HHS Public Access

Author manuscript

Transl Res. Author manuscript; available in PMC 2018 January 01.

Published in final edited form as:

Transl Res. 2017 January ; 179: 199–203. doi:10.1016/j.trsl.2016.09.003.

Microbiome and Food Allergy

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Abstract

Food allergy is a common disease affecting approximately 8% of children and 5% of adults. The prevalence has increased over the last two decades, suggesting an important environmental contribution to susceptibility. Studies have identified mode of birth, pet exposure, and having older siblings as being significant risk modifying factors in the development of food allergy. With the discovery that these factors significantly impact the composition of the intestinal microbiome, which is known to play a critical role in shaping the immune system, recent studies have begun to address the role of the intestinal microbiota in the development of food allergy. Studies in human cohorts support a dysbiosis in food allergy, and limited data suggest that this dysbiosis occurs early in life, preceding the onset of sensitization. Studies from animal models have clearly shown that the composition of the intestinal microbiota confers susceptibility to food allergy, and that there are organisms such as Clostridia species that are protective in the development of food allergy. Our understanding of microbial regulation of food allergy is in its nascency, but the state of the field supports an important contribution of intestinal microbes to susceptibility. Challenges going forward are to identify commensal-derived microorganisms that could be used therapeutically to prevent or perhaps treat food allergy.

Introduction

Food allergy is an adverse reaction to food which can be mediated by IgE or other immune mechanisms. IgE-mediated food allergy is increasing in prevalence [1, 2] for reasons that are not yet clear. Although there is a strong genetic contribution to food allergy, a number of environmental factors that influence the composition of the intestinal microbiota have also been identified as modifiers of food allergy risk. In this review, our aim is to discuss the evidence that the composition of the commensal microbiota regulates the development of food allergy.

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The authors have read the journal's policy on conflict of interest disclosure, and have none to declare. The authors have read the journal's authorship agreement and the manuscript has been reviewed and approved by all named authors.

Epidemiology of Food Allergy

IgE-mediated food allergy affects approximately 5% of adults and 8% of children [3]. Food allergy is a disease with an onset in early life. An unselected cohort of 2848 12-month-old infants in Australia demonstrated a prevalence of food challenge-proven peanut, sesame and egg allergy of 3.0%, 0.8%, and 8.9%, respectively [4]. Studies to test the impact of early food introduction on the development of food allergy have found that infants have sensitization and clinical reactivity to egg or peanut as early as 4 months of age [5, 6]. Therefore, environmental risk factors for food allergy are likely to play a role early in postnatal life or in utero.

Factors that have been associated with modified risk of food allergy include mode of birth, breastfeeding, having older siblings, attending daycare in early life, and exposure to furred pets (comprehensively reviewed in [7]). These findings are supportive of the hygiene hypothesis, which proposes that lack of appropriate microbial exposure in early life drives allergic disease. There is evidence that each of these factors individually alters the composition of the intestinal microbiome [8–12]. Linking these two observations, there is great interest in determining whether an altered microbial colonization is responsible for increased susceptibility to food allergy, and understanding the mechanism of that susceptibility.

Early life bacterial colonization of the human gut

Yatsunenko et al used 16S rRNA data collected from fecal specimens of geographically diverse populations to show that the greatest inter-individual variability in the microbial composition occurs in the first 3 years of life [13]. Although meconium has been reported to have a detectable microbial presence, colonization of the infant starts at birth when microorganisms from the maternal body surfaces and the environment are acquired [14]. There is a preserved pattern of microbial colonization in early life from relatively aerobic to anaerobic. Newborns have a domination of Proteobacteria (Escherichia, Shigella), which progresses to domination by Actinobacteria (e.g. Bifidobacterium), followed by acquisition of an adult-like domination by Firmicutes and Bacteroidetes [9, 15, 16]. The maturation of the infant microbiome is driven primarily by cessation of breastfeeding. The rate of microbial maturation is influenced by environmental factors [17], and has been proposed to be a critical factor in health outcomes such as adiposity [16]. Therefore the microbial composition of the infant gastrointestinal tract is highly dynamic, which adds to the complexity of trying to capture the impact of microbial composition on health outcomes.

Intestinal microbial composition and food allergy

There are a limited number of culture-independent studies that have directly looked at microbial composition associated with food allergy. Cross-sectional studies comparing food allergic to healthy subjects are confounded by differences in the diet, which is particularly problematic when studying foods that contribute significantly to the diet such as cow’s milk. Ling et al used 16S rRNA sequencing to study differences in microbial composition between children with food allergy (n=17 with IgE-mediated food allergy, n=17 with non-IgE-

mediated food allergy) and healthy controls (n=45) [18]. Sampling was performed at time of diagnosis, from age 2 – 11 months. There was no difference in microbial diversity, but they found increased levels of *Clostridium sensu stricto* and *Anaerobacter* and decreased levels of *Bacteroides* and *Clostridium XVIII* in infants with IgE-mediated food allergy [18]. Furthermore, levels of *Clostridium sensu stricto* correlated with levels of IgE. Strengths of the study include the use of food challenge-proven diagnosis, and acquisition of samples prior to diagnosis suggesting that diet may not already have been adjusted. However, as the infants already have symptoms, dietary adjustment is likely. Furthermore, in a cross-sectional design it is not possible to determine if changes in microbial composition preceded the development of food allergy which would support causation.

Hua et al recently reported findings from the publically available American Gut Project [19]. Of 1879 participants (primarily adult, mean age 45 years), 2.5% self-reported allergy to peanuts, 3.2% to tree nuts, 2.6% to shellfish, and 9.1% to other foods. There was a marked reduction in microbial richness and alpha diversity in those self-reporting peanut or tree nut allergy compared to those without peanut or tree nut allergy. There were also significant differences in beta diversity in those with peanut or tree nut allergy. At the level of microbial taxa, there was an increased correlation of peanut and tree nut allergy with *Bacteroides fragilis*, which is somewhat surprising as *B. fragilis* has been shown to promote the generation of regulatory responses in the intestine [20]. There were negative correlations with *Clostridiales*, *Prevotella*, and *Ruminococcaceae*. These compositional differences were not unique to peanut and tree nut allergy, and were found associated with seasonal allergies but not bee sting or shellfish allergy. Strengths of the study include the number of participants. Self-reported food allergies tend to over-estimate the true prevalence of food allergy, particularly in the absence of additional questions about symptoms, however the rate of peanut or tree nut allergy was within reported ranges and over-estimation of true food allergy would likely reduce the effect observed. As with the previous study, in a cross-sectional design it is not possible to assess probability of causation.

The only prospective study to address food allergy was recently reported by Azad et al [21]. Their findings showed that low fecal microbial richness at 3 months preceded food sensitization as measured at 12 months, whereas concurrent richness at 1 year was not associated with food sensitization [21]. Enterobacteriaceae were overrepresented while Bacteroidaceae were underrepresented in food sensitized infants at 3 months of age, suggesting a maturational difference in microbial composition. Strengths of the study were prospective design, while weaknesses include the small number of subjects (166 infants, with 12 having reported food sensitization), and the use of sensitization (skin prick test positive) rather than food allergy.

These findings were consistent with the previous studies where low microbial diversity in early infancy (1 week and 1 month, respectively) was found to predict atopic dermatitis [22, 23].

Bunyavanich and colleagues have recently reported on microbial composition in a cohort of children being followed longitudinally to assess natural history of food allergy. They demonstrated that in a cohort of 226 children with milk allergy that the microbial

composition at 3–6 months of age was predictive of the resolution of milk allergy at 8 years of age. Predictive metagenome analysis indicated that a decrease in fatty acid metabolism was associated with milk allergy resolution [24]. Short chain fatty acids play an important role in the promotion of regulatory T cells [25] and oral tolerance [26], which likely contribute to the resolution of food allergy.

The limited data available support the conclusion that food allergy is associated with changes in microbial composition, and are suggestive that changes in microbial composition may precede development as well as resolution of food allergy. Larger prospective studies are needed with outcomes that include clinical food allergy and not merely sensitization, although the role of microbiome in sensitization to foods is in itself of interest. Furthermore, analysis of biological properties of the microbial community may be more informative than identifying differences at the taxa level.

Impact of intestinal microbiota on experimental models of food allergy

It is difficult to determine causation in human studies, and therefore we turn to murine models of food allergy to examine mechanisms by which the intestinal microbiota may alter susceptibility to food allergy. Antibiotic treatment of mice has been shown to increase the susceptibility of mice to peanut allergy induced by oral feeding of peanut with the mucosal adjuvant cholera toxin [27], suggesting that the intestinal microbiota provide a suppressive effect on food allergy. This was supported by the finding that germ-free mice were also more susceptible to peanut allergy, with a significant increase in peanut-specific IgE and IgG levels compared to specific pathogen free mice with a replete microbiota [28]. Specific colonization of germ-free mice was performed to identify suppressive components of the microbiota.

Monocolonization with *Bacteroides uniformis* did not reduce peanut sensitization or anaphylaxis. However, colonization with Clostridia species was sufficient to reduce peanut allergy (sensitization and symptoms) [28]. Other studies have also shown that Clostridia species effectively suppress symptoms in a model of intestinal allergy [29, 30]. A suppressive role of the microbiota in food allergy is also supported by data showing that colonization of germ-free mice with healthy infant microbiota suppressed sensitization and allergy to cow’s milk [31].

Evidence that an altered microbial composition can increase susceptibility to food allergy has been shown by studies demonstrating that food allergy susceptibility is transmissible [32]. These studies used mice with a gain-of-function mutation in the IL-4 receptor a-chain (*Il4raF709*) that are susceptible to oral sensitization to food allergens including ovalbumin (OVA) or peanut [33]. OVA-sensitized *Il4raF709* mice exhibited a specific microbial signature compared to OVA-sensitized wild-type (resistant) mice, as measured by 16S rDNA phylochip analysis of fecal samples [32]. Reconstitution of germ-free mice with microbiota derived from sensitized susceptible mice, but not sensitized resistant mice, transferred food allergy susceptibility to the recipient mice [32].

Mechanisms of microbial regulation of food allergy

Food allergy symptoms are generated by degranulation of mast cells and basophils through allergen cross-linking of IgE bound to the cell surface through the high affinity IgE receptor FcεRI. The generation of IgE is under the control of Th2 lymphocytes that produce IL-4, a cytokine necessary for B cell class-switching to the IgE isotype. Regulatory T cells can suppress the generation of Th2 immunity and IgE production. Modulation of any of these pathways by the microbiota could contribute to altered susceptibility to food allergy.

In the absence of an intact intestinal microbiota, IgE levels are increased as are circulating basophils [34]. An absence of MyD88 in B cells also led to elevated IgE production, indicating that some of the inhibitory effect of the microbiota could act directly on the B cells. B cells express a wide range of TLRs that regulate immunoglobulin production, including IgE [35]. TLR4 and TLR9 have been shown to modulate IgE production in mice, and their absence results in increased and decreased susceptibility to food allergy, respectively [2, 27]. IgE class-switching has been described to occur in the Peyer's patches of the intestine, and germ-free mice had elevated levels of IL-4 as well as molecular evidence of IgE class-switching in the Peyer's patches [36]. Suppression of IgE production by the microbiota was only possible within an early window of time, again pointing to the importance of early immune imprinting by the intestinal microbiota [36].

Faith et al showed that colonization of germ-free mice with a variety of different intestinal commensal strains could induce an expansion of Foxp3+ Tregs in the colon [37]. Suppression of inflammatory bowel disease and food allergy by Clostridia strains was also shown to be associated with an expansion of Tregs in the colon [29, 30]. The impact of colonization on Tregs is primarily in the colon, rather than the small intestine where food antigens would be sampled and presented. It is not clear if Tregs localized to the colon could be responsible for suppression of immune responses initiated in the small intestine. Ohnmacht recently showed that microbial signals promote the development of induced (antigen-specific) Tregs that express the transcription factor RORγt [38]. These RORγt-expressing Tregs are necessary for the suppression of Th2 responses to fed antigens [38]. Thus the microbiota may play a key role in shaping the Treg/Th2 balance.

In addition to immunologic effects, the microbiota may also influence susceptibility to food allergy through other mechanisms including control of intestinal barrier function. The intestinal epithelium forms a physical barrier between the gut lumen contents and the immune system, and it is the first site of host-microbe interaction. Intestinal epithelial cells (IEC) regulate the passage of antigens from the lumen into the lamina propria (LP) via tight junction proteins. Stefka et al demonstrated that colonization with Clostridia species increased expression of IL-22, a barrier-promoting cytokine, and decreased intestinal permeability as measured by reduced uptake of peanut proteins into the serum after gavage [28]. Neutralization of IL-22 suppressed the uptake of peanut, but surprisingly did not significantly alter sensitization to peanut. Other changes induced by Clostridia colonization included increased IgA, and increased Tregs not only in the colon but in the mesenteric lymph node where antigen-specific responses to fed antigens are initiated. Therefore,

multiple protective mechanisms involving both immune and intestinal barrier function may be induced by commensal microorganisms.

One mechanism by which changes in microbial composition may influence susceptibility to food allergy has recently been shown using a model of high fiber diet in mice [26]. Dietary fiber is fermented in the colon by anaerobic bacteria, releasing short chain fatty acids (SCFA) that can be utilized by intestinal epithelial cells for energy. Mice fed a high fiber diet are protected from food allergy [26]. Mice lacking two of the receptors for SCFA, GPR43 or GPR109A, had enhanced susceptibility to food allergy. High fiber diet enhanced the tolerogenic potential of CD103+ DCs in the intestine, increased Tregs, and increased IgA. The impact of fiber in the diet was also dependent on dietary vitamin A. Thus diet and the microbiome work together to alter susceptibility to food allergy by regulating the tolerogenic tone of the mucosal immune system.

Concluding remarks

Data from human studies and mouse models support a regulatory role of the intestinal microbiota in food allergy, but many questions remain to be answered. Prospective studies on human cohorts using well-defined clinical outcomes of food allergy are needed to definitively establish if changes in microbial composition precede development of food allergy. The dynamic composition of the microbiota in early life demands multiple sampling to assess the role of microbiome in a window of susceptibility. A better understanding of the general features of a protective microbiota (for example propensity to produce short-chain fatty acids), and environmental factors such as diet that could regulate the development of a protective microbiota are needed to realize our goal of manipulating the intestinal microbiota for prevention or treatment of food allergy.

Acknowledgments

This work was supported in part by the NIH grants AI093577 and AI124062, and by the David and Julia Koch Research Program in Food Allergy Therapeutics.

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