine encounters the bulk of the food Ags. As such, the tissue-selective inflammation observed in this model may indicate that the inflammatory response occurs as a result of a breakdown in tolerance to food Ags.

In addition to microbial ligands that can be sensed via pattern recognition receptors, commensal bacteria release metabolites, such as short-chain fatty acids (SCFAs), upon fermentation of insoluble dietary fibers (4). SCFAs, including acetate, propionate, and butyrate, were demonstrated to have immunogenic activity locally, within the intestine, and systemically (23, 60–62). SCFAs are used by colonocytes as an energy source (63, 64), but they also have immunomodulatory properties by signaling through G protein–coupled receptors (65–67) and act to inhibit histone deactelylases (68). One possible consequence of dysbiosis for the development of allergic responses may result from reduced levels of SCFAs (69). There is a body of literature supporting the idea that low levels of SCFAs are associated with an allergic phenotype and that increasing SCFA levels can ameliorate disease (61, 63, 70). One mechanism by which SCFAs protect against allergic disease is through the induction of colonic Tregs (60, 62, 68). The addition of SCFAs to the drinking water of germ-free mice resulted in an increased abundance of colonic Tregs and protected against colonic inflammation (62). Recent work showed that mice fed a high-fiber diet had increased SCFA levels and were protected against food allergy, in part through enhanced induction of Tregs in the mLN (23). DCs isolated from the mLN of the mice fed a high-fiber diet had increased retinal dehydrogenase activity compared with controls, suggesting a link between the protective effects of dietary fiber and vitamin A metabolism (23).

Microbiota-mediated modulation of intestinal barrier function

It is remarkable to think that a single layer of epithelial cells is all that separates the enormous number and variety of food Ags and microbes within the intestinal lumen from the underlying immune cell–rich lamina propria. Specialized epithelial subpopulations have unique properties and functions that are important for enforcing barrier protection to prevent uncontrolled access of Ags to the lamina propria (53). These include the production of antimicrobial peptides by Paneth cells and of mucus by goblet cells, which together form a physical barrier that limits the access of bacteria to the epithelial surface (71). Additionally, intestinal epithelial cells express tight junction proteins that seal off the underlying immune-rich lamina propria from excessive exposure to luminal Ags (72). We found that increased intestinal permeability contributes to food Ag sensitization. Stefka et al. observed that sensitization of mice to peanuts is associated with an increased concentration of the major peanut allergens Ara h 2 and Ara h 6 in the serum (22). Colonization with a consortium of mucosa-associated Clostridia reduced serum Ara h 2 and Ara h 6 and ameliorated allergic sensitization to peanut, suggesting that commensal microbe–driven reinforcement of the epithelial barrier is important for protection against food allergy (22). The improved barrier function observed after colonization by this Clostridia consortium was associated with an increase in goblet cell numbers and mucus production in the intestine, as well as upregulated expression of the antimicrobial peptides Reg3β and Reg3γ, suggesting a broad effect on multiple intestinal epithelial cell types (22). Intestinal mucus and antimicrobial peptide production are regulated by the barrier-protective cytokine IL-22 (73). By using treatment with a neutralizing Ab for IL-22 or depletion of innate lymphoid cells (ILCs), which produce IL-22, Stefka et al. (22) demonstrated that Clostridia-induced IL-22 production by type 3 ILCs was necessary and sufficient to reduce intestinal permeability and prevent food Ag sensitization in this model. This finding highlights the complex role of the microbiota in modulating innate immune responses that, in turn, influence intestinal epithelial cell function and regulate its barrier-protective properties (Fig. 2).

Indeed, the microbiota has a profound influence on mucus production and goblet cell homeostasis (74). Germ-free mice have altered mucus production compared with colonized mice, with differences observed in the small and large intestine (74). These mucus layers have unique features that limit access of bacteria to the intestinal epithelium (74). In the large intestine the mucus forms two layers: an inner sterile layer and a diffuse outer layer that is heavily colonized by bacteria that are thought to feed on mucins and other proteins within the mucus layer (75). The permeability of the mucus layer differs along the length of the intestine. In the distal colon, the inner mucus layer is impenetrable to bacteria-sized beads, whereas some penetrability is observed in the proximal colon (76). In the small intestine, the mucus layer is diffuse and allows limited penetration by bacteria; however, secretion of antimicrobial peptides and high gut motility (which removes this easily detached mucus) helps to limit access to the epithelium (76). Within 5 wk postcolonization of germ-free mice, mucus thickness, impenetrability, and detachment properties in the colon and small intestine resemble those seen in conventional

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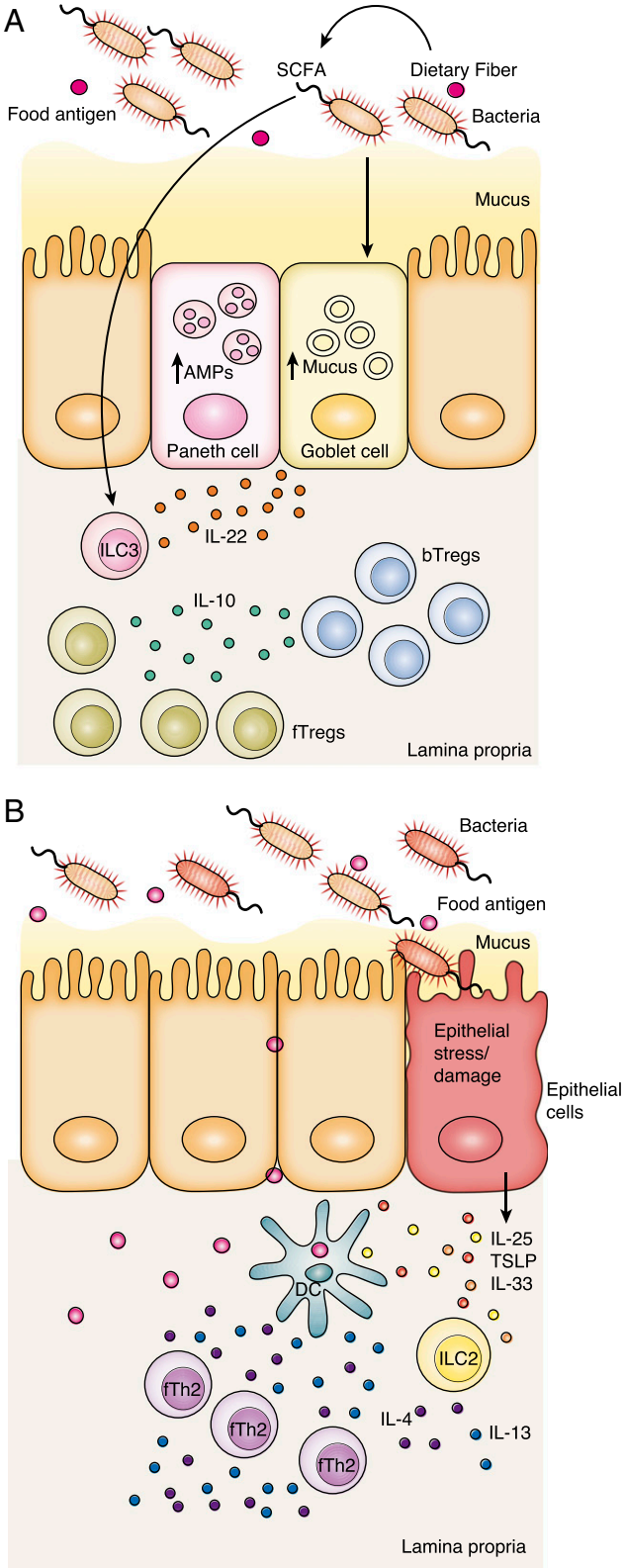

FIGURE 2. The microbiota regulates protective and pathogenic barrier responses in the intestine. (**A**) A healthy microbiota induces a barrier-protective response in the intestine, in part, through production of SCFAs that are most likely to act on type 3 ILCs to produce IL-22. IL-22 induces antimicrobial peptide (AMP) production by Paneth cells and mucus production by goblet cells to reinforce barrier function, controlling the location and composition of the microbiota. This barrier-protective function prevents uncontrolled access to the lamina propria by food Ags to prevent allergic sensitization. (**B**) Dysbiosis fails to induce these protective pathways. Dysregulated epithelial barrier function and a compromised mucus layer allow increased permeability to food Ags. Damaged or stressed epithelial cells release the alarmins IL-25, IL-33, and TSLP that activate type 2 ILCs to produce IL-4 and IL-13, which promotes the development of allergic sensitization to food Ags through the generation of fTh2s.

mice (74). Alteration in mucus properties postcolonization is associated with changes in the microbiota (74). Because treatment with some antibiotics also alters the mucus layer (77), this suggests that changes in the microbiota affect mucus-associated barrier function in the intestine. Stefka et al. (22) demonstrated that one mechanism by which Clostridia protect against allergic sensitization is by eliciting increased mucus production. Additionally, germ-free and antibiotic-treated mice have increased susceptibility to oral Ag sensitization (22, 29). Given the profound defect in mucus production in these mice, it is likely that the altered mucus production and subsequent defects in barrier function contribute to a breakdown in oral tolerance. However, goblet cells and mucus play a role in the maintenance of intestine homeostasis beyond their well-known contributions to barrier function. It was reported that culturing DCs in vitro with the mucus protein Muc2 induces a tolerogenic DC phenotype that induces greater Treg differentiation from naive T cells (78). Tolerance to OVA is impaired in Muc2-deficient mice; mucosal and systemic tolerance is restored when OVA is coadministered in the presence of Muc2 protein (78). Other work identified that goblet cells form goblet cell–associated Ag passages (GAPs) by taking up luminal Ag and delivering it to intestinal DCs (79–81). GAP formation is a regulated process, induced by acetylcholine acting on goblet cells. GAPs form in the steady-state in the small intestine, but goblet cell responsiveness to acetylcholine is inhibited by MyD88-dependent, goblet cell–intrinsic sensing of the gut microbiota in the colon (80). Deletion of MyD88 in goblet cells, or disruption of the microbiota by antibiotics, overrides the normal suppression by the microbiota and allows the formation of colonic GAPs, potentiating inflammation due to uncontrolled exposure to luminal contents (80, 81). Moreover, altering GAP formation in early life results in persistent Th2 responses (R. Newberry, personal communication), providing an additional link between alterations in the gut microbiota in early life and a predisposition to food allergy.

Intestinal epithelial cells also monitor the luminal environment and produce cytokines that direct immune responses in the underlying lamina propria (82). Stressed or damaged intestinal epithelial cells secrete the cytokines IL-33, TSLP, and IL-25, also collectively referred to as alarmins, to induce protective immunity and promote repair (82). These cytokines are associated with the initiation of the protective type 2 immune response that is responsible for the clearance of enteric helminths and repair of epithelial damage induced by infection (83–85). Epithelial alarmins activate type 2 ILCs to produce IL-13 and prime intestinal DCs to promote the differentiation of naive T cells to Th2 cells that mediate worm clearance (82, 86). Recently, the population of epithelial cells that produces IL-25 was revealed to be a specialized lineage known as tuft cells (83–85, 87). Howitt et al. showed that tuft cells release IL-25 in response to activation by parasites via chemosensory receptors (84). Because some chemosensory receptors are G protein–coupled receptors, this raises the

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interesting possibility that, in addition to helminths, these receptors may also respond to microbe-derived signals, such as SCFAs, skewing the immune environment in the intestine. Indeed, there is evidence that the microbiota can directly regulate the expression of epithelial alarmins in the intestine. Intestinal expression of IL-25, IL-33, and TSLP is reduced in germ-free mice and increases following colonization (88–90). Moreover, administration of IL-25 can alter the expression of antimicrobial peptides, changing the composition of the microbiota and demonstrating a role for IL-25 in the host-microbe cross-talk that is essential for homeostasis (91). In the context of allergic inflammation, allergen exposure can induce the release of alarmins and drive the pathogenic type 2 immune response associated with disease (71). Overexpression of IL-25 or IL-33 drives allergic responses to dietary Ags, suggesting that these epithelial-derived cytokines may contribute to the development of dietary allergies by skewing the immune environment in the intestine from tolerogenic to proallergic (92, 93). A dysbiotic microbiome might also elicit epithelial alarmins and prime for allergic sensitization to food (94). In this setting, characterized by IL-4 production by mast cells and Th2 T cells, oral allergen exposure results in reprogramming of Tregs from a tolerogenic to a Th2-like phenotype, further propagating the allergic response (95). IL-33 was shown to drive the expansion of GATA3$^+$ Tregs in the colon (96), which are similar in phenotype to reprogrammed Tregs implicated in the loss of tolerance to food Ags (95), supporting the idea that epithelial alarmin production may alter the immunological environment in the intestine and contribute to allergic sensitization. In further support of this hypothesis, consumption of a low-fiber diet that exacerbates food allergen sensitization is associated with increased expression of IL-33 and TSLP in the intestine (23). Conversely, protection against allergic sensitization following administration of a high-fiber diet alters the microbiota and is associated with reduced expression of IL-33 and TSLP. Taken together, these data suggest that a healthy microbiota may protect against allergic sensitization by reducing the expression of alarmins by intestinal epithelial cells. However, in conditions of dysbiosis, the microbiota may induce elevated levels of these alarmins, resulting in aberrant Th2 responses toward dietary Ags by skewing the immune environment in the intestine toward a type 2, rather than a tolerogenic, response (Fig. 2). Identifying how epithelial cell alarmin production is regulated may help to identify new targets to prevent allergic sensitization to food.

Clinical considerations and potential therapeutics

The findings described above outline the complex interplay between host immunity and the microbiota. Translational studies are beginning to explore a role for microbe-modulating therapeutics for diseases such as food allergy (97). Murine and human studies suggested that dysbiosis early in life contributes to the development of allergic disease and that therapeutic interventions that alter the microbial composition during this time period may be most effective to prevent allergic sensitization (22, 29, 98–106). Recent analysis of one cohort of 319 children emphasized that changes in the microbiota in the first 100 d of life were most likely to be associated with allergic disease (99). During this critical time period, children at risk for developing allergic asthma exhibited marked reductions in the abundance of four key microbial genera (*Faecalibacterium*, *Lachnospira*, *Rothia*, and *Veillonella*) and in SCFA metabolites. Moreover, colonization of germ-free mice with these genera protected from the development of allergic airway inflammation (99). This protection was also associated with increased levels of fecal butyrate, suggesting that one mechanism of protection was via SCFA signaling (99). Most studies have concluded that the efficacy of conventional *Lactobacillus*-based probiotics is limited to infancy. Berni Canani et al. (107) showed that treatment with a *Lactobacillus* GG–supplemented formula accelerated the acquisition of tolerance in infants with cow's milk allergy. Fecal samples collected from a small subset of infants from this study revealed stark differences in the microbiota of cow's milk allergy patients compared with healthy age-matched controls (108). Twelve months of treatment with the tolerance-inducing formula was associated with an increase in butyrate-producing bacteria and fecal butyrate levels, suggesting that one therapeutic effect of probiotic administration was the alteration of intestinal microbial community structure (108). Preclinical mouse data also suggest that dietary manipulation of the microbiota may be effective for the treatment of allergic disease (23, 70). These studies are providing exciting early evidence that the microbiota provides protective and inductive signals to shape the intestinal immune response and tip the balance in favor of tolerance over allergic sensitization.

Collectively, then, there is compelling evidence that allergic phenotypes are associated with alterations in the intestinal microbiome and that this dysbiosis may drive the allergic response. It is also possible that allergic inflammation itself induces changes in the microbiota. Both scenarios may contribute to allergic sensitization at different times and stages of disease, highlighting the complex and dynamic nature of host-microbial interactions. As we learn more about the specific immune-modulating effects of particular members of the microbiota, we may be able to identify microbial signatures that are associated with proallergic responses, such as release of alarmins by epithelial cells, that contribute to allergic sensitization to food. Conversely, identification of a healthy microbiota that reinforces tolerance and barrier function in the intestine will allow for better-targeted treatments to harness the microbiota to restore health.

Conclusions

The marked increase in the incidence and severity of dietary allergies that has occurred in parallel with profound environmental and lifestyle changes suggests a link between alterations in the microbiota and the rising prevalence of allergic disease. Increasing knowledge of how the immune response is influenced by the microbiota is revealing new approaches to treat diseases such as food allergy. Although there is already promising evidence in support of manipulating the microbiota during early life to prevent allergic sensitization, it is not yet clear whether a stably established gut microbiota can be effectively manipulated to treat food allergy (109). We do know that, in adults, the microbiota can be readily altered, even on daily timescales, by changes in components of the diet, particularly fiber (4, 15, 110). Moreover, the use of microbial metabolites to treat complex immune-mediated diseases is starting to generate results (111). These observations lend

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promise to the vision that microbiome-modulating therapeutics will have efficacy later in life, particularly as an adjunctive strategy to potentiate Ag-specific desensitization protocols, and promote long-lasting tolerance (112).

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Disclosures

C.R.N. is president and cofounder of ClostraBio, Inc., a company developing microbiome-modulating therapeutics for the treatment of food allergies. The other author has no financial conflicts of interest.

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