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Microbial Adjuncts for Food Allergen Immunotherapy

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Abstract

Purpose of Review Food allergen immunotherapy may benefit from adjunct therapies to enhance safety and efficacy. We review preclinical studies investigating the effects of probiotics and other microbial-based interventions on oral tolerance, describe the human clinical trial evidence thus far for microbial adjuncts, and discuss steps for translating research findings in this area to clinical therapy.

Recent Findings Murine studies support that microbial-based interventions confer protection against sensitization and may augment treatment efficacy for food allergy. Microbial adjunct therapies can promote regulatory T cells and modulate Th1 vs. Th2 responses. There is a wide array of novel modalities utilizing microbial components. Ongoing efforts are focused on translating preclinical data into potential treatments.

Summary Probiotics, prebiotics, and microbial components have all been examined as microbial adjunct therapies in murine models of food allergy. The effects of probiotics appear to be strain-specific. Prebiotics and bacterial components are innovative modalities to modulate oral tolerance. Better characterization of dysbiosis in human cohorts with food allergy, deeper mechanistic understanding of microbial adjunct therapies, safety evaluation, and careful clinical trial design will be crucial for the development of microbial adjuncts for food allergen immunotherapy. Microbial adjunct therapies have the potential to enhance the efficacy, safety, and durability of food allergen immunotherapy.

Keywords Food allergy · Immunotherapy · Microbiome · Microbiota · Microbial adjunct · Peanut allergy · Probiotics · Prebiotics · Oral tolerance

Introduction

Food allergy has become a major health problem in recent decades, with up to 10% of the population affected in some industrialized countries [1, 2]. Historically, strict allergen avoidance has been advised to prevent potentially life-threatening systemic allergic reactions. In the past decade, however, food allergen immunotherapy has become an area of intense interest, with a series of studies suggesting that the introduction of a tiny quantity of allergen followed by incremental increases in dosage may lead to clinical protection from accidental exposures below a threshold [3, 4••, 5–8]. Recent completion of the first phase 3 trial for peanut oral immunotherapy (OIT) has highlighted the therapeutic benefits, as well as current limitations, of food allergen immunotherapy [4••]. The lack of peanut OIT efficacy in adults (age > 17 years) and up to a third of children (age 4–17 years), as well as remaining concerns about optimizing safety and whether sustained unresponsiveness (Table 1) can be achieved have raised interest in potential roles for adjunct therapy [9].

Concurrent to advances in immunotherapy, we have come to appreciate the immunologic influence of commensal microbiota, (Table 1) on oral tolerance in recent years [10, 11]. As a result, the potential application of microbiota and/or microbial-derived products in food allergy treatment has garnered significant attention. In particular, whether microbial-based therapies can serve as an adjunct to immunotherapy remains an area of active investigation. In this review, we discuss (1) emerging preclinical data on microbial-based manipulations in animal models of food allergy, (2) available clinical data on microbial adjunct therapies in food allergen

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Table 1 Glossary

Microbiota	The community of microbes in a particular environment
Microbiome	The sum of microbes and their genomic elements in a particular environment
Probiotics	Live microorganisms with beneficial effects on the host
Prebiotics	Non-digestible food components that promote the growth and/or function of beneficial microorganisms
Oral tolerance	A state of local and systemic immune suppression to antigens (e.g., food proteins) introduced by the oral route, translating to a lack of clinical reactivity upon oral antigen introduction that is independent of both quantities of antigens ingested and routine antigen exposure
Desensitization	A temporary increase in reactivity threshold to provide a measure of safety that is dependent on continued treatment exposure
Sustained unresponsiveness	A state of clinical unresponsiveness to food allergen that persists despite discontinuation of treatment exposure

immunotherapy, and (3) key knowledge gaps in the field and essential steps necessary to translate what we have learned thus far into potential therapies.

Food Allergen Immunotherapy

The natural development of oral tolerance involves complex interactions between food antigen, mucosal barriers, the innate and adaptive immune systems, microbiota, and diet [12]. Tolerance is an active immunological process. Based on adoptive transfer murine studies, it is clear that regulatory T (Treg) cells are key suppressors cells that actively maintain oral tolerance to food antigens [13], with gut homing and expansion of Treg cells required for intestinal tolerance [14, 15]. In contrast, food allergy is thought to arise from a lack of adequate regulatory response combined with a pathological T-helper (Th) 2 response driving the production of allergen-specific IgE antibodies [16, 17]. The development of oral tolerance vs. food allergy has previously been reviewed [12].

Food allergen immunotherapy commonly involves initial introduction to a small amount of allergen followed by a slow dose-escalation phase (over weeks to months) and then a maintenance phase of stable dosing. Multiple forms of food allergen immunotherapies are currently being investigated, including oral (OIT), sublingual (SLIT), and epicutaneous (EPIT) immunotherapy (reviewed previously in [18]). As data from clinical trials emerge, it has become clear that the "tolerance" induced by immunotherapy differs from naturally developed oral tolerance [19]. True oral tolerance is characterized by a complete lack of clinical reactivity to food antigens regardless of the quantity ingested, and it is independent of continued antigen exposure (Table 1). In contrast, the main outcome of immunotherapy appears to be a state of "desensitization," in which the clinical reactivity threshold to a food antigen is increased with ongoing antigen exposure (Table 1). In addition, it is unclear whether long-term "sustained unresponsiveness," a state of non-responsiveness to a food antigen in the absence of continued antigen exposure, may be achieved (Table 1) [19]. Thus, the "durability" of food allergen immunotherapy in its current forms remains to be seen.

The exact mechanism of food allergen immunotherapy remains to be fully elucidated (reviewed previously in [20]). However, basophil anergy, decreased mast cell reactivity, increased food-specific IgG4 and IgA, and ultimately decreased food-specific IgE (following an initial increase) have been observed [21–23]. Similarly, the immune mechanism of sustained unresponsiveness is currently unknown. It has been postulated that Treg cells may be involved [7, 24, 25]. For instance, there is differential DNA modification in antigen-induced Treg cells between human subjects who develop clinical tolerance vs. those who do not after OIT [26•].

In its current state, the efficacy of food allergen immunotherapy (defined as either successful desensitization or sustained unresponsiveness) varies widely between studies. In OIT, for instance, desensitization rate ranges from 36% to 100%, and sustained unresponsiveness rates range from 27% to 92% under various definition of its duration [18]. The recently completed phase 3 peanut OIT trial showed efficacy in up to two thirds of children (age 4–17 years) [4••]. It has become clear that age is an important factor, with minimal efficacy observed in adults (for OIT) and older children (for EPIT) [4••, 27]. However, it still remains unclear why some children fail to respond to immunotherapy. Another major limitation of food allergen immunotherapy is its safety profile. For OIT, mild allergic adverse events were recorded in most participants during the intervention phase; in addition, systemic reactions or anaphylaxis were also observed in a significant portion of participants (up to 40%) [28–31]. In the recent phase 3 peanut OIT trial, 14% of the actively treated group needed epinephrine at one point and 11.6% withdrew from the trial due to side effects during the intervention period (vs. 2.4% of the control group) [4••]. As limitations of food allergen immunotherapy become apparent, there is interest in adjunct therapies that could improve its efficacy, safety, and durability.

Microbial-Based Interventions in Animal Models of Oral Tolerance

A strong body of preclinical literature supports a role for commensal bacteria in mediating tolerance to food antigens. There are trillions of microbes colonizing our mucosal and skin surfaces. Genetic evidence suggests a symbiotic co-evolution between the bacterial communities and the host, with the microbiome containing genes that are greatly beneficial to the host but of minimal benefit to the microbe itself [32]. This complex ecosystem maintains a meticulous homeostasis with the host immune system, shaping each other's development since birth [33]. In addition, gut microbiota also facilitates primary and secondary metabolism, leading to interactive host-microbiota metabolic, signaling, and immune axes [33, 34]. It is now appreciated that microbiota influence human health and disease [35, 36]. In food allergy, epidemiologic studies have linked differential early-life microbial exposures to atopic risk, while emerging observational studies have suggested the presence of gut dysbiosis in children with food allergy, implicating its role in the pathogenesis of food allergy. [10, 11, 36–38]

Current insights into a potential role for microbial-based interventions in food allergy are mostly derived from gnotobiotic murine models. While the majority of studies thus far have focused on *allergic sensitization* and *prevention*, and not *desensitization* or *treatment*, the available evidence is that "healthy" human microbiota promote oral tolerance in murine models. Rodriguez et al. previously demonstrated that the transfer of healthy human infant gut microbiota into germ-free mice confers protection against cow's milk allergy development [39]. More recently, Feelhey et al. took this a step further by showing that consistent with Rodriguez et al., fecal transfer from healthy human infants to germ-free mice confers protects against cow's milk allergy, but additionally, fecal transfer from *cow's milk allergic* infants does *not* protect against anaphylactic response to cow's milk challenge [40]. Specifically, both germ-free mice and mice colonized with cow's milk allergic infant bacteria had significant core body temperature drop (a measure of anaphylactic response in mice) during oral food challenges [40]. In comparison, mice colonized with healthy infant bacteria did *not* have core body temperature drop during oral food challenges [39, 40].

Microbial-based interventions come in several forms, and thus far, probiotics, prebiotics (Table 1), and microbial components have all been examined in murine models of food allergy (Table 2). As the major theme, many of these treatment modalities were selected based on the ability of commensal microbes and their components to (1) mediate Treg cells and (2) modulate the balance between T-helper (Th) type 1 vs. atopic Th2 responses [10, 11]. These two key capabilities have made microbial-based manipulation a compelling adjunct to food allergen immunotherapy. A summary of mechanisms by which microbial adjunct therapies may promote oral tolerance can be seen in Fig. 1.

Probiotics

Probiotics are live microorganisms that are thought to have beneficial effects on the host when used as a dietary supplement. In line with this concept of probiotics, the protective effects of "healthy" human microbiota may be attributable to specific bacterial strains. Based on the finding that the spore-forming component of the indigenous gut microbiota promoted Treg cell development, Atarashi et al. demonstrated that compared to untreated specific pathogen-free (SPF) mice, oral administration of *Clostridium* during early life to conventionally reared SPF mice led to a significant reduction of egg ovalbumin (OVA)-specific IgE production after allergen sensitization [41]. In addition, splenocytes from OVA-alum-immunized, *Clostridium*-treated mice showed significantly lower type 2 cytokine (IL-4) secretion after *in vitro* OVA re-stimulation compared to untreated SPF mice [41]. Stefka et al. independently reported that selective colonization of gnotobiotic mice with *Clostridium*-containing microbiota protected against sensitization to peanut-cholera toxin, with reduced peanut-specific and total IgE in the serum (compared to germ-free controls) [43]. Upon oral peanut challenge, mice colonized with *Clostridium* had an absence of core body temperature drop, while germ-free controls developed anaphylactic response [43]. Narushima et al. subsequently isolated the protective effect of *Clostridia* to 17 specific strains within clusters IV, XIVa, and XVIII—a group that demonstrated high potency in enriching Treg cells while lacking prominent toxins and virulence factors [42••]. Oral supplementation with these 17 *Clostridia* strains in mice similarly led to a reduction of egg OVA-specific IgE production after sensitization attempts (compared to SPF control mice) [42••]. In an allergic diarrhea model, colonization with these 17 *Clostridia* strains led to reduced diarrhea scores after allergen (egg OVA) challenge [42••].

In a manner synergistic to the postulated mechanism of immunotherapy, the beneficial effects of supplementation with select *Clostridia* strain appear, in part, to be due to their influence on Treg cell number and function in the gut. Atarashi et al. showed that *Clostridium* colonization significantly increased the local production of TGF-β, a key cytokine for the differentiation and expansion of Treg cells, in the colon [41]. In addition, colonic intestinal epithelial cells from *Clostridium*-colonized mice also expressed high levels of indoleamine 2,3-dioxygenase (IDO), another regulator of Treg induction [41, 52]. Indeed, gnotobiotic mice colonized with *Clostridium* were found to have a robust accumulation of Tregs in the colonic lamina propria compared to SPF and germ-free mice [41]. Furthermore, these colonic Treg cells were found to produce IL-10, a key suppressor cytokine that

Table 2 Preclinical evidence from murine models for microbial-based interventions that modulate oral tolerance

Treatment	Category	Experimental model	Outcomes
Select *Clostridia* strains	Probiotics	Egg ovalbumin (OVA) sensitization model	Lower OVA-specific IgE, reduced allergic diarrhea score to oral OVA challenge compared to specific pathogen-free controls [41, 42••]
		Peanut sensitization model	Lower peanut-specific IgE and total IgE, absence of core body temperature drop to oral peanut challenge compared to germ-free controls [43]
Bifidobacterium infantis	Probiotics	Egg OVA sensitization model	Lower OVA-specific IgE, reduced allergic diarrhea score to oral OVA challenge compared to germ-free controls [44]
Lactobacillus rhamnosus GG	Probiotics	Cow's milk-sensitized model, adjunct therapy to hydrolyzed formula	Lower cow's milk-IgE, reduced acute allergic skin reaction, reduced anaphylactic symptom score to oral cow's milk challenge compared to formula-alone group [45]
High-fiber diet	Prebiotics	Peanut sensitization model	Lower total IgE, reduced anaphylactic score to oral peanut challenge compared to control diet group [46••]
Butyrate, acetate, and propionate (BAP)	Bacterial metabolites	Airway allergy-prone antibiotic-treated model	Lower total IgE, reduced airway infiltration of inflammatory cells compared to no BAP supplementation [47]
CpG oligonucleotides	Bacterial components: TLR agonist (TLR9)	Peanut sensitization model	Lower peanut-specific IgE compared to no CpG DNA supplementation [48]. Reduced symptom score, decreased body temperature drop to allergen challenge by injection compared to sham treatment [49]
		Treatment model of peanut allergy, adjunct to injection peanut immunotherapy	No reduction of peanut-specific IgE. Decreased symptom score and decreased body temperature drop to allergen challenge by injection in mice with established peanut allergy compared to peanut immunotherapy-alone group and placebo treatment group [49].
Glucopyranosyl lipid A (GLA)	Bacterial components: TLR agonist (TLR4)	Treatment model of peanut allergy, adjunct to sublingual peanut immunotherapy	Lower anaphylaxis score to oral peanut challenge compared to sublingual peanut immunotherapy-alone group and no treatment group [50]
Family 5 extracellular solute-binding proteins (*Bifidobacterium longum*)	Bacterial components: extracellular vesicles	Egg OVA sensitization model	Lower diarrhea occurrence to oral OVA challenge compared to no treatment or other component proteins of extracellular vesicles [51•]

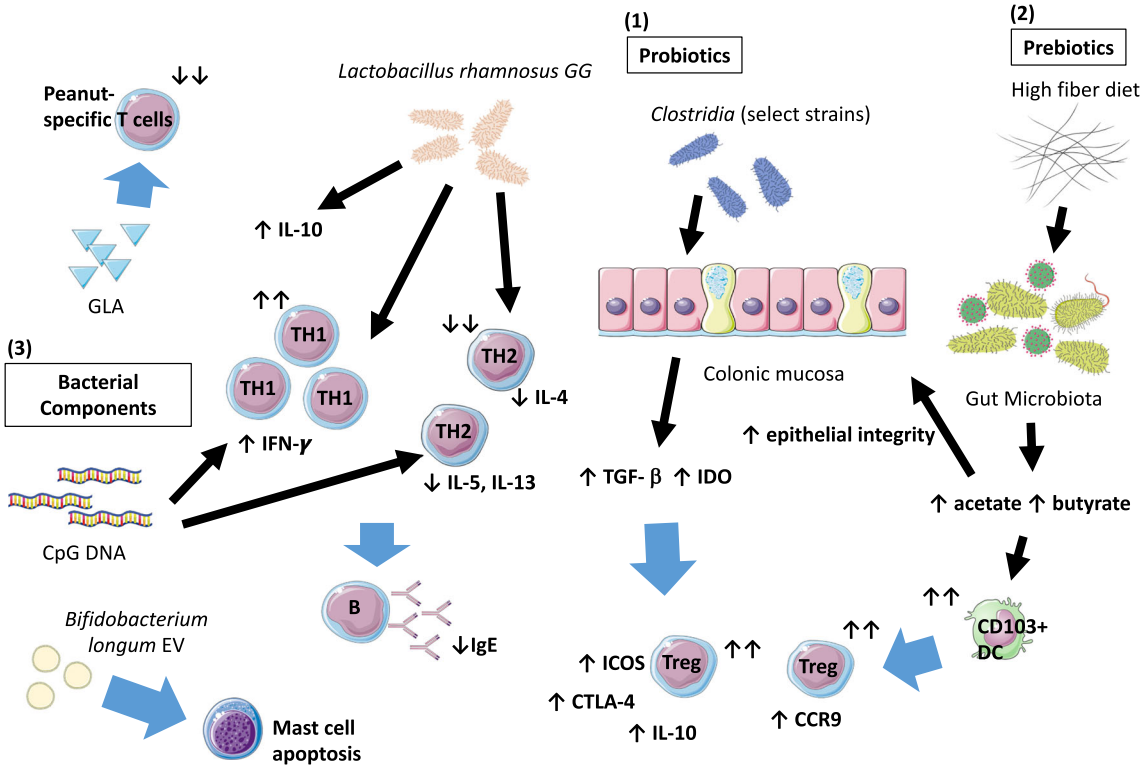

Fig. 1 Mechanisms by which microbial adjunct therapies can promote oral tolerance. The immunologic impacts of multiple probiotics, prebiotic, and bacterial components have been investigated in murine models of food allergy. *(1) Probiotics, such as select Clostridia strains and Lactobacillus rhamnosus GG (LGG), modulate regulatory T (Treg) cell and T-helper (Th) 1 vs. Th2 to food allergens*. Select *Clostridia* strains enhance colonic TGF-β and indoleamine 2,3-dioxygenase (IDO), leading to increased accumulation of Treg cells in the colonic lamina propria. These colonic Treg cells produce IL-10, a key suppressor cytokine, and express cytotoxic T lymphocyte antigen-4 (CTLA-4) and inducible T cell co-stimulator (ICOS), which are surface molecules central to Treg inhibitory effects. LGG supplementation reduces IL-4 and increases IFN-γ expression, thus promoting a shift away from an atopic Th2 response. In addition, IL-10 secretion from T cells is also increased. LGG supplementation resulted in reduced cow's milk-specific IgE production when used in conjunction with hydrolyzed formula. *(2) Prebiotics, such as a high-fiber diet, also promote Treg cell development*. A high-fiber diet alters gut microbial composition and increases short chain fatty acids (SCFA; acetate, butyrate) levels. Increased SCFA levels activate corresponding receptors on dendritic cells and epithelial cells, leading to the development of tolerogenic CD103+ dendritic cells and enhanced epithelial integrity, respectively. CD103+ T cells travel to regional lymph nodes and stimulate the development of gut-homing CCR9+ Treg cells. *(3) Bacterial components, such as toll-like receptors (TLRs) 4 and 9 agonists, and extracellular vesicles (EV) have potent effects on T cells and mast cells.* CpG DNA (TLR9 agonist) promotes the production of IFN-γ (Th1 cytokine) and suppresses the production IL-5 and IL-13 (Th2 cytokines), resulting in reduction of food allergen-specific IgE in a sensitization model. Glucopyranosyl lipid A (GLA) is a synthetic version of monophosphoryl lipid A (MPL), a lipopolysaccharide derived from bacteria and a potent TLR4 agonist. Combination of GLA with sublingual peanut immunotherapy leads to the reduction of peanut-specific T cells in a preliminary report. *Bifidobacterium longum* EVs have also been found to reduce clinical symptoms to food allergen challenge. Specifically, protein components on these EVs have been found to induce mast cell-specific apoptosis without affecting other cell types

is known to inhibit the production of pro-inflammatory cytokines, chemokines, and chemokine receptors [41, 53]. Similarly, select *Clostridia* strains were found to expand the percentage of ICOS+ cells within the Treg population, as well as cytotoxic T lymphocyte antigen-4 (CTLA-4) expression—both of which are surface molecules central to Treg inhibitory effects [54].

Additional bacterial species, though less characterized mechanistically in murine models of food allergy, may also provide protections against food allergy. For instance, colonization of germ-free mice with *Bifidobacterium infantis* has been shown to significantly reduce egg OVA-IgE production upon sensitization, with reduced allergic diarrhea scores upon oral challenge (compared with germ-free mice) [44]. The effects of *Lactobacillus* strains on cow's milk allergy have also been examined in conjunction with hydrolyzed formulas in mice. In a conventionally reared cow's milk-sensitized model, Aitoro et al. showed the addition of *Lactobacillus rhamnosus GG* (LGG) to extensively hydrolyzed casein formula further reduced anaphylactic symptom scores and cow's milk-IgE productions compared to animals fed with the formula alone [45]. The authors similarly reported that LGG supplementation further reduced IL-4 expression, while enhancing the expression of IL-10 and IFN-γ, supporting the ability of LGG "probiotic" to mediate a shift away from pathogenic Th2 response [45]. In the aforementioned human infant microbiota

transfer studies, Rodriguez et al. attributed the protective effects of the "healthy" infant microbiota to *Bifidobacterium* and *Bacteroides* based on a composition analysis, while Feehley et al. attributed it to *Anaerostipes caccae* (a clostridial species) based on a correlation analysis between ileal bacteria abundances and local transcriptional changes in the recipient mice [39, 40]. However, the full effects of these bacterial species/strains have yet to be specifically evaluated in murine models of food allergy. Overall, inquiry on key bacterial strains central to the development and maintenance oral tolerance remains an area of active investigation, and comprehensive human data remain lacking [11]. It should also be noted that many murine studies thus far have utilized germ-free controls, instead of SPF controls, and thus the results may require careful interpretation, as it is less clear whether the protective effects observed may be attributable to specific bacterial strains or to the presence of common bacterial components.

Prebiotics

In addition to the administration of live bacteria (i.e., probiotics), use of prebiotics (Table 1) has also been shown to promote oral tolerance in murine models. Prebiotics are substances that can stimulate the growth and/or function of beneficial gut microorganisms. Tan et al. reported enhanced oral tolerance in mice fed with a high-fiber diet compared to those fed with a zero-fiber diet [46••]. In particular, high-fiber diet protected mice against peanut allergy, with reduced anaphylactic scores upon oral challenge, and overall reduction of IgE production [46••]. This effect appeared to be mediated through altering microbial composition and a corresponding increase in short chain fatty acids (SCFAs), such as acetate and butyrate. These SCFAs have direct immunological impacts on the gut mucosa. Specifically, through the activation of SCFA-sensing G protein-coupled receptors (GPR43, GPR109A) on immune cells and epithelial cells, the potency of tolerogenic CD103+ dendritic cells and the integrity of epithelium were increased in the high-fiber diet treatment group [46••]. With diet high in prebiotics vs. zero-fiber diet, there was a greater proportion of tolerogenic CD103+ dendritic cells in the regional lymph nodes, in conjunction with enhanced Treg induction, and increased proportion of gut-homing CCR9+ Treg cells in the context of peanut allergen challenges [46••]. At the same time, there were decreased total cell numbers in the regional lymph nodes of mice receiving a high- vs. zero-fiber diet, suggesting a diminished inflammatory response during peanut allergen challenges [46••]. In addition, this feeding regimen also boosted IgA production and thus enhanced gut mucosal barrier function [46••]. The protective effect of a high-fiber prebiotic intervention was also thought to be dependent on the presence of vitamin A [46••].

Bacterial Metabolites—Short Chain Fatty Acids

While the tolerogenic effect of a high-fiber diet is mediated, in part, through increased gut SCFA production and subsequent colonic Treg cell actions [46••, 55, 56], the effects of direct SCFA supplementation have not been evaluated in murine models of food allergy. However, in an airway allergy-prone dysbiosis mouse model, dietary supplementation with SCFA (butyrate, acetate, and propionate (BAP)) attenuated the infiltration of inflammatory cells, including eosinophils, into the airway mucosa and abolished serum IgE elevation [47]. Additional indirect support for targeting SCFAs is also found in the probiotic literature. When the effects of probiotics were evaluated, several of the tolerance-promoting bacterial strains were found to alter gut SCFA metabolism. For instance, quantitative analysis of SCFAs in the cecal contents of mice revealed higher concentration of acetate, propionate, butyrate, and isobutyrate when they were given the ideal mix of tolerance-promoting *Clostridia* strains (vs. those receiving selective strains only) [54]. In food-allergic infants, LGG-supplemented formula was also found to expand butyrate-producing bacterial strains [57].

Bacterial Components—CpG Oligonucleotides (TLR9 Agonists)

Microbial components, such as bacterial DNA, lipoglycans, and surface proteins, may induce similar beneficial immunomodulatory effects on their own. In particular, bacteria and viruses express pathogen-associated molecular patterns (PAMPs) that are known to activate specific toll-like receptors (TLRs) and shape the characteristics of adaptive immune response (e.g., modulating Th2 response) [58]. The effects of multiple bacterial components have been investigated in murine models of food allergy. Early investigations have focused on CpG oligonucleotides, which are DNA segments with unmethylated cytosine-phosphate-guanine that are commonly found in bacteria and virus. CpG oligonucleotides can stimulate TLR9 and modulate allergic responses by promote Th1 instead of atopic Th2 response [59]. Using synthetic DNA containing this CpG motif, Zhu et al. showed that CpG DNA supplementation resulted in decreased peanut-specific IgE in a sensitization murine model (compared to controls receiving peanut sensitization alone) [48]. Co-administration of CpG DNA with peanut during sensitization promoted the production of Th1 cytokines (IFN-γ) and reduced the production of Th2 cytokines (IL-5 and IL-13) [48]. Compared to those who did not receive CpG DNA, oral administration of CpG DNA after peanut sensitization protected mice against anaphylactic reactions, with significantly decreased symptom scores upon allergen challenge [48].

CpG DNA has been used as an adjunct to peanut immunotherapy in mice. Peanut immunotherapy plus CpG DNA adjunct treatment appeared to be superior to peanut immunotherapy alone in reducing the severity of allergic reactions. Specifically, Kulis et al. reported that compared to peanut immunotherapy alone, the co-administration of CpG DNA with peanut protein subcutaneous immunotherapy was more effective in reducing anaphylaxis symptom scores, with reduced body temperature drop and reduced mast cell degranulation during allergen challenge in mice [49]. Treatment response to peanut immunotherapy with CpG DNA was accompanied by increased Th1 cytokine (IFN-γ) and decreased Th2 cytokines (IL-5 and IL13) compared to peanut immunotherapy alone [49]. Not all types of CpG DNA were equally efficacious; Type 2 CpG DNA, which is known for modulating B cell proliferation and antibody secretion, was the most effective as an adjunct therapy [49]. Of note, there was no change in peanut-specific IgE level following CpG DNA adjunct treatment with peanut immunotherapy (compared to peanut immunotherapy alone), though this may be related to the timing of the experimental design given the kinetics of the initial rise and ultimate fall of IgE levels during allergen immunotherapy [18, 60].

Bacterial Components—Monophosphoryl Lipid A and Glucopyranosyl Lipid A (TLR4 Agonists)

Lipopolysaccharides (LPS) are commonly found in the outer membrane of gram-negative bacteria, and they are known to be immunomodulatory through the activation of TLR4. Monophosphoryl lipid A (MPL) is a LPS derived from attenuated *Salmonella*, and the addition of MPL to environmental allergen immunotherapy has been reported to induce higher response rates in a short period compared to allergen immunotherapy alone [61]. Glucopyranosyl lipid A (GLA), a synthetic version of MPL, is therefore currently being investigated in a treatment model of peanut allergy, with some preliminary results reported. Soo et al. compared the effect sublingual peanut immunotherapy with or without GLA after 4 weeks of treatment in peanut-sensitized mice [50]. Upon subsequent peanut challenge, preliminary results showed attenuated anaphylaxis in the group receiving both peanut immunotherapy and GLA compared to placebo group. In contrast, mice receiving peanut immunotherapy alone in this short time course had similar anaphylaxis score as the placebo group. In an in vitro experiment, Soo et al. reported that GLA inhibited peanut allergen-specific T cell proliferation in a dose-dependent manner when given to peripheral blood cells from peanut allergic individuals [50].

Bacterial Components—Extracellular Vesicles

Extracellular vesicles (EVs) are known to contain crucial effector molecules, and they have been found to be capable of mediating immune regulations on its own in an animal model of colitis [62]. Kim et al. found that the administration of *Bifidobacterium longum* led to a reduction of diarrhea occurrence in an OVA-alum-induced allergic diarrhea model [51•]. They questioned whether this effect may be replicated by administrating *B. longum* EVs alone. In an in vitro analysis, the group found that *B. longum* EVs induce mast cell-specific apoptosis without affecting dendritic cells, eosinophils, B cells, and T cells [51•]. They further isolated family 5 extracellular solute-binding protein from the EVs and evaluated its effect in an allergic diarrhea murine model. Interestingly, the injection of this EV-isolated protein alone similarly reduced diarrhea occurrence [51•].

Microbial Adjuncts in Clinical Trials of Food Allergen Immunotherapy

While various forms of microbial-based interventions have been evaluated in preclinical models with promising results, human clinical trials assessing their efficacy as an adjunct therapy have been limited. Tang et al. compared the effects of peanut OIT with the probiotic Lactobacillus rhamnosus GG (LGG) vs. placebo in a double-blinded randomized trial [63•]. In this study, children with peanut allergy were defined by positive history and positive skin prick testing. The active treatment arm received 8 months of peanut dose-escalation phase and 10 months of maintenance at 2000 mg of peanut protein. The primary outcome investigated was sustained unresponsiveness 2–5 weeks after discontinuation of treatment. The group reported significantly higher sustained unresponsiveness in the active treatment group (82.1% vs. 3.6% in the placebo group) [63•]. This positive outcome was associated with reduced peanut skin prick test responses, peanut-specific IgE, and increased peanut-specific IgG4 in the group receiving combination peanut OIT and probiotics. Even though the "remission" rate reported in this study was higher than those of other peanut oral immunotherapy studies to date [18], the effect of LGG supplementation itself was unclear due to the lack of comparison arms with an OIT-only treatment group. In addition, cross-study comparison is difficult due to varied definition of sustained unresponsiveness (e.g., 2 vs. 4 weeks post-treatment cessation) between available studies. Lastly, the results are limited by the lack of confirmatory food challenges during enrollment. In a 4-year follow-up report with 48 participants (24 in each group) from the initial study, children in the active treatment arm (peanut OIT + LGG) were more likely to continue to ingest peanuts *ad libitum* (67% vs. 4% in the placebo group) [64]. Four children from the active

treatment group had reported non-anaphylactic adverse reaction to peanut ingestion during the follow-up period (vs. six children in the placebo group) [64]. When 27 participants were asked to stop peanut ingestion and be evaluated for sustained unresponsiveness, 7 of 12 (58%) participants from the active treatment arm attained 8-week sustained unresponsiveness (vs. 1 of 15 [7%] participants in the placebo group, absolute difference 52% [95% CI 21–82], $p = 0.012$) [64]. While this rate is higher than the natural history of outgrowing peanut allergies (~ 20%), the limitations of the original study design prevent conclusive results to be derived for the efficacy of LGG probiotic adjunct therapy. A follow-up study with direct comparison to OIT without probiotics is currently being undertaken by the same group [65].

Conclusions and Future Directions

Despite growing preclinical data supporting a role for microbial adjunct therapies in food allergen immunotherapy, multiple areas of research are still needed for the successful translation of preclinical data into clinical therapies. First, there is a need to better understand the nature of dysbiosis in foodallergic subjects to identify the modulations that will be highest yield to target. The longitudinal dynamics of dysbiosis in food allergy subjects is not currently well understood. Characterization at finer taxonomic levels may facilitate translation since the immunomodulatory effects of probiotics are strain-specific [66]. Second, further dissection of the mechanisms by which commensal microbiota promote oral tolerance may reveal additional therapeutic targets. For instance, multiple lines of recent research have highlighted the immunomodulatory properties of bacterial metabolites [67]. However, the pathways activated by such bacterial metabolites and subsequent immune effect in humans remain to be fully elucidated. Third, while the safety profile of multiple commercial probiotics is known, safety of newly identified probiotic strains and novel applications of bacterial components will need to be evaluated. Many bacterial components have been highlighted as potential therapy based on their ability to promote a Th1, instead of an atopic Th2, response, but there exist theoretical concerns whether such treatment may carry autoimmune potential [68]. Fourth, the effect sizes of microbial-based manipulations may differ based on differential food allergy endotypes and severity. In OIT, for instance, a favorable response is determined by food allergy severity [69], pretreatment IgE level [70, 71], and comorbid atopic conditions, such as asthma and allergic rhinitis [71]. Better characterization of food allergy endotypes may help identify subjects more likely to respond favorably to microbial-based adjunct therapy. Future clinical trials involving microbial-based adjunct therapies will also benefit from comparisons with immunotherapy-only arms in order to conclusively measure the effect size of adjunct therapy. With a wide variety of modalities and convincing preclinical data, there is strong potential for microbial-based interventions to be utilized as adjuncts to enhance the efficacy, safety, and durability of food allergen immunotherapy.

Compliance with Ethical Standards

Conflict of Interest Dr. Bunyavanich reports grants from the National Institutes of Health, during the conduct of the study. Dr. Hsi-en Ho declares no conflicts of interest relevant to this manuscript.

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