# Food allergy and the microbiome: Current understandings and future directions

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**Growing evidence points to an important role for the commensal microbiota in susceptibility to food allergy. Epidemiologic studies demonstrate associations between exposures known to modify the microbiome and risk of food allergy. Direct profiling of the gut microbiome in human cohort studies has demonstrated that individuals with food allergy have distinct gut microbiomes compared to healthy control subjects, and dysbiosis precedes the development of food allergy. Mechanistic studies in mouse models of food allergy have confirmed that the composition of the intestinal microbiota can imprint susceptibility or resistance to food allergy on the host and have identified a unique population of microbially responsive RORγt-positive FOXp3-positive regulatory T cells as critical for the maintenance of tolerance to foods. Armed with this new understanding of the role of the microbiota in food allergy and tolerance, therapeutics aimed at modifying the gastrointestinal microbiota are in development. In this article we review key milestones in the development of our current understanding of how the gastrointestinal microbiota contributes to food allergy and discuss our vision for the future of the field. (J Allergy Clin Immunol 2019;144:1468-77.)**

***Key words:*** *Food allergy, microbiome, microbiota, dysbiosis, prebiotic, probiotic, symbiotic, regulatory T cells, short-chain fatty acids*

*Abbreviations used*
FMT: Fecal microbiota transplantation
Foxp3: Forkhead box P3
MyD88: Myeloid differentiation response gene–88
OFC: Oral food challenge
OR: Odds ratio
RORγt: Retinoic acid–related orphan receptor γt
SCFA: Short-chain fatty acid
sIgE: Specific IgE
Treg: Regulatory T

The dynamics and functions of the ***microbiota*** that inhabit our bodies contribute to both health and disease.[1,2] In recent years, there has been increasing interest in how dysregulation of resident microbial communities (ie, ***dysbiosis***) might be associated with allergy risk.[3-7] The sum of microbes, their genomic elements, and their interactions in a given ecological niche (ie, ***microbiome***) differs by body site.[2] Accumulating evidence supports a potential role for the gut microbiome in the pathogenesis and course of food allergy.[3-17] Next-generation sequencing, including ***16S rRNA sequencing*** and shotgun ***metagenomic sequencing***, has advanced microbiome research in recent years by enabling more comprehensive and culture-free profiling of **taxa** in a given sample.[3,4] Building on our previous discussion of this topic,[3-6] here we review the role of the gut microbiome in food allergy.

## OBSERVATIONAL HUMAN COHORT STUDIES SUPPORT ASSOCIATIONS BETWEEN MICROBIAL EXPOSURES AND ALLERGY RISK

A potential role for microbiota in allergy risk was put forth as the hygiene hypothesis in 1989, with the epidemiologic observation that children from larger households had lower rates of allergic rhinitis and eczema.[18] The hypothesis is that infection in early childhood as a result of contact with siblings or acquired prenatally through maternal contact with older siblings protects against allergy.[18] Thinking on this has evolved over recent decades to suggest that the increased incidence of allergic disease might not be specifically due to reduced infections, but rather, due to the modern Western living environment, diet, and lifestyle in which exposures to human microbiota are different from prior generations.[19] Observational studies in support of this include those showing that maternal prenatal exposures to pets,[20] birth by vaginal versus cesarean section delivery,[21] childhood spent in rural environments,[22] and childhood exposure to pets[23] are associated with reduced allergy risk.[3,24] The "old friends hypothesis" and "biodiversity hypothesis" posit that the increase in allergies is from the loss of commensal relationships with parasites and bacteria that had previously exerted beneficial effects on human evolution.[19,25,26]

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Terms in boldface and italics are detailed in the glossary on page 1469.

With regard to food allergy specifically, a few observational studies have provided concordant evidence that conditions leading to differential microbial exposures are associated with food allergy risk. An Australian birth cohort study of 5276 infants, 453 of whom were confirmed to have egg allergy by oral food challenge (OFC) or recent reaction plus evidence of IgE-mediated sensitization, found that having older siblings and dog ownership were each associated with decreased egg allergy risk (adjusted odds ratio [OR], 0.72 [95% CI, 0.62-0.83] and 0.72 [95% CI, 0.52-0.99], respectively).[27] Within a Norwegian birth cohort of 2803 infants, cesarean delivery was not associated with confirmed reactions to egg (based on OFCs or on history of reaction plus increased egg-specific IgE levels) by age 2.5 years, but stratified analyses demonstrated a 4-fold increased risk (OR, 4.1; 95% CI, 0.9-19; $P = .08$) of confirmed egg allergy in children with atopic mothers.[28] Although conflicting results for delivery mode and food allergy risk exist in the literature,[27-29] a meta-analysis of 6 studies on this topic showed an increased summary OR of OFC-confirmed food allergy or food sensitizations (OR, 1.32; 95% CI, 1.12-1.55) in children who were born by cesarean delivery.[21] Collectively, these observational findings support the notion that differential microbial exposure and colonization in early childhood might modify the risk for development of food allergy.

## GUT MICROBIOTA DIFFER IN HUMAN SUBJECTS WITH FOOD ALLERGY

Although the hygiene, old friends, and biodiversity hypotheses and findings from epidemiologic studies support that circumstances linked to differential microbial exposures are associated with allergy and food allergy risk, studies that have directly measured microbial ***diversity*** and composition in populations with and without food allergy provide direct evidence that gut microbiota differ in individuals with food allergy (Fig 1). Culture-based studies of gut microbiota from subjects with and without food allergy provided early findings on this,[13,30] although because the vast majority of microbiota cannot be cultured, these cultured-based studies could provide only partial insight.

More recent studies have used next-generation sequencing, including 16S rRNA sequencing and shotgun metagenomic sequencing, which enable more comprehensive and culture-free profiling of taxa in a given sample.[31] Sequencing of the 16S rRNA gene, which has highly conserved primer binding sites and contains hypervariable regions that can provide species-specific signature sequences for bacterial identification, allows for more comprehensive bacterial identification without the constraints of culture-based methods. Shotgun metagenomic sequencing, in which the total DNA of an ecosystem is sequenced, provides broad and deep characterization of all microbiota in addition to bacteria, as well as better resolution at lower taxonomic levels and potential for functional annotation. However, shotgun metagenomic sequencing has not yet been widely implemented in studies of food allergy.[3]

Findings from 16S rRNA sequencing–based studies have shown that children with food allergy have distinct gut microbiome compositions compared to those without food allergy (Fig 1). Furthermore, results thus far suggest that the gut microbiota can differ by individual food allergy. In a study of 141 children with egg allergy and control subjects without food allergy from the multicenter, US-based Consortium for Food Allergy Research, where egg allergy was strictly defined based on OFC results, convincing history, and positive allergy test results or flare of atopic dermatitis associated with egg ingestion and an egg-specific IgE (sIgE) level of 2 $kU_A/L$ or greater, genera from the *Lachnospiraceae, Streptococcaceae,* and *Leuconostocaceae* families were found to be differentially abundant in the gut microbiome of children with egg allergy versus control children.[9] Independent studies of gut microbiota in subjects with milk allergy demonstrate overlapping as well as contrasting gut microbial compositions. A study of 39 Italian children showed that compared to healthy control

## GLOSSARY

**DESENSITIZATION:** A temporary increase in reactivity threshold to provide a measure of safety that is dependent on continued treatment exposure.

**DIVERSITY:** A calculated index incorporating measures of richness and taxa distribution.

**DYSBIOSIS:** A state of imbalance in a microbial ecosystem.

**FECAL MICROBIOTA TRANSPLANTATION:** The administration of fecal matter from a donor to a recipient.

**GATA-3$^+$ Treg CELLS:** Also referred to as $T_H2$-reprogrammed regulatory T (Treg) cells, these Foxp3$^+$ Treg cells express the transcription factor GATA-3 and produce IL-4. These cells are poorly suppressive of type 2 immunity and, in contrast, contribute to $T_H2$-mediated pathology.

**METAGENOMIC SEQUENCING:** Whole-genome sequencing performed on genomic DNA from a mixed microbial community.

**MICROBIOME:** The sum of microbes and their genomic elements in a particular environment.

**MICROBIOTA:** The community of microbes in a particular environment.

**OPERATIONAL TAXONOMIC UNITS (OTU):** A group of organisms with similar sequence homology according to DNA sequence similarity of a specific taxonomic marker gene.

**ORAL TOLERANCE:** A state of local and systemic immune suppression to antigens introduced through the oral route, translating to a lack of clinical reactivity on oral antigen introduction that is independent of both quantities of antigens ingested and routine antigen exposure.

**PREBIOTIC:** Nondigestible substrates that promote the growth, function, or both of beneficial microorganisms.

**PROBIOTIC:** Live microorganisms with beneficial effects on the host.

**RICHNESS:** The number of taxa found in a given ecological niche.

**ROR$\gamma$t$^+$ Treg CELLS:** Also referred to as type 3 regulatory T (Treg) cells, these Foxp3$^+$ Treg cells coexpress the transcription factor ROR$\gamma$t. These cells are generated in response to the intestinal microbiota and are essential for suppression of type 2 immunity.

**SHORT-CHAIN FATTY ACID:** Fatty acids with fewer than 6 carbon atoms.

**16S rRNA SEQUENCING:** A method for culture-free classification and identification of bacteria present in a sample based on sequencing the hypervariable regions of the 16S ribosomal RNA gene that is present in all bacteria.

**SYNBIOTIC:** Products that contain both prebiotics and probiotics.

**TAXA:** A group of organisms scientifically classified as a unit based on shared characteristics. The unit might be at any taxonomic level of the biological system of classification of organisms (eg, phylum, order, family, genus, and species).

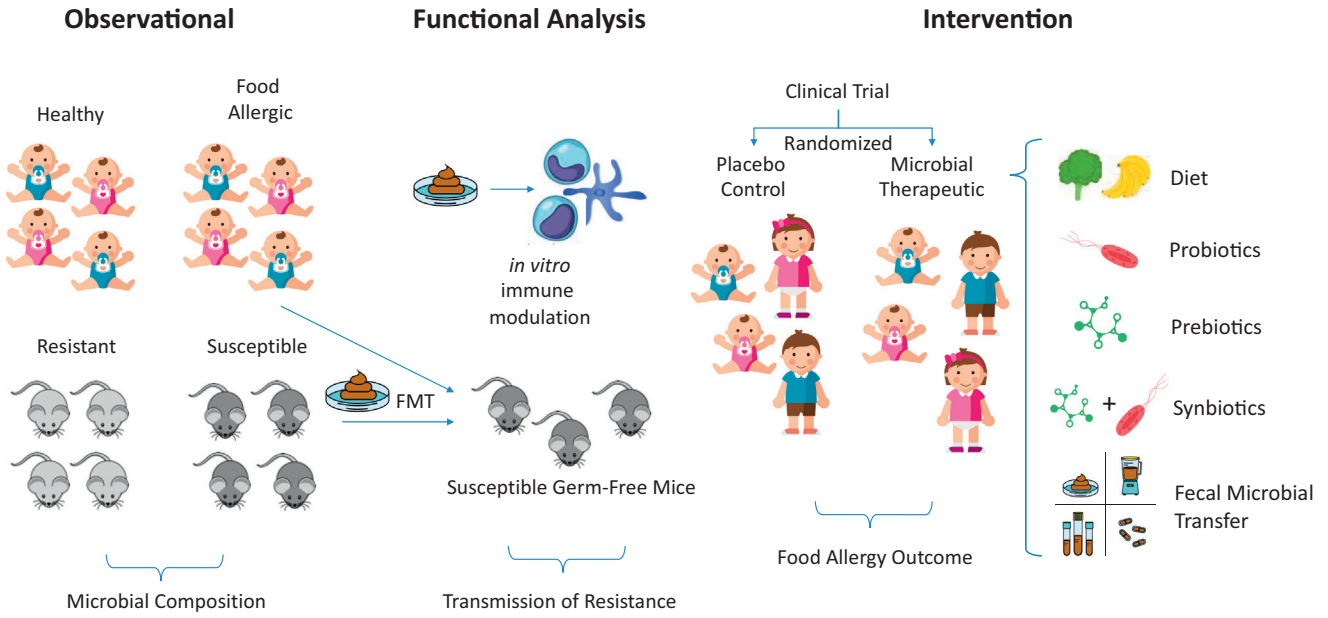



**FIG 1.** Pathways to the development of microbial therapeutics for food allergy. Evidence for an important contribution of the gut microbiota to the pathogenesis of food allergy is derived from observational studies of distinct microbial composition in allergic and healthy human cohorts, and in mice with susceptibility versus resistance to food allergy. Evidence that changes in microbial composition lead to meaningful changes in host immunity come from studies using FMT into germ-free mice with susceptibility to food allergy. Additional functional evidence can be derived from *in vitro* culture systems examining the effect of stool extracts on immune phenotype. Based on the weight of this preclinical evidence, clinical trials using defined microbial transfer, probiotics, prebiotics, synbiotics, and FMT are in process to determine the efficacy of microbial therapeutics for the treatment of food allergy.

subjects, the 19 infants with milk allergy studied had a higher Shannon diversity index and gut microbial community structures dominated by *Lachnospiraceae* and *Ruminococcaceae*.[14] Differences in the implicated taxa across these studies of egg and milk allergies might have been due to the individual food allergies targeted in each study, although additional explanations include differences in population characteristics, phenotyping approach, and/or analytic methodology. The characterization and comparison of microbial profiles of individual food allergies remains an area for continued exploration.

## GUT MICROBIOTA ARE ASSOCIATED WITH THE CLINICAL TRAJECTORY OF FOOD ALLERGY

Sequencing-based studies of gut microbiota in children with food allergy have shown that gut microbiome composition is associated with the clinical trajectory of food allergy, and the timing of host-microbe interactions in early life appears to be important. The gut microbiome changes during the course of life, with the most rapid changes occurring in early life.[32]

To examine the relationship between the infant gut microbiome and the clinical course of food allergy, investigators studied 226 infants with milk allergy (strictly defined based on OFC results, a convincing history, and positive allergy test results or flare of atopic dermatitis associated with milk ingestion and milk sIgE level ≥5 $kU_A/L$) from the multicenter Consortium for Food Allergy Research, whose clinical courses were followed longitudinally up to age 8 years.[8] The investigators found that taxa from the *Firmicutes* phylum, including *Clostridia*, were enriched in the gut microbiome of infants with milk allergy age 3 to 6 months whose milk allergy later resolved by age 8 years, whereas this was not the case in infants with milk allergy of the same age who continued to have persistent milk allergy.[8] Among infants with milk allergy older than 6 months, there was no association between gut microbiome composition and later milk allergy resolution.[8] Based on these results, it is possible that (1) early infancy (ie, age 3-6 months) is a specific developmental window during which gut microbiota can shape the course of food allergy, and gut microbiota at later ages (eg, >6 months age) have little to no effect on food allergy course; and/or (2) gut microbiome composition might be associated with food allergy course later in life as well, but factors that appear during these older ages obscure this signal.

Separately, a study of 56 Japanese infants found evidence that the gut microbiota at age 2 months is associated with the development of self-reported food allergy by age 2 years. Specifically, among 14 infants with egg, cow's milk, soy, and/or wheat allergy based on questionnaire by age 2 years, the investigators found that the genera *Leuconostoc*, *Weissella*, and *Veillonella* were underrepresented in their fecal samples compared to 27 of their counterparts who did not develop any food allergies by age 3 years.[33] We note that although self-reported and/or questionnaire-based assessments of food allergy are often used in studies of food allergy, they are limited by the likelihood of reporting inaccuracy, and supervised oral challenge–based assessments of food allergy remain the gold standard for accurate diagnosis.[34]

Additional studies have similarly found associations between the early-infancy gut microbiome composition and the development of food allergen sensitization, an intermediate phenotype to food allergy. Among children from the Canadian Healthy Infant Longitudinal Development cohort, each quartile increase in gut microbial ***richness*** at age 3 months was associated with a 55% reduced risk for food sensitization (by skin prick test) by age 1 year (adjusted OR, 0.45; 95% CI, 0.23-0.87), whereas there was no association between gut microbial characteristics at age 12 months and food sensitization.[35] In terms of gut microbiome composition, *Enterobacteria* were overrepresented and *Bacteroidaceae* were underrepresented in food-sensitized infants at age 3 months and 1 year.[35] Separately, among 225 US children participating in the Vitamin D Antenatal Asthma Reduction Trial, lower relative abundances of *Haemophilus*, *Dialister*, *Dorea*, and *Clostridium* in stool samples collected at age 3 to 6 months was associated with sensitization (sIgE, $\geq$0.10 $kU_A$/L) to at least 1 food allergen among milk, egg, peanut, soy, and wheat.[10]

Findings from murine models also support age-sensitive contact with the microbiota.[36] Colonization of gnotobiotic mice with a diverse microbial population early but not late in life suppresses IgE and prevents mice from development of food allergy.[37] These collective findings suggest that commensal microbial exposures during early immune system development could play a role in the natural history of food allergy.

## CAUSATION AND MECHANISMS: LESSONS FROM MOUSE MODELS OF FOOD ALLERGY

Studies of human cohorts are observational, and even strong supporting evidence, such as documented dysbiosis preceding development of food allergy, does not demonstrate causation. Mechanistic studies in mice have been essential for supporting the link between the composition of the intestinal microbiota and development of food allergy. The first approaches to test the role of the microbiota in food allergy used broad-spectrum antibiotics to reduce the bacterial load in the intestine or germ-free mice completely lacking a normal commensal microbiota. These tools showed that the microbiota provided a suppressive signal to prevent sensitization and development of peanut or milk allergy.[38-41] In the absence of microbiota, there was a polyclonal hyper-IgE response that was $CD4^+$ T-cell dependent and was associated with increased IL-4 production and IgE class-switching in the Peyer patches of the small intestine.[37] There was a need for diverse microbial colonization during a critical window in early life to suppress polyclonal IgE production and prevent susceptibility to allergic sensitization.[37]

## IDENTIFICATION OF PROTECTIVE ORGANISMS

Germ-free mice or antibiotic treatment demonstrated that the microbiota as a whole provided suppressive signals preventing food allergy, but these approaches do not model the more subtle alterations in microbial composition that are observed in subjects with food allergy. Noval Rivas et al[15] used a mouse model susceptible to food allergy (the $Il4ra^{F709}$ mouse, which has a gain-of-function mutation in the IL-4 receptor) to study how the microbiota differs between mice with susceptibility or resistance to food allergy and anaphylaxis. The composition of the fecal microbiota was significantly different between resistant (wild-type) and susceptible ($Il4ra^{F709}$) mice. Differences were observed between the strains at steady state, and oral sensitization to the egg allergen ovalbumin induced significant changes in the microbial composition of susceptible but not resistant mice. Bacterial families, including Lachnospiraceae, Lactobacillaceae, Rikenellaceae, and Porphyromonadaceae, were overrepresented in the microbiota of mice with food allergy.

When ***fecal microbiota transplantation*** (FMT) was performed from resistant or susceptible mice to germ-free mice, FMT from susceptible sensitized mice conferred greater susceptibility to food allergy than FMT from resistant sensitized mice onto the colonized mice. This was the first experimental model to show that food allergy susceptibility could be transmitted by the gastrointestinal microbiota.[15] It was not clear from these studies whether resistant mice harbored a protective microbe or susceptible mice harbored a microbe that could drive allergy susceptibility.

Atarashi et al[42] identified a group of Clostridia species harvested as chloroform-resistant spores from the fecal contents of healthy mice as being protective against colitis and IgE production when administered to mice with a normal microbiota. Stefka et al[41] used this approach to demonstrate that Clostridia species are protective against experimental peanut allergy when transferred into germ-free mice. Monocolonization with a single *Bacteroides* species *(Bacteroides uniformis)* was not as effective as colonization with the Clostridia consortium in suppressing peanut-specific IgE and anaphylaxis, suggesting unique regulatory properties of the Clostridia consortium.

It was important to identify similarly protective organisms derived from human subjects with a capacity to colonize the human gastrointestinal tract to translate these findings to human disease. Atarashi et al[43] selected 17 strains of healthy human fecal matter–derived Clostridia species based on their ability to colonize mice, induce regulatory T (Treg) cells, enhance IL-10 production, and increase inducible T-cell costimulator expression on Treg cells. The strains were derived from Clostridia clusters IV, XIVa, and XVIII and lacked toxins or other virulence factors. This cocktail of Clostridia strains was able to suppress gastrointestinal manifestations of food allergy in mice.

More recent approaches have focused on functional analyses of fecal organisms by performing FMT, transferring samples derived from subjects with food allergy and healthy subjects to mice. Rodriguez et al[16] was the first to report that FMT from a healthy infant could protect susceptible germ-free mice from milk allergy. Abdel-Gadir et al[12] studied the stool microbial composition of 154 infants from 1 to 15 months of age: 56 infants with food allergy and 98 healthy control subjects. Significant differences were identified in 77 ***operational taxonomic units***, including several species of Clostridiales. The functional implications of these differences were tested by transferring the microbiota into germ-free susceptible ($Il4ra^{F709}$) mice. Seven specimens from allergic subjects and 7 from healthy control subjects were tested, and fecal microbiota from the healthy control subjects but not the subjects with food allergy protected these susceptible recipient mice from food allergy.

A series of transfers with defined microorganisms was then performed to identify protective organisms. Germ-free mice colonized with a cocktail of 6 Clostridiales strains or 5 Bacteroidales strains, including *B fragilis*, were protected from food allergy, whereas colonization with a cocktail of 4 Proteobacteria showed no protection. A single Clostridiales

strain, *Subdoligranulum variabile*, was sufficient to suppress food allergy, although less effectively than the 6-strain consortium. Importantly, as a proof of concept for therapeutic use, these microbes were also protective in conventional mice with a normal microbiota that had been treated with antibiotics to facilitate microbial colonization.[12] Feehley et al[17] used a similar approach to test the functional consequences of compositional differences in the fecal microbiota of infants with or without milk allergy. They added a factor of dietary modification, which was necessary for maintaining the original microbial composition in recipient mice. Mice receiving FMT from donors with milk allergy were given hydrolyzed casein formula, whereas mice receiving FMT from healthy control subjects were given milk formula before sensitization to the milk allergen β-lactoglobulin. Mice receiving FMT from healthy control subjects plus milk formula were protected from milk allergy, whereas mice receiving FMT from infants with milk allergy plus hydrolyzed casein formula had symptoms to β-lactoglobulin after sensitization.[17] The abundance of the Clostridiales species *Anaerostipes caccae* was significantly associated with regulatory gene expression in the intestinal epithelium and, when this organism was administered to germ-free mice together with milk formula, was able to protect germ-free mice from milk allergy. With this design, it is challenging to parse out the effects of the microbiota from the effects of dietary exposure. Germ-free mice receiving milk formula alone were not protected from food allergy; however, ***oral tolerance*** to dietary antigens is known to be impaired in germ-free mice.[44-46]

## MECHANISMS OF PROTECTION AGAINST FOOD ALLERGY BY THE INTESTINAL MICROBIOTA

The weight of evidence indicates that the microbiota, and Clostridia strains in particular, protect against food allergy through the generation of peripheral antigen-experienced Treg cells. Monocolonization studies have shown that a diverse set of microbes can support expansion of forkhead box P3 $(Foxp3)^+$ Treg cells in the mouse intestine.[47] However, it is becoming clear that Treg cells are a highly heterogenous population comprised of subsets that differ in regulatory activity. For example, a subset of $Foxp3^+$ Treg cells that express GATA-3 and IL-4 and have been referred to as "$T_H2$-reprogrammed Treg cells" are increased in food allergy and have reduced suppressive activity.[48] Ohnmacht et al[49] identified a subset of antigen-experienced $Foxp3^+$ Treg cells that express the transcription factor retinoic acid–related orphan receptor γt $(ROR\gamma t)^+$, are dependent on the presence of the intestinal microbiota and vitamin A metabolism, and effectively suppress $T_H2$-skewed immune responses. As shown by Abdel-Gadir et al,[12] protection of susceptible germ-free mice from food allergy by defined microbial transfer was associated with expansion of ***RORγt$^+$ Treg cells***. The effect of the microbiota on Treg cells was likely direct on Treg cells because deletion of the bacteria-sensing molecule myeloid differentiation response gene–88 (MyD88) under control of the Foxp3 promoter (and therefore deleting MyD88 only in Treg cells) abolished the protective effect of the microbiota in susceptible mice.[12] $ROR\gamma t^+$ Treg cells express high levels of cytotoxic T lymphocyte–associated antigen 4 and suppress $T_H2$ immunity by modifying the phenotype of intestinal dendritic cells.[49] Downstream of the induction of $ROR\gamma t^+$ Treg cells is suppression of IgE and enhancement of IgA responses. Intriguingly, an IgE response was detected against the gut microbiota in allergic human subjects and mice,[12] but the function of antimicrobial IgE in food allergy pathogenesis or IgE persistence has not yet been explored. In addition to direct effects on Treg cells, the microbiota can alter epithelial gene expression directly and through activation of type 3 innate lymphoid cells, leading to enhanced barrier function.[41]

The link between the microbiota and protection from food allergy is not well understood. ***Short-chain fatty acids*** (SCFAs), including butyrate, propionate, and acetate, are generated by the microbiota through the metabolism of dietary fiber. A high-fiber diet has been shown to protect mice from food allergy through SCFAs acting on the receptors GPR43 and GPR109a.[50] Feeding of exogenous SCFAs to mice can expand $ROR\gamma t^+$ Treg cells[49] and protect against food allergy[50] and atopic sensitization.[51] However, not all studies have found that administration of exogenous SCFAs is protective in the context of food allergy.[12] In addition to SCFAs, the microbiota can produce a wealth of products that are active on the human immune system. The monohydroxy fatty acid 12,13-diHOME is produced by intestinal bacteria, is increased in neonates at risk of asthma, and drives $T_H2$ skewing by acting on dendritic cells and T cells.[52,53] The microbiota can metabolize tryptophan into ligands active on the aryl hydrocarbon receptor present on epithelial cells, intraepithelial lymphocytes, innate lymphoid cells, and dendritic cells.[54] Aryl hydrocarbon receptor ligands promote development of Treg cells[55] and suppress experimental peanut allergy.[56] The intestinal microbiota can also produce histamine that can act on histamine receptors on T cells, dendritic cells, and epithelial cells, and levels of histamine-producing bacteria have been shown to be increased in subjects with asthma.[57,58] Functional analysis of bacterial products identified through metabolomics will be an important parallel strategy alongside functional studies of candidate organisms to advance our understanding of the mechanisms of microbial regulation of food allergy risk. Fig 2 reviews the main immune mechanisms by which the healthy intestinal microbiota generates immune tolerance rather than allergic sensitization.

## MICROBIAL THERAPEUTICS FOR FOOD ALLERGY

The accumulating evidence from clinical and murine studies that the gut microbiota can affect the pathogenesis and course of food allergy inspires the search for microbial therapeutics that might prevent and/or treat food allergy. Diet, ***probiotics***, ***prebiotics***, ***synbiotics***, and FMT represent candidate interventions to shape the gut microbiome toward beneficial outcomes (Fig 1).[4-6]

### Diet

The immune-modulating and allergy-protective effects of many gut microbiota are thought to be mediated by metabolites produced from their fermentation of dietary fiber.[50,59,60] These metabolites, such as SCFAs, regulate the size and function of the colonic Treg cell pool.[50,59,60] In human cohorts, significant associations have been reported between the composition of dietary intake and SCFA levels as measured in stool, suggesting that diet can be used to modulate microbial production of SCFAs.[51] For example, in a subset study of 301 children from the Protection Against Allergy Study in Rural Environments birth

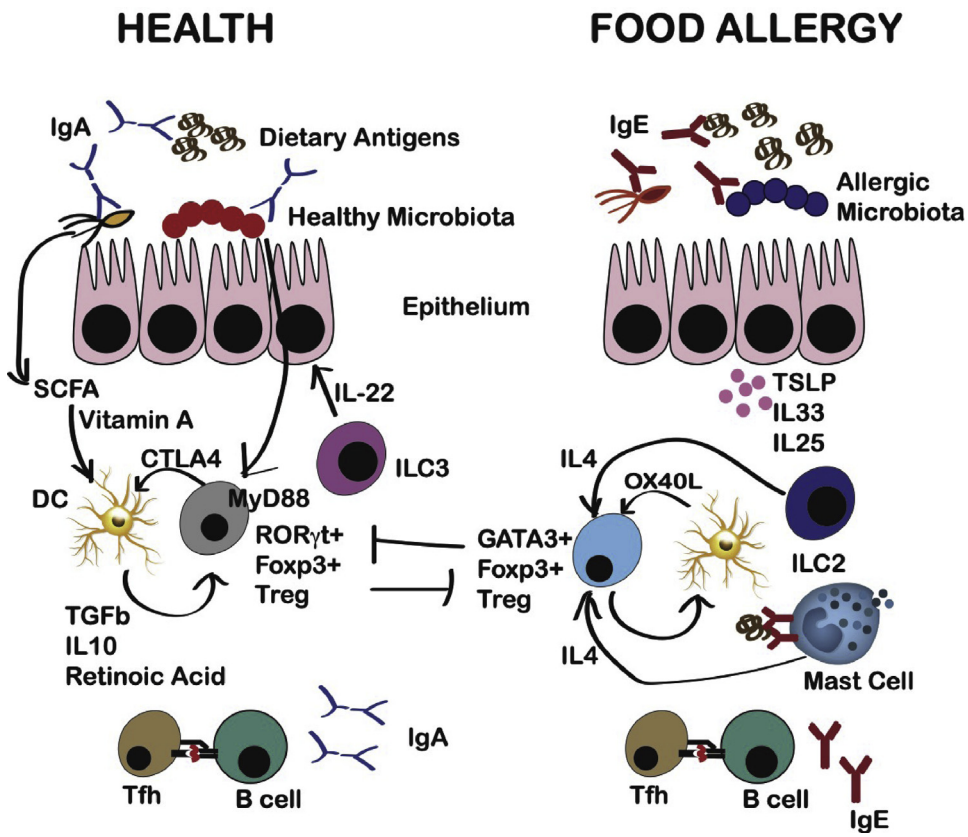



**FIG 2.** Contribution of the microbiota to immune mechanisms of tolerance and allergy to foods. Healthy microbiota, or consortia composed of Clostridiales strains, Bacteroidales strains, or individual microorganisms, including *S variabile* or *A caccae*, protect susceptible germ-free mice from food allergy. Mechanisms of protection include generation of RORγt$^+$ Foxp3$^+$ Treg cells by MyD88 signaling in Treg cells, which enhances antimicrobial and anti–food allergen–specific IgA production while suppressing IgE production and suppresses generation of GATA-3$^+$ $T_H2$-reprogrammed Treg cells. SCFAs derived from the healthy microbiota drive a change in the mucosal dendritic cell *(DC)* phenotype to a more tolerogenic phenotype. RORγt$^+$ Treg cells suppress DCs through increased cytotoxic T lymphocyte–associated antigen 4 *(CTLA-4)* expression to suppress costimulatory-driven priming. In mice susceptible to food allergy, production of IL-4 from type 2 innate lymphoid cells *(ILC2)* and IgE-activated mast cells cooperates with DCs to promote the generation of $T_H2$-reprogrammed Treg cells that express GATA-3, produce IL-4, and contribute to rather than protect against food allergy. Epithelium-derived cytokines contribute to the immune milieu that drives this deviation of the normal regulatory response. *ILC3*, Type 3 innate lymphoid cells; *OX40L*, OX40 ligand; *Tfh*, follicular helper T cells; *TSLP*, thymic stromal lymphopoietin.

cohort, consumption of yogurt, fish, vegetables, and fruits within the first year of life was found to be associated with increased butyrate in stool samples at age 1 year.[51] Furthermore, children with stool butyrate and propionate levels of the 95th percentile or greater at age 1 year had reduced sensitization to any environmental or food allergen between ages 3 and 6 years compared to those with levels less than the 95th percentile (butyrate adjusted OR, 0.25 [95% CI, 0.08-0.82]; propionate adjusted OR, 0.20 [95% CI, 0.05-0.74]).[51] Female BALB/c mice receiving oral administration of the SCFAs examined (acetate, propionate, or butyrate) during sensitization and challenge demonstrated reduced airway hyperresponsiveness after methacholine challenge compared to mice receiving vehicle.[51] Administration of dietary fiber or exogenous SCFAs has been shown to suppress food allergy in mice in some studies[50] but not in others.[12] Clinical trials of fiber and/or SCFA supplementation for food allergy prevention, treatment, or both have yet to be undertaken.

Conversely, other murine models have shown that a high-fat diet induces changes to the gut microbiota that increase food allergy risk.[61] After a high-fat diet, mice had obesity, decreased gut bacterial diversity, and dysregulated intestinal effector mast cell responses.[61] Transfer of the high-fat diet–associated microbiome from obese mice to germ-free mice conferred enhanced food allergy susceptibility to the recipient mice, suggesting a pathogenic role for the high-fat diet–associated microbiota independent of obesity.[61] If such results can be extrapolated to human biology, avoidance of high-fat diets common to Western lifestyles might have benefits to food allergy risk in addition to those for overall health.

## Probiotics

In theory, intake of beneficial microbiota could be a direct way to shape gut microbiota. Probiotics are live microbes whose intake is thought to be beneficial for health. A few clinical trials

have examined probiotics for treatment of challenge-proved food allergy. Among 119 infants with cow's milk allergy, supplementation with *Lactobacillus casei* and *Bifidobacterium lactis* for 12 months did not accelerate milk allergy resolution.[62] However, a subsequent trial of 55 children with milk allergy receiving supplementation with extensively hydrolyzed casein formula containing a different probiotic, *Lactobacillus rhamnosus* GG, demonstrated increased rates of milk allergy resolution after 6 and 12 months compared to a control group receiving the formula alone.[63] Comparison of stool from 20 healthy infants and 19 infants with cow's milk allergy before and after treatment with extensively hydrolyzed formula with (n = 12) or without (n = 7) *L rhamnosus GG* revealed that the only gut microbe that differed in gut abundance between tolerant and allergic infants was *Oscillospira*, although *Blautia* and *Roseburia* were enriched in samples from tolerant infants with higher fecal butyrate levels.[14] The same investigators followed 220 infants with milk allergy randomized to extensively hydrolyzed formula with or without *L rhamnosus GG* for 3 years, finding that those receiving the formula plus probiotic combination had greater incidences of cow's milk tolerance at ages 12, 24, and 36 months.[64]

*L rhamnosus GG* has also been studied as a microbial adjunct to peanut oral immunotherapy for peanut allergy. In a clinical trial in which 62 children were randomized to receive *L rhamnosus GG* with peanut oral immunotherapy versus placebo for 18 months, 89.7% of subjects who received the combination treatment achieved ***desensitization*** compared to 7.1% of those receiving placebo ($P < .001$).[65] However, effects caused by the probiotic itself were not clear from this study given the absence of probiotic-only or oral immunotherapy–only control groups. Four years after treatment cessation, 67% of subjects who received the combination treatment during the 18 months were eating peanut compared to 4% of those who had taken placebo ($P = .001$), and more participants from the treated group had smaller peanut skin prick test response size (mean, 8.1 vs 13.3 mm; $P = .035$) and higher peanut $sIgG_4$/sIgE ratios compared to placebo-treated control subjects (geometric mean, 67.3 vs 5.2; $P = .031$).[66]

Although results from some of the above trials suggest that probiotics might be useful for food allergy, the most recent Cochrane review on this topic[67] concluded that the data are insufficient thus far to recommend probiotic supplementation for food allergy. More recent meta-analyses on the subject,[68] as well as statements from the World Allergy Organization,[69,70] comment on the limited and "very low quality evidence" on this topic thus far and concur with the Cochrane conclusion.

## Prebiotics

Another approach to shape the gut microbiota is the provision of components that foster the growth of desired microbial community members. This is the idea behind prebiotics, nondigestible food components that specific commensal microbes can use to selectively foster their growth and activity. Frequently added to infant formulas, prebiotics are typically oligosaccharides that are not digested or absorbed in the gastrointestinal tract before they reach the large intestine, where specific microbiota can use them for improved growth and activity.[71] Although prebiotic supplementation in infants was found to be associated with decreased risk of asthma and eczema in some investigations,[71-73] there have been no significant effects on the development of food allergy found thus far.[71-73]

## Synbiotics

Synbiotics are combinations of probiotics and prebiotics. It is thought that they might be more effective than probiotics or prebiotics alone because the provision of both together delivers a synergistic combination of desired microbes and the components they need to thrive and drive the host community. A prospective, randomized, double-blind controlled study of 110 full-term infants with cow's milk allergy receiving either amino acid–based formula or amino acid–based formula with synbiotics showed that subjects in both arms have normal and similar growth.[74] The synbiotics used in this trial included the probiotic *Bifidobacterium breve* M-16V in combination with the prebiotics oligofructose, long-chain inulin, and acidic oligosaccharides. The same company is now performing a clinical trial in which 851 young infants at high risk for allergy have been randomized to take partially hydrolyzed infant formula with synbiotics versus standard infant formula (NCT03067714). The children will be assessed for the primary outcome of fecal levels of *Bifidobacteria* at age 17 weeks and secondary outcomes of *Bifidobacteria* levels at age 1 year and IgE-mediated allergic manifestations, including food allergy, up to age 1 year (NCT03067714).

## FMT

Because microbes function not singly but as interacting members of communities, the provision of one or a few probiotic bacterial strains might be insufficient to meaningfully alter the human gut microbiome toward an observable phenotype (eg, food allergy prevention). Transferring a microbial community associated with health might be more effective. FMT refers to the transfer of communities of microbes from a donor to a recipient. FMT has been successfully used to treat patients with *Clostridium difficile* colitis, with communities from healthy donors transferred to those with the condition.[75,76] FMT is an intriguing gut microbiome manipulation strategy to consider given findings from murine models supporting that fecal transfer is an effective mode to alter allergy outcomes. A small phase I open-label trial to evaluate the safety and tolerability of oral encapsulated FMT administered in an open-label manner over 2 days for the treatment of peanut allergy in 10 adult subjects is underway (NCT02960074). The agent being used is a screened-donor inoculum of frozen fecal material.

A study of next-generation microbial transfer of rationally selected strains based on preclinical findings by Atarashi et al[43] is in a phase I clinical trial for treatment of peanut allergy (NCT03936998). VE416 is a combination of dormant bacteria given as a capsule to subjects pretreated with vancomycin to facilitate colonization of the transferred bacteria. In this ongoing study VE416 or placebo will be given with peanut oral immunotherapy (NCT03936998).

The pending results from these interventional clinical studies will lend insight into FMT for food allergy treatment, although additional questions will remain about defining the optimal donor material or strains, methods to ensure establishment of the transferred community, and safety and acceptability of the procedure for food allergy treatment.

## FUTURE DIRECTIONS

Our understanding of the relationship between the gut microbiome and food allergy has grown in recent years with knowledge gained from observational human cohort studies, murine model experiments, and clinical trials. The aggregate evidence thus far supports that gut dysbiosis likely precedes the development of food allergy, and the timing of such dysbiosis is critical. Murine models have enabled valuable mechanistic insight into how microbiota modulate type 2 immunity.[3,4] With multiple clinical trials of microbial therapeutics underway, we are also likely to gain key information from these interventional studies that will inform next-level trials to design and execute. The challenges ahead are to build on this knowledge base to delve deeper into current research foci and to pursue novel directions that will further our understanding, management, and treatment of food allergy.

Although findings from human cohort studies support that subjects with food allergy have distinct gut microbiota (that might further differentiate by individual food allergy), and that the early-life gut microbiome has the greatest effects on food allergy outcomes, additional cohort studies are needed to deepen our understanding of the temporality of such effects and to dissect the web of causal relationships between microbiota, host, environment, and food allergy outcomes. Importantly in this regard, human cohort studies with rigorous longitudinal phenotyping, robust criteria for food allergy characterization, parallel immune profiling, and best-use practices for microbiome profiling will allow for the most reliable results and optimal cross-study learning. Our current understanding of the gut microbiome in individuals with food allergy has been bacteria centric, likely driven by the relative ease of profiling bacteria by culture-based methods in early days, PCR subsequently, and 16S rRNA sequencing more recently. It is possible that the virome and mycobiome have effects on food allergy course that we have not yet uncovered. Shotgun metagenomic sequencing, which is being increasingly used in prominent studies of the microbiome,[77-79] can enable microbiome research beyond the bacterial microbiome while also offering better resolution at the species and strain levels and more accurate community diversity estimates than 16S rRNA sequencing, although at higher cost.[31,80] Additionally, studies to date have focused on the gut microbiome, but the role of the microbiota at other sites, such as the skin and airway, might be of importance because of the role of extraintestinal sites in allergic sensitization.[81]

Multidimensional profiling and systems biology approaches will be needed to parse the complex interactions between the microbiota, our bodies' molecular processes, and environmental exposures that influence food allergy risk.[31,82,83] Food allergy is a complex and heterogeneous disease, and it is unlikely that the microbiota implicated in its pathobiology or even the microbiome in its entirety can capture the interdependent dynamics of the molecular networks involved in individuals with food allergy.[31] Our understanding of food allergy has been advanced not only by studies of the microbiome, but also by findings from genome-wide association,[84-87] transcriptomic,[88] epigenomic,[89,90] and metabolomic[31,91,92] studies of food allergy. Integrating these types of system-wide data is critical if we are to construct models that are predictive of complex biological interactions and systems, a necessary step to developing a more complete understanding of food allergy.[31,82,83] By moving forward research on the microbiome in food allergy and integrating our findings with our knowledge on food allergy, we can further our understanding of food allergy and derive new approaches for its prevention and therapy.

**Key concepts and therapeutic implications**

- **The gut microbiome likely plays a role in the pathogenesis and course of food allergy.**
- **Individuals with food allergy have different gut microbiomes compared to healthy control subjects.**
- **Imbalances in the gut microbial ecosystem precede the development of food allergy, and the timing of such dysbiosis is a key factor.**
- **Microbial transfer studies in mice have demonstrated that food allergy–associated dysbiosis transmits susceptibility to food allergy.**
- **Consortia composed of either *Clostridiales* or *Bacteroidales* strains inhibit food allergy in mice through induction of RORγt$^+$ Treg cells that suppress *GATA-3*$^+$ *Treg cells* and IgE and enhance IgA.**
- **Diet, probiotics, prebiotics, synbiotics, and fecal microbiota transfer represent potential microbial therapeutics for food allergy prevention and treatment.**
- **Future studies of the microbiome and food allergy are needed to deepen our understanding of the temporality of the microbiome's effects and to dissect the web of causal relationships between the microbiota, host, environment, and food allergy outcomes.**

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