



The role of the commensal microbiota in the regulation of tolerance to dietary allergens

Roberto Berni Canani^a, Jack A. Gilbert^{b,c,d,e,f}, and Cathryn R. Nagler^g

Purpose of review

We review the evidence that environmental stimuli that perturb naturally selected host–microbe interactions are driving the increasing prevalence of food allergy and examine the mechanisms by which commensal bacteria regulate tolerance to dietary allergens.

Recent findings

Antibiotic use and the consumption of a high-fat/low-fiber diet have a major and rapid impact on gut bacterial populations, with long-term consequences for both overall microbial community structure and the regulation of host immunity. Recent work emphasizes the role of mucosa-associated commensal bacteria in eliciting a barrier-protective response critical to preventing allergic sensitization to food. Murine model studies are informing the development of novel live biotherapeutic approaches as an adjunctive therapy to enhance antigen-specific oral desensitization and to promote lasting tolerance in patients with food allergy.

Summary

Strategies based on modulating the composition and/or functionality of the gut microbiome hold promise for the treatment of food allergy.

Keywords

commensal bacteria, epithelial barrier, food allergy, gut microbiota, probiotics, short-chain fatty acids

INTRODUCTION

Allergic responses to food are increasingly prevalent in industrialized societies, rising by as much as 20% in the recent 10-year period [1–3]. We have proposed that this dramatic generational upsurge is a consequence of environmentally induced alterations in the composition of the commensal bacteria that normally populate the gastrointestinal tract [4–6]. During the past decade, our understanding of the commensal microbiota – the collection of microbes that reside on our skin and mucosal surfaces – has been transformed by the introduction of culture-independent methods of analysis. The numbers alone are staggering. Our bodies contain 10 times more microbes than eukaryotic cells, which collectively encode 100–1000 times more genetic information, and are referred to as the microbiome [7,8]. Over the course of millions of years of co-evolution, commensal bacteria have taken on many physiological functions essential to our health, including, but not restricted to, the production of vitamins and the digestion of insoluble dietary fibers [9]. Other constituent microbes including bacteriophage and viruses are at least as numerous but much less well understood or characterized.

What is becoming clear, however, is that 21st Century lifestyle practices have shifted the composition of our commensal bacteria away from that which populated our ancestors; this modern-day bacterial community is increasingly correlated with disease [9]. Some environmental influences that can affect the microbiome include pervasive antibiotic use, consumption of a ‘Western’ diet high in fat and sugar and low in fiber, the elimination of previously common enteropathogens, vaccination/reduced

^aDepartment of Translational Medical Science and European Laboratory for The Investigation of Food Induced Diseases and CEINGE Advanced Biotechnologies, University of Naples ‘Federico II’, Naples, Italy, ^bInstitute for Genomic and Systems Biology, Biosciences Department, Argonne National Laboratory, Argonne, ^cDepartment of Ecology and Evolution, ^dDepartment of Surgery, University of Chicago, Chicago, Illinois, ^eMarine Biological Laboratory, Woods Hole, Massachusetts, USA, ^fCollege of Environmental and Resource Sciences, Zhejiang University, Hangzhou, China and ^gCommittee on Immunology and Departments of Pathology, Medicine and The College, University of Chicago, Chicago, Illinois, USA

Correspondence to Cathryn R. Nagler, PhD, University of Chicago, 924 East 57th Street, JFK R120, Chicago, IL 60637, USA. Tel: +1 773 702 6317; e-mail: cnagler@bsd.uchicago.edu

Curr Opin Allergy Clin Immunol 2015, 15:243–249

DOI:10.1097/ACI.0000000000000157

KEY POINTS

- The trillions of bacteria that populate our skin and mucosal surfaces critically regulate key physiological functions.
- Environmentally induced changes in commensal bacterial communities have created a dysbiosis that is linked to the increasing prevalence of complex immune-mediated disease.
- Understanding how indigenous bacterial communities interact with the innate and adaptive immune system will inform the development of novel live biotherapeutics to prevent or treat food allergy.

exposure to infectious disease, Caesarean birth, and formula feeding (reviewed in [6]).

Commensal bacterial community composition varies by anatomic site [8,9]. We are populated with our founder microbiota at birth which, until comparatively recently, occurred for most humans by natural delivery. Enterobacteria and vaginally derived lactic acid-producing bacteria initially predominate; breast milk, which harbors its own microbiome, favors the emergence of Bifidobacteria, which extract nutrients from human milk glycans [10,11]. Subsequent microbial successions eventually result in a diverse and unique microbiota [12]. Surgical delivery disturbs this process; founder bacterial populations in infants born by Caesarean section are derived from the skin of the mother or caregiver [13]. The neonatal period is a time of great plasticity for the emerging microbiome, which is critically intertwined with the maturation of the immune system of its host. Recent work has highlighted the profound influence of environmental factors on the developing microbiome.

ANTIBIOTIC USE AND DIET SHAPE THE MICROBIOTA AND INFLUENCE SUSCEPTIBILITY TO FOOD ALLERGY

Beyond the targeted depletion of particular bacterial taxa, the effects of antibiotics on microbial community structure often persist long after cessation of the treatment [14]. Medical exposure is only one source; the widespread usage of antibiotics in agriculture, especially for their growth-promoting properties for livestock, has contributed to their increasing low-residue presence in the food chain [15]. In the United States (and many other developed countries), most infants receive multiple courses of antibiotics during the first 2 years of life [16]. Murine model studies [4,17,18,19^{***}] have demonstrated that early-life exposure to orally

administered broad-spectrum antibiotics is associated with aberrant immunity to respiratory and dietary antigens. Recent work from our laboratory showed that neonatal antibiotic treatment reduced microbial diversity and bacterial load in both fecal and ileal samples, and enhanced food allergen sensitization [19^{***}]. Other recent studies support the idea that the neonatal period is particularly critical. Even low-dose early-life antibiotic exposure can lead to long-lasting effects on metabolic and immune responsiveness [20^{***}]. Data emerging from human studies link the use of antimicrobial agents to the increasing prevalence of food allergy. Maternal use of antibiotics before and during pregnancy, as well as antibiotic courses during the first month of life, is associated with an increased risk of cow's milk allergy in infants [21]. Higher urinary levels of the common antibacterial agent triclosan are detected in children sensitized to food and aeroallergens [22].

Given that its components serve as a source of nutrition for both the host and its microbial inhabitants, it is not surprising that intestinal microbial community structure is strongly influenced by the composition of the diet. The gut microbiota of children, consuming a Western-style diet of processed food high in sugar and fat and low in fiber content, differs markedly from that of children from a rural African community consuming a low-fat, high-fiber plant-based diet (potentially resembling that of Neolithic subsistence farmers from 10 000 years ago) [23]. However, the rapidity and extent with which diet can alter human microbial communities has not been well understood; a recent study [24^{***}] suggests that marked changes can be observed on daily time-scales, particularly in response to fiber intake. Moreover, rapid and reproducible alterations in particular gut bacterial taxa can be detected after a short-term dietary intervention [25^{***}]. Shifts in the abundance of fecal microbial communities were noted in human volunteers after only 5 days of consumption of a plant-based diet (high in fruits, vegetables, grains, and legumes) or an animal-based diet of meat, eggs, and cheese [25^{***}]. Of particular interest, in individuals consuming an animal-based diet, a reduced abundance of *Firmicutes* genera that ferment plant polysaccharides was evident even in this short time-frame. These results suggest that the timing of introduction of solid food and the types of food consumed may influence the development of food allergy by changing the composition of the intestinal microbiota. A recent study [26^{***}] examining the influence of dietary patterns during the first year of life on the development of food allergy at 2 years of age provides some support for this hypothesis. Principal component analysis of prospective food

diary data in a nested, case-control, within-cohort study [26[■]] showed that nonallergic infants had an ongoing diet that was high in fruits, vegetables, and home-prepared (nonprocessed) foods when compared with the diet of their challenge-proven food-allergic counterparts.

A new study [27[■]] also suggests that microbial transmission patterns have changed with urbanization as humans have become a predominantly indoor species, and with this lifestyle change we, and our children, are being predominantly exposed to our own microbiome. The controlled indoor environment, which has only arisen in the past 100 years, has fundamentally altered our exposure to the microbial world. These environments are designed, through controlled temperature, humidity, and light, to be 'antimicrobial' [28]. We spend approximately 90% of our lives in this 'clean' ecosystem. It is likely this significantly reduces our microbial exposure, which may serve to limit the development of our immune system, our associated microbial diversity, and the ability of our commensal microbiota to rebound from composition-altering exposures. Whether the built environment contributes to allergic susceptibility (or might be manipulated to prevent disease) is an idea that is only beginning to be explored.

ALTERATION OF THE INTESTINAL MICROBIOTA IN FOOD ALLERGY

Given the profound influence of multiple environmental stimuli on the composition of the microbiota (and their confounding effects on its analysis), the available data characterizing the microbiota of patients with food allergy are still quite preliminary [29]. Most of the data come from pediatric patients. 16S rRNA profiling suggests that potentially negative alterations in the gut microbiome composition (dysbiosis) may precede the occurrence of allergic manifestations. Azad *et al.* [30[■]] found that an increased Enterobacteriaceae/Bacteroidaceae ratio and low Ruminococcaceae abundance, in the context of low gut microbiota richness in early infancy, are associated with subsequent food sensitization, suggesting that early gut dysbiosis contributes to subsequent development of food allergy. Particular bacterial phylotypes, but not the overall gut microbial diversity, were significantly altered in a cohort of Chinese infants with food allergy [31]. When sampled at 5 months of age, the fecal microbiota of the food-allergic infants was characterized by increased relative abundance of *Clostridium* cluster I and *Anaerobacter*, and a decreased relative abundance of *Bacteroides* and *Clostridium* XVIII [31]. We have examined the intestinal microbiota of infants

allergic to cow's milk at the time of diagnosis (by double-blind oral food challenge) in comparison to age-matched healthy 4-month-old controls [32[■]]. We found that the microbiota of allergic infants in our study was significantly more diverse than that of healthy controls. Strikingly, although the healthy infants' microbiota was dominated by Bifidobacteriaceae, Enterobacteriaceae, and Enterococcaceae, the microbiota of the allergic infants demonstrated a significant increase in the abundance of Ruminococcaceae and Lachnospiraceae, which typically predominate in the adult gut [32[■]].

TREATMENT OF FOOD ALLERGY WITH CURRENTLY AVAILABLE PROBIOTICS

The evidence reviewed thus far suggests that therapeutic modulation of the commensal microbiota may be beneficial for the prevention or treatment of food allergy. Probiotics are defined as microorganisms that, when ingested, confer health benefits to the host [33]. Studies examining the efficacy of currently available probiotics in treating food allergy have yielded conflicting results. Differences in the study design, populations, probiotic strains, and dosages may be responsible for the discrepancies observed [33]. A meta-analysis of clinical trials concluded that administration of probiotics prenatally, or during the period shortly after birth, reduced total IgE levels and the risk of atopic sensitization, but not asthma or wheezing [34]. Recently published guidelines for atopic disease prevention from the World Allergy Organization concluded that there is a likely net benefit in using probiotics for eczema prevention [35]. However strain selection is important. Administration of *Lactobacillus acidophilus*, for example, was associated with a significantly increased risk of atopic sensitization when compared with other strains [34]. Allen *et al.* [36] demonstrated that high-dose administration of multiple strains of *Lactobacilli* and *Bifidobacteria* to mothers during late pregnancy and to their infants from birth to 6 months of age did not prevent eczema or reduce the frequency of asthma in early childhood, but did promote a reduced frequency of sensitivity to food antigens. A Japanese study [37] showed that both prenatal and postnatal supplementation with *Bifidobacteria* was associated with a significantly reduced risk of eczema/atopic dermatitis during the first 18 months of life. Studies investigating the therapeutic effect of probiotics on challenge-confirmed food-allergic infants are scant. In one randomized, double-blind, placebo-controlled study [38] of infants with challenge-proven cow's milk allergy, administration of *Lactobacillus casei* CRL431 and *Bifidobacterium lactis* Bb12 for 12 months did not

affect the acquisition of tolerance to cow's milk. In contrast, Berni Canani *et al.* [39,40] demonstrated in two different prospective clinical trials that an extensively hydrolyzed casein formula containing *Lactobacillus rhamnosus* GG (LGG) accelerated the development of tolerance acquisition in infants with cow's milk allergy. When we compared the fecal microbiota of infants receiving this tolerance-inducing probiotic-supplemented therapy to that obtained from infants receiving an extensively hydrolyzed casein formula (EHCF) alone, we found statistically significant positive correlations between the abundance of genera with the potential for producing butyrate and the concentration of fecal butyrate in the infants that received EHCF supplemented with LGG [32[■]]. Strain-level demarcations for butyrate-producing genera (including *Roseburia*, *Coprococcus*, and *Blautia*) identified in infants that acquired tolerance to cow's milk suggest that LGG treatment contributes to acquisition of tolerance by altering the strain-level community structure of taxa with the potential to produce butyrate [32[■]].

Oral immunotherapy (OIT) has shown promise in eliciting desensitization in patients with food allergy, but its ability to induce long-lasting tolerance in the absence of ongoing allergen administration has not been reliably demonstrated [41]. A double-blind, placebo-controlled randomized trial evaluated the efficacy of 18 months of therapy, combining oral peanut desensitization with administration of the probiotic *L. rhamnosus* CGMCC in 1–10-year-old children with peanut allergy [42[■]]. Although 'possible sustained unresponsiveness' 2–5 weeks after cessation of OIT was noted in most (82%) of the children receiving the probiotic and OIT, no comparison was made to treatment groups receiving either OIT or probiotic alone. The effect of daily *L. rhamnosus* CGMCC treatment on microbial community structure was not evaluated.

DEVELOPMENT OF NOVEL LIVE BIOTHERAPEUTICS FOR THE PREVENTION AND TREATMENT OF FOOD ALLERGY

Increasing appreciation of the role of the microbiota in regulating complex immune-mediated diseases has led to the emergence of a number of biotechnology companies seeking to commercialize novel live biotherapeutics with microbiome-modulating properties [43]. The efficacy of fecal transplantation in resolving disease in patients with relapsing *Clostridium difficile* infection has provided proof of principle for therapeutic approaches based on oral administration of constituents of the normal commensal microbiota [44]. Preclinical studies have been facilitated by the use of germ-free mice, which

lack commensal microbes. Both the cell-mediated (Treg) and humoral (IgA) arms of the immune system are dramatically under-developed in these mice [45]. Honda and colleagues have identified mucosa-associated Firmicutes in the Clostridia class as the anaerobic component of the indigenous microbiota critical for the induction of Tregs in the colonic lamina propria [46]. Atarashi *et al.* [47[■]] showed that Tregs are also induced when spore-forming *Clostridia* isolated from human feces are transferred into germ-free mice. A Treg-inducing mixture of human isolates that can be cultured *in vitro* has been selected for development as a novel biotherapeutic for the treatment of inflammatory bowel diseases [48].

New work from our laboratory shows that this type of approach may also hold promise for the treatment of food allergy. We found that sensitization to a food allergen is enhanced in germ-free mice and in mice that have been treated by neonatal antibiotic administration [19[■]]. Selective colonization of germ-free mice demonstrated that the allergy-protective capacity is contained within the *Clostridia* class. Moreover, re-introduction of a *Clostridia*-containing microbiota to antibiotic-treated mice blocked sensitization to a food allergen [19[■]]. *Clostridia* colonization of germ-free mice restored both the Treg and IgA compartments. Microarray analysis of the isolated intestinal epithelial cells led to the identification of a novel innate mechanism by which *Clostridia* protect against sensitization to dietary antigens. Prior work has implicated defects in intestinal permeability to aberrant allergic responses to food [49]. Our discovery that *Clostridia* colonization induces the production of the barrier-protective cytokine interleukin (IL)-22 provided mechanistic insight into how commensal bacteria regulate intestinal barrier permeability. We used a sensitive capture ELISA to demonstrate that IL-22 acts to reduce the concentration of orally administered dietary antigen detectable in the systemic circulation [19[■]]. Our data therefore suggest that *Clostridia* stimulate both innate and adaptive immune-signaling pathways to maintain tolerance to food. This is a paradigm shift since oral tolerance has typically been attributed primarily to an antigen-specific Treg response [50,51]. Our work suggests a new model in which tolerance to dietary antigen requires both food antigen-specific Tregs and a bacteria-induced barrier-protective response [19[■],52].

It is not yet clear whether *Clostridia* modulate host immunity by direct cell-to-cell contact, secreted metabolites, or both. The ability of particular bacterial taxa to ferment dietary fiber for the production of short-chain fatty acids (SCFAs) helps to explain the health-promoting role of bacterially

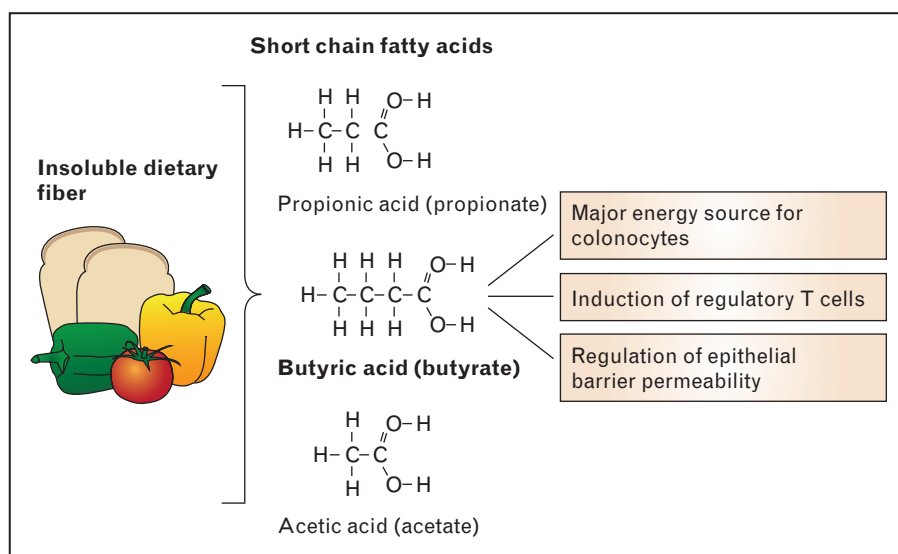


FIGURE 1. Commensal bacteria ferment insoluble dietary fiber to produce short-chain fatty acids (SCFAs), the most abundant of which are propionate, butyrate, and acetate. Colonocytes metabolize butyrate as their primary energy source. The production of immunoregulatory metabolites is one way that commensal bacteria interact with the host immune cells to promote nonresponsiveness to innocuous luminal antigens. Recent work suggests that SCFAs, particularly butyrate, contribute to mucosal homeostasis through the induction of regulatory T cells and the regulation of epithelial barrier permeability.

produced metabolites [53]. Of the major SCFAs, butyrate is the preferred energy source for colonocytes (Fig. 1). Its abundant presence in the gut is often considered a sensor of intestinal health [54]. Butyrate-producing bacteria comprise a functional, rather than a taxonomic group, although the Clostridial families Lachnospiraceae and Ruminococceae are among its most prominent producers [55]. Bacteria-produced SCFAs have been implicated in the regulation of both the proportions and functional capabilities of colonic Tregs [56,57], which, in some studies, has been specifically attributed to butyrate production by spore-forming Clostridiales [58^{***}]. Preliminary data from our laboratory also link

butyrate, but not other SCFAs, to regulation of epithelial barrier permeability (Feehley *et al.*, personal communication). The clinical relevance of this mouse model work is highlighted by a recent study [59^{***}] which correlated the severity of atopic disease with intestinal microbial diversity and the abundance of butyrate-producing bacteria.

CONCLUSION

Taken together, these findings provide a compelling rationale for exploring the administration of novel *Clostridia*-based biotherapeutics or the administration of butyrate as adjunctive therapies to

Table 1. Analysis of the role of the commensal gut microbiome in the regulation of allergic responses to food

Study participants	Comparative evaluation of a well characterized patient population with a definitive diagnosis of food allergy (clinical history, positive screening test for immune response against food allergens) prior to any therapeutic intervention to healthy individuals matched for age, sex, and exposure to environmental risk factors that can affect the composition of the microbiome, including: antibiotic use, birth order, bacterial and viral infections, delivery and feeding modes, degree of social exposure (child care), vaccinations and exposure to animals (endotoxin), smoking and gastric acidity inhibitors
Methods	Stool samples will be collected from patients at initial diagnosis and/or in longitudinal studies of samples collected over time for each patient to capture variation in the microbial composition under conditions where food intake and environmental interactions are monitored. These data will then be supplemented with periodic samples and questionnaires collected in an unsupervised setting to improve data density. Bacterial DNA will be extracted from stool using standard techniques outlined by the Earth Microbiome Project (http://www.earthmicrobiome.org/) [64]. The DNA will then be processed for either 16S rRNA amplicons and Illumina sequencing [65], or for shotgun metagenomic analysis with Illumina sequencing [66]. Sequence data will be initially analyzed using published methods (e.g. refs. [27 ^{***} ,65]), followed by a suite of nonparametric statistical tests to identify taxa or genes that show significant differences between cohorts, through time, or with treatment [66]. By leveraging publically available databases of human microbiome data (e.g. hmpdacc.org or microbio.me/americangut/) it will be possible to determine if observed trends show any statistical pattern within a larger population

promote tolerance to food allergens. Protocols which analyze the efficacy of OIT based on multi-parameter flow cytometric analysis of peripheral blood may have limited utility; an elegant recent study [60^{***}] has shown that most T-effector/memory cells are tissue-resident. In this regard, evaluation of allergen concentration in the bloodstream in the hours following gavage may provide a more clinically (and immunologically) relevant form of assessment. Moreover, it is tempting to speculate that poor digestibility and the access of undigested protein to the bloodstream with B-cell epitopes intact may be a distinguishing feature of food allergens (see refs. [61–63]) for peanut, β -lactoglobulin, and wheat). Whether this is in fact the case, and whether commensal bacteria regulate the systemic concentration of allergens other than peanut, is now readily testable in preclinical murine models. Variations of this assay can be adapted to clinical trials to evaluate the efficacy of administered live biotherapeutic agents/butyrate in modulating allergen concentrations in serum during double-blind oral food challenge. Several methodological challenges to the understanding of the gut microbiome in food allergy must be considered in future studies and will require multidisciplinary teams of immunologists, clinicians, microbial ecologists, and bioinformaticians (Table 1) [27^{**},64–66].

Acknowledgements

We thank Taylor Feehley and Sandeep Pawar for critical review of this manuscript.

Financial support and sponsorship

The work was supported by NIAID AI106302, Food Allergy Research and Education (FARE) and UChicago Digestive Diseases Center Core Grant P30DK42086 (C.R.N.), the Italian Ministry of Health PE-2011-02348447 (R.B.C.), and the US Dept. of Energy under Contract DE-AC02-06CH11357.NS (J.A.G.).

Conflicts of interest

There are no conflicts of interest.

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