

Review

The gut microbiome in food allergy

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Key Messages

- Growing evidence supports a role for the gut microbiome in the pathogenesis and course of food allergy.
- Gut dysbiosis may precede the development of food allergy, and the timing of such dysbiosis is critical.
- Gut microbiota associated with individual food allergies may be distinct.
- Murine models suggest that gut microbiota affect food allergy susceptibility by modulating type 2 immunity, influencing immune maturation and tolerance, regulating basophil populations, and promoting intestinal barrier function.
- Ongoing and future interventional clinical trials of probiotics, prebiotics, synbiotics, and fecal microbiota transfer can help translate our understanding of the gut microbiome in food allergy to clinical practice.
- Robust and consistent study design, multidimensional profiling, and systems biology approaches will further advance our understanding of the microbiome in food allergy.

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ABSTRACT

Objective: To review observational human, murine, and interventional trial studies that have examined the gut microbiome in food allergy, and to provide perspective on future investigations in this field.

Data Sources: A review of the published literature was performed with PubMed, and clinical studies catalogued at [ClinicalTrials.gov](https://clinicaltrials.gov) were also reviewed.

Study Selections: The most recent relevant studies, seminal works, and topical clinical trials were selected.

Results: Gut dysbiosis likely precedes the development of food allergy, and the timing of such dysbiosis is critical. Gut microbiota associated with individual food allergies may be distinct. Murine models support the importance of gut microbiota in shaping immune maturation and tolerance. Gut microbiota may affect food allergy susceptibility by modulating type 2 immunity, influencing immune development and tolerance, regulating basophil populations, and promoting intestinal barrier function. Ongoing and future interventional trials of probiotics, prebiotics, synbiotics, and fecal microbiota transfer will help translate our understanding of the gut microbiome in food allergy to clinical practice. Future work in this area will include deepening of current research foci, as well as expansion of efforts to include the virome, mycobiome, and interactions between the microbiome, host, and environment. Robust and consistent study designs, multidimensional profiling, and systems biology approaches will enable this future work.

Conclusion: By advancing research on the microbiome in food allergy, we can further our understanding of food allergy and derive new approaches for its prevention and therapy.

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Introduction

Food allergy is a clinical and public health problem that affects up to 10% of the US population.¹ Defined as an adverse health effect arising from a specific immune response that occurs reproducibly

on exposure to a food, immunoglobulin (Ig) E-mediated food allergy encompasses relatively immediate symptoms affecting the skin, respiratory, gastrointestinal, or cardiovascular systems.² The cause of food allergy involves deviation from a default state of immune tolerance that is likely driven by antigen exposure, commensal microbiota, and their interactions.³

Resident microbial communities vastly outnumber human cells and genes, motivating interest in how their dysregulation (ie, dysbiosis) may influence host immunologic development and risk for allergic disorders.⁴ The sum of microbes, their genomic elements, and interactions in a given ecologic niche (ie, microbiome) differs by body site.⁵ Growing evidence supports a potential role for the gut microbiome in the pathogenesis and course of food allergy.^{3–14} 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.⁴

Building on our previous address of this topic,³ here we review the potential role of the gut microbiome in the development and course of food allergy through a targeted discussion of results from observational human, murine, and interventional trial studies. We additionally discuss the directions in which we envision future investigations of this exciting area will move.

Observational Human Studies

Studies of the microbiome in human subjects with food sensitization and food allergy thus far have yielded variable findings, likely because of several factors, including heterogeneity of study populations, variable definitions of food sensitization and food allergy, differences in profiling and study designs, and varying sample sizes.

Early studies of gut microbiota and food allergy were culture-based, targeting groups of and individual bacteria of interest to explore hypotheses regarding the relationship between gut microbial characteristics and food allergy in early childhood. For example, a culture-based study of Spanish children with milk allergy (based on sensitization by skin prick test [SPT] > 3 mm, sIgE ≥ 0.35 kU_A/L, and milk formula challenge) showed that milk-allergic infants had higher total bacteria and anaerobic counts compared with healthy controls; after 6 months of differential formula intake, the 46 milk-allergic infants had higher proportions of lactobacilli and lower proportions of enterobacteria and bifidobacteria observed in bacterial cultures.⁶ Although culture-based methods can quantify counts of specific bacteria, the approach is tied to the specific culture conditions of individual bacteria of a priori interest, and most bacteria cannot be cultured. Culture-based approaches are therefore limited in the number and diversity of microbes that can be profiled.

More recent studies of gut microbiota in food-allergic individuals have employed sequencing of the 16S rRNA gene, which encodes a component of the prokaryotic ribosome.¹⁵ The 16S rRNA gene has highly conserved primer binding sites and contains hypervariable regions that can provide species-specific signature sequences for bacterial identification, thus enabling more comprehensive bacterial identification without the constraints of culture-based methods.¹⁵

Findings from 16S rRNA sequencing-based studies of food sensitization and food allergy defined broadly suggest that gut dysbiosis may precede the development of food allergy. Among a cohort of 225 US children, lower relative abundances of *Haemophilus*, *Dialister*, *Dorea*, and *Clostridium* in stool samples collected at ages 3 to 6 months was associated with sensitization (sIgE ≥ 0.10 kU_A/L) to at least 1 food allergen among milk, egg, peanut, soy, and wheat.⁷ Additionally, the investigators found lower relative abundances of *Citrobacter*, *Oscillospira*, *Lactococcus*, and *Dorea* in stool collected at ages 3 to 6 months in children who had food allergy by

age 3 years, defined by sensitization and convincing allergic symptom.⁷ Separately, a longitudinal study of 166 infants at ages 3 and 12 months from the Canadian Healthy Infant Longitudinal Development study showed that lower gut microbial richness at age 3 months was associated with increased likelihood of food sensitization (SPT wheal ≥ 2 mm than negative control) by age 12 months.¹⁶ Each quartile increase in richness at 3 months was associated with a 55% reduced risk for food sensitization by age 12 months (adjusted odds ratio [OR], 0.45; 95% confidence interval [CI], 0.23, 0.87).¹⁶ Enterobacteriaceae were overrepresented and Bacteroidaceae were underrepresented in food-sensitized infants at 3 months and 1 year.¹⁶ These associations between early gut microbiome composition and later food allergen sensitization or food allergy suggest a possible role for gut dysbiosis in the development of food allergy.

Studies of the gut microbiome in food allergy and related phenotypes additionally suggest a critical role of timing. The gut microbiome is known to change with time, with the most rapid change occurring in early life.¹⁷ In the Canadian Healthy Infant Longitudinal Development cohort, gut microbial richness at age 3 months was associated with increased likelihood of food sensitization by age 1 year, whereas no association was seen between gut microbial characteristics at age 12 months and food sensitization.¹⁶ Beyond food allergen sensitization, a multicenter, longitudinal study of 226 milk-allergic children in the United States from the Consortium for Food Allergy Research examined the relationship between infant gut microbiome and food allergy resolution, with milk allergy defined based on oral food challenge, convincing history, and positive allergy testing, or flare of atopic dermatitis associated with milk ingestion and milk sIgE ≥ 5 kU_A/L. The investigators found that Firmicutes including Clostridia were enriched in the gut microbiome of infants ages 3 to 6 months whose milk allergy resolved by age 8 years.⁸ However, in older infants, no association was seen between gut microbiome composition and milk allergy resolution.⁸ Findings from murine models also support age-sensitive contact with microbiota.¹⁸ 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.¹⁹ These collective findings support the notion that microbial effects on early immune system development play a role in subsequent food allergy development.

Given differences in the presentations and natural histories of specific food allergies, gut microbiota associated with individual food allergies also might be distinct. In a study of 141 children, genera from Lachnospiraceae, Streptococcaceae, and Leuconostocaceae were differentially abundant in the gut microbiome of US children with egg allergy vs non-food-allergic controls from the multisite Consortium for Food Allergy Research. In this study, egg allergy was defined based on oral food challenge, convincing history, and positive allergy testing, or flare of atopic dermatitis associated with egg ingestion and egg sIgE ≥ 2 kU_A/L ($n = 141$).⁹ Studies of gut microbiota in milk-allergic individuals demonstrate some overlapping but also contrasting gut microbial compositions. For example, a study of 39 Italian children showed that, compared with healthy controls, milk-allergic infants (diagnosed based on history, oral food challenge, and milk sIgE level) had gut microbial community structures dominated by Lachnospiraceae and Ruminococcaceae.¹² Although differences in the implicated taxa reported in these studies may have been attributable to the specific food allergy targeted, additional explanations include differences in other population characteristics, phenotyping details, or analytic strategy, including assessment of microbiota at varying taxonomic levels. The characterization and comparison of microbial signatures of different food allergies remains an area that merits further investigation.

Murine Studies

Murine models of food allergy have provided insightful experimental dimensions to understanding the role of the microbiome in food allergy, with intriguing results to suggest that risk for food allergy can be altered by gut microbiota. For example, food allergy-prone mice with a gain-of-function mutation in the interleukin (IL)-4 receptor- α chain have differential gut abundances of Lachnospiraceae, Lactobacillaceae, Rikenellaceae, and Porphyromonadaceae compared with wild-type mice.¹⁴ Transfer of gut microbiota from these food allergy-prone mice to germ-free mice seemed to transfer disease susceptibility, with reconstituted mice demonstrating OVA-specific IgE responses and symptoms consistent with anaphylaxis on OVA challenge.¹⁴ Although therapy of these food allergy-prone mice with Treg cells specific for the immunodominant OVA peptide suppressed allergen sensitization and correlates of anaphylaxis, and this Treg therapy altered the gut microbiota, their gut microbiota did not shift back to baseline, suggesting that immunomodulatory mechanisms beyond Treg cells are involved.¹⁴ Another study demonstrated the converse—that transfer of gut microbiota from healthy mice to allergy-prone mice is protective. Specifically, colonization of milk-sensitized, germ-free mice with bacteria from healthy infant stool, which contains abundant taxa from *Bifidobacterium* and *Bacteroides*, reduced signs of systemic allergic response (eg, lower magnitude of rectal temperature drop) on challenge to cow's milk protein compared with their uncolonized murine counterparts.²⁰ Additionally, wild-type mice induced to have altered gut flora by antibiotic treatment also become more susceptible to systemic anaphylaxis on oral challenge compared with mice not treated with antibiotics.²¹ When the gut flora of these mice are allowed to repopulate, allergen-specific IgE levels in these mice decrease.²¹ Collectively, these findings from murine models suggest that microbial dysbiosis plays a role in food allergy pathogenesis.

The mechanisms by which gut microbiota contribute to food allergy risk are not yet completely understood. Based on observations from murine studies, we have previously described the potential mechanisms by which gut microbiota may influence allergic diathesis,³ which we summarize in Figure 1 and below under the following categories: (1) modulation of type 2 immunity, (2) influence on immune maturation and tolerance, (3) regulation of basophil populations, and (4) promotion of intestinal barrier function.

Modulation of Type 2 Immunity

Evidence from germ-free mouse models supports a role for microbiota in modulating type 2 immunity. Compared with wild-type mice, germ-free mice exhibit elevated serum IgE levels at baseline. This is thought to be attributable to isotype switching of B cells to IgE at mucosal sites in a CD4 T-cell and IL-4-dependent manner.¹⁹ Germ-free mice are also more likely to become sensitized to cow's milk protein and ovalbumin through oral exposure.^{19,20} When challenged, milk-sensitized germ-free mice exhibit exacerbated systemic anaphylaxis with reduced rectal temperatures, higher levels of blood mouse mast cell protease-1, beta lactoglobulin-specific IgG1, and a systemic Th2-skewed response compared with sensitized wild-type controls.²⁰ In the absence of microbial signals, increased expression of IL-33, a type 2 cytokine, also has been observed in the intestinal epithelium.²² The presence of microbiota can induce IL-6 and IL-23 production toward a type 3 response that suppresses type 2 responses.²² Microbiota also may regulate type 2 responses by inducing retinoic acid receptor-related orphan nuclear receptor gamma positive (ROR γ t+) Tregse in a retinoic acid-dependent manner. ROR γ t+ Tregs regulate coactivator functions of dendritic cells by expressing high levels of CTLA4, thereby regulating the generation of Th2 cells

in the intestine. Mice deficient in ROR γ t+ Tregs produced higher amounts of the type 2 cytokines IL-4 and IL-5.²²

Immune Maturation and Tolerance

Conventional immune maturation in mice requires colonization by mouse-specific microbiota.²³ In contrast, germ-free mice and mice populated with human microbiota have low levels of CD4+ and CD8+ T cells, low rates of T cell proliferation, few dendritic cells, and low antimicrobial peptide expression.²³ These differences in immune maturation are possibly attributable to the decreased ability of human microbiota in mice to induce antigen uptake from the gut lumen by dendritic cells, thereby decreasing downstream T cell activation and proliferation.²³ Colonizing germ-free mice with segmented filamentous bacteria, which are commensal taxa in mice, partially restores CD4+ ROR γ t+ T cell counts, while failing to increase CD4+ Foxp3+ cell numbers.²³ These results suggest that individual components of host-specific microbiota may be responsible for specific aspects of immune maturation.

In murine models, microbial colonization promotes the expansion of Treg cells, which are essential for immune tolerance and modulation of adaptive immunity.²⁴ Oral colonization of mice with certain Clostridia strains promotes colonic Treg cell population by stimulating the secretion of transforming growth factor beta from intestinal epithelial cells.²⁵ In addition, strain-specific Clostridia colonization induced key anti-inflammatory molecules (IL-10 and ICOS) in Treg cells, leading to an attenuated phenotype in an allergic disease model.²⁵ *Bacteroides* strains similarly promoted Treg cells and functions, although to a lesser degree compared with strains within Clostridia.²⁴

The presence of microbiota overall also promotes IL-1 β secretion by intestinal macrophages, which leads to granulocyte-macrophage colony-stimulating factor release by type 3 innate lymphoid cells and subsequent release of retinoic acid and IL-10 by dendritic cells and macrophages. Retinoic acid and IL-10, in turn, promote the expansion of local Treg cells.²⁶ In 1 study aimed at identifying CD4+ FOXP3+ Treg-inducing bacterial strains from human microbiota, 17 strains of bacteria from human stool were identified as enhancing Treg cell abundance, with genome sequencing revealing that all 17 strains were within Clostridia.²⁵ In another study, colonization of gnotobiotic mice with *Clostridium* clusters was found to be protective against sensitization to peanut/cholera toxin. Compared with germ-free controls, the mice colonized with *Clostridium* clusters showed reduced levels of peanut-specific and total IgE and no drop in rectal temperature on allergen challenge.²⁷ Although many studies in murine models have found that taxa from Clostridia confer an overall protective effect on the host, their association with atopic risk has been more variable in human studies.^{5,8,9,11,28,29}

Regulation of Basophil Populations

Gut microbiota may modulate allergic responses by modulating basophil populations. In mice treated with broad-spectrum antibiotics, increased numbers of steady-state circulating basophil populations were observed along with exaggerated Th2 cell responses and elevated IgE concentrations.³⁰ Depletion of basophils in these mice attenuated Th2 cell responses, suggesting that basophils contributed to the observed allergic inflammation. Microbially derived signals also were observed to influence proliferation of bone-marrow resident precursor populations for basophil development.³⁰

Promotion of Intestinal Barrier Function

Gut microbiota can promote IgA secretion, contribute to immune exclusion, and reduce allergen uptake.²⁷ For example, fecal IgA levels in germ-free mice can be restored with colonization by

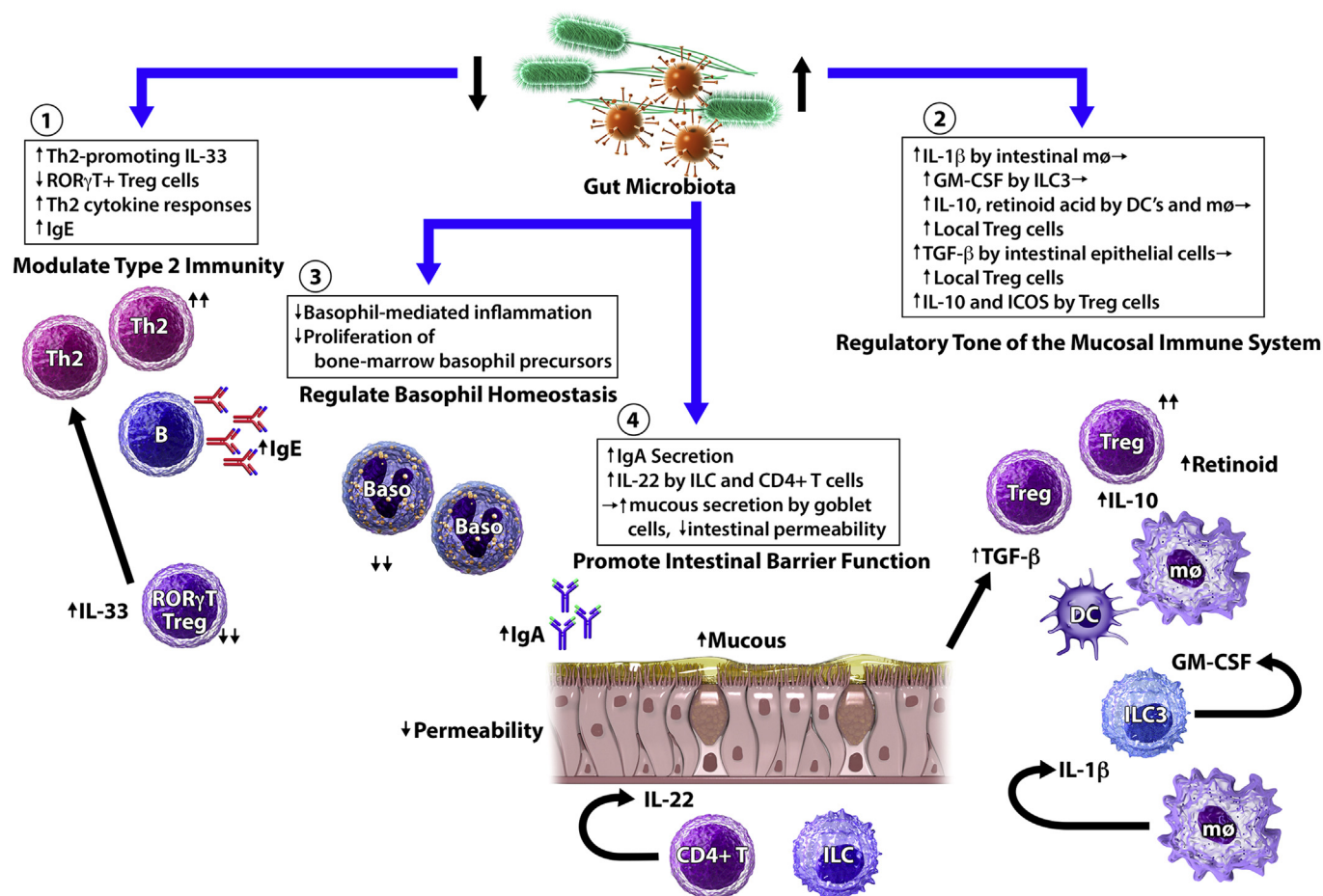


Figure 1. Mechanisms by which gut microbiota may affect food allergy susceptibility. Murine models of food allergy have shown that gut microbiota interact with multiple aspects of the intestinal mucosa, innate immunity, and adaptive immunity. (1) Gut microbiota modulate type 2 immunity. In germ-free mice, expression of Th2-promoting cytokine IL-33 by epithelial cells is increased. In contrast, Th2-inhibiting ROR γ t⁺ Treg cells are profoundly reduced in both germ-free and antibiotic-treated mice. As a result, Th2-associated pathology is exacerbated, and IgE levels are elevated in the absence of key microbial signals. (2) Gut microbiota influence immune development and tolerance. Microbial colonization promotes the expansion of Treg cells. Microbial signals promote IL-1 β secretion by intestinal macrophages (m ϕ), which leads to GM-CSF release by type 3 innate lymphoid cells (ILC3). As a result, IL-10 and retinoid acid secretions by dendritic cells (DC) and m ϕ are elevated, which leads to the expansion of local Treg cells. Strain-specific Clostridia colonization of gnotobiotic mice stimulates the secretion of transforming growth factor beta from intestinal epithelial cells, leading to the expansion of colonic Treg cells. Strain-specific Clostridia colonization also induces key anti-inflammatory molecules (IL-10 and ICOS) in Treg cells. (3) Gut microbiota regulate basophil populations. Circulating basophil levels increase in antibiotic-treated mice. Conversely, the presence of microbial signals limits the proliferation of bone-marrow basophil precursors, and reduces basophil-mediated allergic inflammation. (4) Gut microbiota promote intestinal barrier function. Selective colonization of germ-free mice with certain strains of Clostridia and Bacteroides promotes intestinal IgA secretion, which can contribute to immune exclusion and reduce allergen uptake. Strain-specific Clostridia colonization of germ-free mice also induces IL-22 production by innate lymphoid cells (ILC) and CD4⁺ T cells. IL-22, in turn, promotes mucous secretion by goblet cells and reduces intestinal permeability to dietary allergens. Adapted from Ho and Bunyavanich. *Curr Allergy Asthma Rep.* 2018;18(4):27. Abbreviations: ICOS, ; Ig, immunoglobulin; IL, interleukin; ROR γ t⁺, ; TGF- β , transforming growth factor beta.

Clostridia clusters (predominantly clusters XIVa, XIVb, and IV), and partially restored with *Bacteroides uniformis* colonization.²⁷ Gene expression of IL-22, a cytokine that protects intestinal epithelial barrier by promoting mucus secretion by goblet cells, is also up-regulated in lamina propria lymphocytes of germ-free mice colonized with Clostridia clusters (but not *B uniformis*), with concomitant increases in the numbers of mucus-producing goblet cells.²⁷ This is thought to affect permeability of the intestinal barrier to dietary allergens, such as peanut Arah2 and Arah6 proteins, because circulating concentrations of these proteins after intragastric peanut gavage were reduced in Clostridia-colonized (but not *B uniformis* colonized) mice.²⁷

Although murine models provide insight into potential mechanisms that characterize microbiome interactions with immune development and tolerance in humans, such mechanisms may be distinct in human beings, and the extent to which findings from murine models can be observed in human beings remains to be seen. In human beings, evidence supporting a causal role for gut

microbiota in the development and course of food allergy remains largely unexplored, in large part because of the challenges of performing interventional trials in this area.

Interventional Trials

Findings that gut microbiota can modulate food allergy susceptibility suggest potential therapeutic utility from the manipulation of the gut microbiome to the host's advantage. This is an area of great interest given the potential benefits and possible opportunities to shape food allergy development and treatment. Potential modalities for gut microbiome manipulation include diet, probiotics, prebiotics, synbiotics, and fecal microbiota transplantation (Fig 2).

Probiotics

A limited number of clinical trials of probiotics for the prevention or treatment of challenge-proven food allergies have

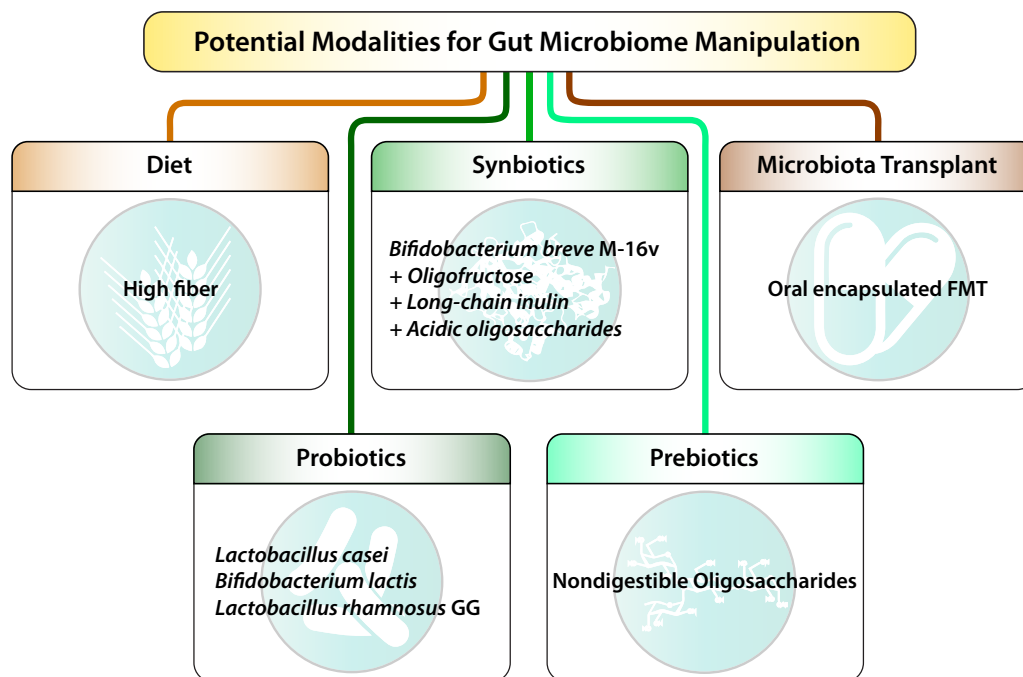


Figure 2. Potential modalities for gut microbiome manipulation. Potential modalities for gut microbiome manipulation include diet, probiotics, prebiotics, synbiotics, and fecal microbiota transplantation. Probiotics are live microorganisms that, when administered, confer a health benefit to the host. Prebiotics are nondigestible substrates that can be selectively used by host microorganisms. Synbiotics are products that contain both prebiotics and probiotics. Fecal microbiota transplantation (FMT) is the administration of fecal matter from a donor to a recipient. Examples of each based on existing or planned studies are provided.

been published. Probiotics refer to live microorganisms that can confer benefits to the host when administered. Clinical trials of probiotic supplementation with *Lactobacillus casei* and *Bifidobacterium lactis* in children with milk allergy for 12 months showed no effect on milk allergy resolution.^{13,31} However, another trial studying supplementation with *Lactobacillus rhamnosus* GG combined with extensively hydrolyzed casein formula in milk-allergic children demonstrated increased rates of milk allergy resolution after 6 and 12 months compared with a control group receiving the formula alone.^{13,31} At 12 months, the absolute risk difference in cow's milk tolerance was 0.20 (95% CI, 0.05–0.35) between the trial arms. A subsequent study comparing stool from healthy infants and from cow's milk-allergic infants before and after treatment with extensively hydrolyzed formula with or without *Lactobacillus rhamnosus* GG showed that *Blautia* and *Roseburia* were enriched in the gut microbiome of tolerant infants with higher concentrations of the short-chain fatty acid butyrate, leading the investigators to hypothesize that the acquisition of specific strains in these genera is associated with tolerance.¹² Consistent with observations from other studies of gut microbiota, the effects of probiotics are likely strain-specific, and thus careful attention to strain-level effects and interventions is merited.

Much of the work on probiotics and food allergy has focused on individuals with cow's milk allergy, with far fewer studies of other food allergies. However, the rationale for an effect of probiotics on other food allergies is analogous, and *Lactobacillus rhamnosus* GG has also been studied in the context of peanut allergy. In a clinical trial, *Lactobacillus rhamnosus* GG was administered with peanut oral immunotherapy for 18 months. Subjects receiving the combination treatment had higher rates of desensitization to peanut compared with placebo (82.1% vs 3.6%, respectively).³² However, because there was no OIT-only or probiotic-only group, efficacy attributable to the probiotic itself was unclear. A follow-up study of 48 of the 56 children who participated in this combined probiotic and oral immunotherapy trial 4 years after treatment cessation found that individuals who had been treated were more likely to

have continued eating peanut compared with those who had taken placebo (67% vs 4%, $P = .001$), and more participants from the treated group had smaller peanut skin prick test size and higher peanut sIgG4:sIgE ratios compared with placebo-treated controls.³³

Given the limited aggregate literature on probiotics and food allergy thus far, the most recent Cochrane review³⁴ states that more data are needed before recommendations can be made to support probiotic supplementation for food allergy. This is supported by more recent meta-analyses on the subject,³⁵ as well as recognition by the World Allergy Organization that there is "very low quality evidence" on this topic at present.³⁶

Prebiotics

Prebiotics are nondigestible food components that selectively foster the growth and activity of selected commensal microbiota in the host. For example, fiber is a prebiotic that many gut bacteria use as a nutrient and in turn produce short-chain fatty acids, which are thought to beneficially inhibit allergic inflammation.^{37,38} Prebiotics are frequently added to infant formulas. Effective prebiotics are not digested or absorbed in the upper gastrointestinal tract and reach the large intestines, where they are selectively used by microorganisms, with health-promoting effects.³⁹ Clinical trials of prebiotic supplementation in infants have shown no effect on the development of food allergy.^{39–41} However, risk of asthma and eczema was reduced in some individual studies.^{39–41}

Synbiotics

Combinations of probiotics and prebiotics are termed synbiotics, and clinical trials of these for the prevention of allergy are underway. A prospective, randomized, double-blind, controlled study had 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.⁴² The synbiotics used in this trial included *Bifidobacterium breve* M-16V, oligofructose, long-chain inulin, and acidic

oligosaccharides. A planned but not yet recruiting randomized, double-blind clinical trial of children at high risk for allergy sponsored by the same company will compare partially hydrolyzed infant formula with synbiotics vs standard infant formula (NCT03067714) for the primary outcome of doctor-diagnosed IgE-mediated allergic manifestations.

Fecal Microbiota Transplantation

Fecal microbiota transplantation (FMT) refers to the transfer of communities of microbes from a donor to a recipient, such as has been used for patients with *Clostridium difficile* colitis.^{43,44} This is an intriguing option to consider, given findings from murine models supporting that fecal transfer can be an effective mode to shape allergy outcomes. A small phase I open-label trial to evaluate the safety and efficacy of oral encapsulated FMT for the treatment of peanut allergy is underway (NCT02960074). However, until further work is done in this area, significant questions will remain about FMT for food allergy treatment, including the optimal community to transfer, how best to ensure establishment of the donated community, and safety of the procedure.

Future Directions

Knowledge about the gut microbiome's role in food allergy is an emerging area with ample opportunities to deepen current foci as well as develop new directions. Findings in this area of research have been relatively heterogeneous, reflecting the variability of study designs, diverse phenotyping criteria, and differences in profiling and analytic approaches. Especially for human studies of the gut microbiome of food allergy, thoughtful prospective planning of study design and execution in robust sample sizes are needed to enable reliable findings that allow for valid cross-study comparisons. Attention to clinical phenotyping will ensure rigorous and reproducible definitions of food sensitization and food allergy, and more studies should be conducted to assess the extent to which associations between the gut microbiome and food allergy differ by specific food allergy. Although murine studies have yielded many insights, additional work will be needed to assess the degree to which murine findings are translatable to the human context. Longitudinal studies of the gut microbiome in human populations, involving sampling and phenotyping at multiple time points, will also further our understanding of temporal sequence, causality, and how host–microbe interactions may shape the development and course of food allergy.

Because most current investigations of the microbiome in food allergy have focused on bacterial microbiota, future research could assess contributions from the virome and mycobiome. Sequencing methods, reference databases, and analytic tools to assess the virome and mycobiome are less developed than those for the bacterial microbiome.⁵ However, increasingly available tools for the analysis of data from shotgun metagenomic sequencing (ie, whole genome sequencing performed on genomic DNA from a mixed microbial community) are enabling microbiome research efforts beyond the bacterial microbiome.^{4,45} For characterizing the bacterial microbiome, shotgun metagenomic sequencing also can offer better resolution than 16S rRNA sequencing at the species and strain level and more accurate community diversity estimates by avoiding polymerase chain reaction biases.^{4,45} For these reasons, shotgun sequencing is increasingly used in prominent studies of the microbiome,^{46–48} although its relative higher cost is a consideration. Because both human and murine studies have shown that strains within the same bacterial species can have differing immunologic effects,^{25,27} the capacity to decipher microbiota at the strain level will likely become increasingly desired.

Better characterization of interactions between microbiota and host, and the specific pathways and effects by which microbiota

exert their influence, is another worthwhile research direction that will be enabled by multidimensional profiling and systems biology.^{4,49,50} Because food allergy is a complex and heterogeneous disease, it is unlikely that the microbiota implicated in its pathogenesis and disease course, or even the microbiome in its entirety, can capture the interdependent dynamics of the molecular networks involved in food allergy.⁴ Our understanding of food allergy has been advanced not only by studies of the microbiome, but also by data generated through genome-wide association,^{51–54} transcriptome,⁵⁵ epigenome,^{56,57} and metabolome studies.^{4,58,59} Integrating these types of systemwide 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.^{4,11,49,50}

Likely bacterial, viral, and fungal biomes interact with the human genome in complex ways to influence food allergy.^{4,36,49,50} System biology approaches have been used to examine relationships between microbiota and host genomic profiles in other disease areas such as inflammatory bowel disease.^{60,61} To make accurate disease predictions, we need to apply a systems biology approach to create the networks capable of representing causal relationships among molecular features within a given cell or tissue type,⁵⁵ between different tissues,⁶² and between host and environment, including the microbiome.⁴

Conclusion

Our understanding of the role of the gut microbiome in food allergy has evolved with knowledge gained from observational human, murine, and interventional trial studies. The evidence thus far suggests that gut dysbiosis likely precedes the development of food allergy, and the timing of such dysbiosis is critical. Gut microbiota associated with individual food allergies may be distinct, but further research in this area is needed. Murine models have provided important experimental dimensions to our study of the microbiome in food allergy. Mechanistically, gut microbiota may affect food allergy susceptibility by modulating type 2 immunity, influencing immune maturation and tolerance, regulating basophil populations, and promoting intestinal barrier function. Ongoing and future clinical trials of probiotics, prebiotics, synbiotics, and fecal microbiota transfer will help translate our enhanced understanding of the gut microbiome in food allergy to clinical practice. Future work in this area will include the deepening of current research foci as well as expansion to considerations of the virome, mycobiome, and interactions between the microbiome, host, and environment through systems biology approaches. By moving forward research on the microbiome in food allergy, we can further our understanding of food allergy and derive new approaches for its prevention and therapy.

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