



The role of the gut microbiota in food allergy

Rima Rachid^{a,b} and Talal A. Chatila^{a,b}

Purpose of review

The rise in the prevalence of food allergy over the past decades has focused attention of factors that may impact disease development, most notably the gut microbiota. The gut microbial communities play a crucial role in promoting oral tolerance. Their alteration by such factors as Cesarean section delivery, diet and antibiotics may influence disease development. This review highlights recent progress in our understanding of the role of the gut microbiota in the development of food allergy.

Recent findings

Food allergy is associated with alterations in the gut microbiota or dysbiosis early in life that may be predictive of disease persistence versus tolerance acquisition. Evidence for the benefits of adjunct therapy with probiotics for the prevention of food allergies and for potentiating oral immunotherapy remains circumstantial, with further studies needed to validate its use. Studies in murine models of food allergy suggest that microbial therapy with protolerogenic bacteria such as certain *Clostridial* species holds promise in future applications for prevention or therapy of food allergy.

Summary

Progress in understanding the role of dysbiosis in food allergy and the factors that promote its development, such as antibiotic therapy, diet, modes of infant delivery, and environmental exposures, offer windows of opportunity for both preventive and therapeutic interventions to stem the rising tide of the food allergy epidemic.

Keywords

dysbiosis, food allergy, innate lymphoid cells, microbiota, regulatory T cells, T helper cells type 2

INTRODUCTION

Food allergy is a major public health concern, whose prevalence has grown dramatically over the past decade. Food allergy now affects 6% of children under 5 years, as well as 3% of teens and adults, and peanut allergy has tripled in prevalence during the same period in children under the age of 18 years, from 0.4% in 1997 to 1.4% in 2008 [1,2].

The rise in food allergy prevalence, and that of other atopic diseases such as asthma and eczema, suggests an important role for environmental factors in early childhood. Originally, the hygiene hypothesis postulated by Strachan in 1989 proposed that increased cleanliness, decreased family size and decreased childhood infections could lead to an increase prevalence of allergic diseases [3]. Since then, it has been modified to propose that the modern Western life style and environment limits general microbial exposure in early infancy hence affecting microbial gut colonization, which leads to alteration in the natural development of immune tolerance which subsequently leads to allergic diseases [4]. There is an estimated 10^{13} to 10^{14} bacteria populating the gut, consisting of at least 400 species.

The structure of microbial community is affected by factors such as diet, probiotic intake, antibiotic treatment, and environmental exposures such as pets, farming environment [5[¶]]. The role of intestinal viruses and bacteriophages are much less understood [6,7]. The inherent plasticity of the intestinal microbiota suggests that unfavorable early alterations affecting gut microbial communities promote underlying dysbiosis, which raises the host's susceptibility to allergic and inflammatory responses.

Several studies have shown that factors that impact gut microbial colonization and composition early in life influence the development of atopic

^aDivision of Immunology, Boston Children's Hospital and ^bDepartment of Pediatrics, Harvard Medical School, Boston, MA, USA

Correspondence to Talal A. Chatila, MD, MSc, Division of Immunology, Boston Children's Hospital and the Department of Pediatrics, Harvard Medical School, Karp Family Building, Room 10-214, 1 Blackfan Street, Boston, MA 02115, USA. Tel: +1 617 919 3529; e-mail: talal.chatila@childrens.harvard.edu

Curr Opin Pediatr 2016, 28:748–753

DOI:10.1097/MOP.0000000000000427

KEY POINTS

- Several factors impact the developing gut microbiome in early life, including diet, mode of delivery, antibiotic treatment, and pet and environmental exposures.
- Experimental food allergy is associated with dysbiosis that may contribute to disease pathogenesis.
- Dysbiosis has also been noted in some human studies on food allergic subjects, although its reproducibility and role in disease pathogenesis remains to be established.

disease [8–11]. Environmental exposure to more diverse microbial communities, or to certain bacteria in those diverse microbial communities, may protect against the development of atopy [12–15]. In contrast, factors that may adversely affect microbial communities include increased rate of birth by Cesarean section, formula feeding, drugs such as antibiotic and gastric acidity inhibitors and low fibers/high fat diet, controlled light, humidity, and temperature of indoor environment [16,17]. Cesarean section delivery is associated with dominance of skin-derived bacterial populations in the gut microbiota [18]. Living in farm environments, in contact with a more diverse pool of microbes, reduces the risk of developing asthma and allergy [19–23]. Similarly, exposure to a larger number of pets in the first year of life is associated with protection against atopic disease [24], and is associated with more diverse environmental microbiota in house dust samples compared to that of individuals unexposed to pets with fur [25].

EVIDENCE IN MURINE STUDIES THAT GUT MICROBIOME AFFECTS ALLERGIC DISEASE

Substantial evidence exists that a ‘healthy’ intestinal microbiota facilitates the development of immune tolerance [26,27]. Earlier studies showed that gut associated lymphoid tissues (GALT) including Peyer’s patches are poorly developed or absent in germ-free mice [28,29]. It has been shown that introduction of *Bacteroides fragilis* into the lower gut of germ-free mice can lead to a redevelopment of GALT and induction of tolerance if performed in the neonatal period [30]. Inability to establish and maintain immune tolerance early in life increases the host’s risk of developing allergic and inflammatory diseases [31]. For example, mice raised in a sterile environment cannot be tolerized to antigens given orally [32], and have reduced

immunoglobulin A (IgA) [33] and reduced interleukin (IL)-10 producing T regulatory (T_{reg}) cells [34]. Furthermore, the presence of segmented filamentous bacteria (SFB) and *Clostridium* species, particularly clusters IV and XIVa, promotes the development of IL-17 producing T cells and T_{reg} cells, respectively [35,36]. Atarashi *et al.* [37] isolated 17 strains within Clostridia clusters XIVa, IV and XVIII from human feces and demonstrated in mice that these strains affect T_R differentiation and function in the colon. Our own studies in a food allergy-prone mouse model with a gain of function mutation in the IL-4 receptor alpha (IL-4Rα) chain (*Il4raF709* mice) established that acquisition of food allergy is associated with a gut microbiota signature that is distinct from that of food allergy-resistant mice, including changes in relative abundance of taxa of different bacterial families, such as Lachnospiraceae, Lactobacillaceae, Rikenellaceae, and Porphyromonadaceae. Furthermore, the gut microbiota of food allergic but not those of tolerant mice transmitted susceptibility to food allergy when transferred into germ-free mice [38]. Enforcement of oral tolerance in food allergic mice by means of allergen-specific T_{reg} cell therapy was associated with restructuring of the gut microbiota away from the disease-associated signature. Stefka *et al.* [39] found that neonatal exposure to antibiotics alters the commensal gut microbiota and enhances food allergen sensitization. Colonization with a *Clostridia* consortium consisting primarily of Clostridium clusters XIVa, XIVb and IV protected against sensitization to peanut [39].

EVIDENCE OF GUT DYSBIOSIS IN HUMANS WITH FOOD ALLERGY

Several factors complicate analysis of dysbiosis in subjects with food allergy. Comparison in-between studies is difficult, as there have been considerable heterogeneity in study design, sample size and age at time fecal collection, methods of analysis of gut microbiome, geographic location among others. Nevertheless, evidence of gut dysbiosis in food allergy is evolving with time, aided by increasing availability of very sensitive techniques. Earlier studies relying on bacterial cultures showed that infants allergic to cow’s milk had higher total bacteria and anaerobic counts [40]. There was no association between culturable bacteria and food sensitization by 18 months of age in three cohorts of European infants [41]. Kendler *et al.* [42] found no association between culturable gut bacteria and sensitization to food including milk, casein, egg, peanut, and hazelnut.

A recent study by Azad *et al.* [43^{***}] found that, in 166 infants participating in the population-based Canadian Healthy Infant Longitudinal Development (CHILD) study, 12 (7.2%) became sensitized to 1 or more common food allergens at 1 year of age. Food sensitization was defined as a food-specific wheal size of 2 mm or greater compared to the one elicited by the negative control. 16S rRNA sequencing was performed by Illumina MiSeq on stool samples collected at 3 and 12 months of age. The authors found that gut microbiota richness but not diversity at 3 months of age was statistically significantly lower in food-sensitized patients compared with control infants, though this difference did not persist at 1 year of age. Enterobacteriaceae were overrepresented and Bacteroidaceae were underrepresented in the fecal samples at 3 and 12 months in sensitized patients. Each quartile increase in richness at 3 months was associated with a 55% reduction in risk of sensitization to food at 1 year, while each quartile increase in Enterobacteriaceae/Bacteroidaceae ratio was associated with a two-fold increased risk of sensitization.

Bunyavanich *et al.* [44^{***}] have compared gut microbiome composition differences in infancy in patients with cow's milk allergy whose allergy to milk either persisted or resolved by the age of 8 years. 16S rRNA sequencing was performed on Illumina MiSeq platform. In subjects who were sampled at age 3–6 months and later became tolerant to milk, there was an enrichment of *Firmicutes* phylum and *Clostridia* class in their infant gut microbiome. *Bacteroidetes* and *Enterobacter* were characteristic of subjects whose milk allergy did not resolve by age 8 years. This was not noted when samples were taken in older patients age 7–12 or 13–16 months. Although there was a trend toward increased bacterial diversity mostly in subjects sample at age 3–6 months shown recently that in patients whose milk allergy resolved by age 8 years, gut microbiome composition. The authors speculated that either the gut microbiome in early infancy shapes the course of food allergy, or that cofounders obscure difference in gut microbiome in older age groups. Fatty acid metabolism was decreased in subjects who were sampled at age 3–6 months and whose milk allergy resolved.

A major limitation for all of these studies included that the number of patients with sensitization or food allergy was small, not allowing for robust statistical analysis of the effect of potential confounding variables, such as Cesarean delivery, diet, antibiotic intake, number of siblings, infections, and pets [45]. In addition, several studies have focused on sensitization to food, rather than evaluating patients with a history of an allergic reaction

to food or a confirmatory food challenge coupled with a documented by skin and/or serum immunoglobulin E (IgE) food-specific testing.

MECHANISMS OF FOOD ALLERGY PROMOTION BY GUT DYSBIOSIS

The commensal gut microbiota target different cellular components of the innate and adaptive immune compartments to promote oral tolerance. One mechanism by which the commensal microbiota influence the outcome of the allergic response is by modulating the innate lymphoid cells (ILC). Colonization of germ-free mice with consortia of Clostridial taxa protects from oral allergen sensitization by inducing IL22⁺ ILC type 3 (ILC3) at the intestinal mucosa [39]. IL-22 promotes mucosal integrity, preventing seepage of protease-resistant allergens through the intestinal mucosal barrier [39].

The commensal microbiota also target the adaptive immune response to promote tolerance, in part by directly acting on T_{reg} cells through their toll-like receptors (TLRs). Commensal microbiota promote the differentiation of induced T_{reg} (iT_{reg}) cells from naïve CD4⁺ T cells by a T_{reg} cell intrinsic, TLR- and MyD88-dependent mechanism [46^{*},47]. Studies in mice have revealed that food antigens normally elicit iT_{reg} cell responses in the gut, and that this process is defective in antibiotic-treated mice and in food allergic mice [46^{*},48,49^{***}]. Therapy with food allergen-specific iT_{reg} cells suppresses the food allergic response and reverse the dysbiosis in experimental mouse model of food allergy [38,49^{***}]. TLR/MyD88 signaling in T_{reg} cells also enables further iT_{reg} cell differentiation into T follicular helper cells (T_{FH}) in the Peyer's patches and lamina propria, which act to support local IgA production [46^{*},50]. In turn, luminal IgA shapes the intestinal microbiota and supports commensalism [51^{*}]. Another target of the commensal microbiota is the B cells [52]. Depletion of the commensal flora by antibiotic treatment of mice is associated with the development of T helper type 2 (Th2) cell-type allergic responses and higher serum IgE levels [52]. Th2 cell-type allergic immune responses were held in check by MyD88-dependent microbial sensing by B cells, which suppressed the IgE responses [52].

Another mechanism by which the commensal flora promote tolerance is through the production of short chain fatty acids (SCFAs), generated by bacterial fermentation of dietary fibers. SCFA act on T cells via a G-protein-coupled receptor (GPR43) and protect mice from intestinal inflammation by expanding colonic T_{reg} cells [53]. SCFAs also promote the generation of intestinal iT_{reg} cells

from naïve CD4⁺ T cells by T-cell intrinsic epigenetic mechanisms [54,55]. Butyrate, a SCFA known as an histone deacetylase inhibitor, increases Foxp3 protein acetylation conferring thereby increased stability and enhanced suppressive function on de-novo generated intestinal iT_{reg} cells [55]. A high fiber diet protects against allergic airway inflammation by altering the composition of the flora, leading to increased Bacteroidetes and decreased Firmicutes abundances, and resulting in increased circulating levels of SCFAs [56].

ROLE OF PROBIOTICS IN FOOD ALLERGY TREATMENT AND PREVENTION

The role of probiotics in the treatment and prevention of allergic diseases in general and food allergy in particular remains inconclusive. Probiotics are defined by the World Health Organization as 'live micro-organisms that when administered in adequate amount, confer a health benefit to the host'. The use of probiotics in atopic conditions stems from the possibility that by protecting against colonization with pathogenic bacteria, probiotic may protect intestinal barrier and decrease susceptibility allergens. The pharmacokinetics and the dosage required for adequate implantation and gut colonization with probiotic bacteria has not been well quantified [57]. Studies on prevention of eczema or food hypersensitivity lead to conflicting outcome. For example, a prospective randomized trial in infants with cow's milk allergy found that those who were randomized to receive extensively hydrolyzed milk formula (EHMF) supplemented with *Lactobacillus* GG (LGG) acquired tolerance to cow's milk evaluated by a double-blind placebo-controlled food challenge faster than those who were only consuming or EHFM [58]. On the other hand, in a double-blind placebo-controlled trial with 119 infants with cow's milk allergy fed either EHF alone, or EHF supplemented with *Lactobacillus casei* and *Bifidobacterium lactis* CMA infants fed EHF plus probiotics acquired tolerance to cow's milk at the same rate as infants given EHF alone [59]. In a different study, children considered at high-risk for developing atopic disease were fed standard infant formula or a nonhydrolyzed fermented infant formula containing heat-killed *Bifidobacterium breve* C50 and *Streptococcus thermophilus* 065. There was no statistically significant difference in the incidence of cow's milk allergy between the two groups based on history and oral milk challenge. However, infants fed the fermented formula with heat-killed probiotics were more likely to have negative skin prick tests to milk [60]. A recent systemic review and meta-analysis of 17 randomized-controlled trials,

involving 2947 infants showed that when probiotics were administered either only prenatally or only postnatally, no effects of probiotics on atopy and food hypersensitivity were observed. When administered especially to pregnant mothers prenatally and postnatally to the child, pooled analysis indicated that probiotics could reduce the risk of atopy and the risk of food sensitivity defined as positive skin or food IgE testing [61^{*}]. Taken together, these studies suggest that probiotics may modulate development of allergic sensitization to foods as measured by allergy skin prick testing, but the administration probiotic does not necessarily translate into food allergy prevention per se. Further evaluation of LGG as a therapeutic modality in food allergy is warranted. Differences in these studies may be related to the study design, the dose and duration of probiotic intake as well as the type of probiotic (s) administered.

A recent double-blind randomized placebo-controlled trial evaluated the effect of 18 months of daily *Lactobacillus rhamnosus* CGMCC combined with peanut oral immunotherapy (OIT) on sustained unresponsiveness. 82% of subjects in the peanut OIT/probiotic were noted to have possible sustained unresponsiveness after interrupting OIT for 2–5 weeks after interruption. The study, however, did not have a peanut OIT arm or probiotic arm alone [62]. A prior multisite study on peanut OIT studies without probiotics have shown that the rate of sustained unresponsiveness was ~50%, after at least 4 weeks of OIT interruption [63]. It is therefore cannot be excluded that the elevated rate of sustained unresponsiveness compared with other studies was at least in part secondary to a shorter duration of interruption of therapy in some patients. The effect of probiotic therapy on gut microbiota community structure in these patients was not evaluated.

CONCLUSION

The role of the intestinal microbiota in the development of immune tolerance to food is increasingly appreciated. Although there is a substantial evidence in murine models that gut microbiome plays a major role in food allergy and tolerance, human studies have led to inconsistent findings. Larger studies, preferably longitudinal birth cohort with more homogenous designs are needed to further evaluate for the presence or absence of a defined dysbiotic food allergy signature. In addition, larger interventional trials in infants are required to evaluate the role of different probiotic in prevention or treatment of food allergy.

Acknowledgements

This work was supported by the National Institutes of Health (SR01AI065617 and 1R56AI117983), to T.A.C.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Branum AM, Lukacs SL. Food allergy among children in the United States. *Pediatrics* 2009; 124:1549–1555.
2. Sicherer SH, Sampson HA. Food allergy: epidemiology, pathogenesis, diagnosis, and treatment. *J Allergy Clin Immunol* 2014; 133:291–307; quiz 8.
3. Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; 299:1259–1260.
4. Wold AE. The hygiene hypothesis revised: is the rising frequency of allergy due to changes in the intestinal flora? *Allergy* 1998; 53 (46 Suppl):20–25.
5. Tamburini S, Shen N, Wu HC, Clemente JC. The microbiome in early life: ■ implications for health outcomes. *Nat Med* 2016; 22:713–722. Excellent review on the microbiome in early life.
6. Gill SR, Pop M, Deboy RT, *et al.* Metagenomic analysis of the human distal gut microbiome. *Science* 2006; 312:1355–1359.
7. Steinhoff U. Who controls the crowd? New findings and old questions about the intestinal microflora. *Immunol Lett* 2005; 99:12–16.
8. Koplin J, Allen K, Gurrin L, *et al.* Is caesarean delivery associated with sensitization to food allergens and IgE-mediated food allergy: a systematic review. *Pediatr Allergy Immunol* 2008; 19:682–687.
9. Bjorksten B. Disease outcomes as a consequence of environmental influences on the development of the immune system. *Curr Opin Allergy Clin Immunol* 2009; 9:185–189.
10. West CE, Jenmalm MC, Prescott SL. The gut microbiota and its role in the development of allergic disease: a wider perspective. *Clin Exp Allergy* 2015; 45:43–53.
11. Garn H, Neves JF, Blumberg RS, Renz H. Effect of barrier microbes on organ-based inflammation. *J Allergy Clin Immunol* 2013; 131:1465–1478.
12. Kalliomaki M, Salminen S, Arvilommi H, *et al.* Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001; 357:1076–1079.
13. Forno E, Onderdonk AB, McCracken J, *et al.* Diversity of the gut microbiota and eczema in early life. *Clin Mol Allergy* 2008; 6:11.
14. Penders J, Thijs C, van den Brandt PA, *et al.* Gut microbiota composition and development of atopic manifestations in infancy: the KOALA Birth Cohort Study. *Gut* 2007; 56:661–667.
15. Penders J, Stobbering EE, van den Brandt PA, Thijs C. The role of the intestinal microbiota in the development of atopic disorders. *Allergy* 2007; 62:1223–1236.
16. Di Costanzo M, Amoroso A, Canani RB. Gut microbiota as a target for food allergy. *J Pediatr Gastroenterol Nutr* 2016; 63 (Suppl 1):S11–S13.
17. Kembel SW, Meadow JF, O'Connor TK, *et al.* Architectural design drives the biogeography of indoor bacterial communities. *PLoS One* 2014; 9:e87093.
18. Dominguez-Bello MG, Costello EK, Contreras M, *et al.* Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A* 2010; 107:11971–11975.
19. Illi S, von Mutius E, Lau S, *et al.* Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. *BMJ* 2001; 322:390–395.
20. Braun-Fahrlander C, Riedler J, Herz U, *et al.* Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002; 347:869–877.
21. Ege MJ, Mayer M, Normand AC, *et al.* Exposure to environmental micro-organisms and childhood asthma. *N Engl J Med* 2011; 364:701–709.
22. Stein MM, Hrusch CL, Gozdz J, *et al.* Innate immunity and asthma risk in Amish and Hutterite farm children. *N Engl J Med* 2016; 375:411–421.
23. Chatila TA. Innate immunity in asthma. *N Engl J Med* 2016; 375:477–479.
24. Ownby D, Johnson C, Peterson E. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA* 2002; 288:963–972.
25. Fujimura KE, Johnson CC, Ownby DR, *et al.* Man's best friend? The effect of pet ownership on house dust microbial communities. *J Allergy Clin Immunol* 2010; 126:410–412; 2 e1-3.
26. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science* 2012; 336:1268–1273.
27. Rudensky AY, Chervonsky AV. A narrow circle of mutual friends. *Immunity* 2011; 34:697–699.
28. Furrie E, Turner MW, Strobel S. Failure of SCID mice to generate an oral tolerance after a feed of ovalbumin: a role for a functioning gut-associated lymphoid system. *Immunology* 1994; 83:562–567.
29. Sudo N, Sawamura S, Tanaka K, *et al.* The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* 1997; 159:1739–1745.
30. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 2005; 122:107–118.
31. McLoughlin RM, Mills KH. Influence of gastrointestinal commensal bacteria on the immune responses that mediate allergy and asthma. *J Allergy Clin Immunol* 2011; 127:1097–1107; quiz 108-9.
32. Sudo N, Sawamura S, Tanaka K, *et al.* The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* 1997; 159:1739–1745.
33. Fritz JH, Rojas OL, Simard N, *et al.* Acquisition of a multifunctional IgA+ plasma cell phenotype in the gut. *Nature* 2012; 481:199–203.
34. Geuking MB, Cahenzli J, Lawson MA, *et al.* Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity* 2011; 34:794–806.
35. Ivanov II, Atarashi K, Manel N, *et al.* Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 2009; 139:485–498.
36. Atarashi K, Tanoue T, Shima T, *et al.* Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science* 2011; 331:337–341.
37. Atarashi K, Tanoue T, Oshima K, *et al.* Treg induction by a rationally selected mixture of *Clostridia* strains from the human microbiota. *Nature* 2013; 500:232–236.
38. Noval Rivas M, Burton OT, Wise P, *et al.* A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. *J Allergy Clin Immunol* 2013; 131:201–212.
39. Stefa AT, Feehley T, Tripathi P, *et al.* Commensal bacteria protect against food allergen sensitization. *Proc Natl Acad Sci U S A* 2014; 111:13145–13150.
40. Thompson-Chagoyan OC, Vieites JM, Maldonado J, *et al.* Changes in faecal microbiota of infants with cow's milk protein allergy – a Spanish prospective case-control 6-month follow-up study. *Pediatr Allergy Immunol* 2010; 21 (2 Pt 2):e394–400.
41. Adlerberth I, Strachan DP, Matricardi PM, *et al.* Gut microbiota and development of atopic eczema in 3 European birth cohorts. *J Allergy Clin Immunol* 2007; 120:343–350.
42. Kendler M, Uter W, Rueffer A, *et al.* Comparison of fecal microflora in children with atopic eczema/dermatitis syndrome according to IgE sensitization to food. *Pediatr Allergy Immunol* 2006; 17:141–147.
43. Azad MB, Konya T, Guttman DS, *et al.* Infant gut microbiota and food ■ sensitization: associations in the first year of life. *Clin Exp Allergy* 2015; 45:632–643. Evidence for early life dysbiosis and its association with food sensitization.
44. Bunyavanich S, Shen N, Grishin A, *et al.* Early-life gut microbiome composition ■ and milk allergy resolution. *J Allergy Clin Immunol* 2016; pii: S0091-6749(16)30268-8. [Epub ahead of print] Evidence for early life dysbiosis and its association with food allergy outcome.
45. Molloy J, Allen K, Collier F, *et al.* The potential link between gut microbiota and IgE-mediated food allergy in early life. *Int J Environ Res Public Health* 2013; 10:7235–7256.
46. Wang S, Charbonnier LM, Noval Rivas M, *et al.* MyD88 adaptor-dependent ■ microbial sensing by regulatory T cells promotes mucosal tolerance and enforces commensalism. *Immunity* 2015; 43:289–303. Evidence for a direct effect of the microbiota on T_{reg} cells via the TLR-MyD88 pathway and its role in regulating intestinal tolerance.
47. Round JL, Lee SM, Li J, *et al.* The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* 2011; 332:974–977.
48. Kim KS, Hong SW, Han D, *et al.* Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. *Science* 2016; 351:858–863.
49. Noval Rivas M, Burton OT, Wise P, *et al.* Regulatory T cell reprogramming ■ toward a Th2-cell-like lineage impairs oral tolerance and promotes food allergy. *Immunity* 2015; 42:512–523. Provides a mechanism for how T_{reg} cell reprogramming into Th2 like cells in food allergy, promotes disease.
50. Kawamoto S, Maruya M, Kato LM, *et al.* Foxp3 (+) T cells regulate immunoglobulin a selection and facilitate diversification of bacterial species responsible for immune homeostasis. *Immunity* 2014; 41:152–165.
51. Macpherson AJ, Koller Y, McCoy KD. The bilateral responsiveness between ■ intestinal microbes and IgA. *Trends Immunol* 2015; 36:460–470. Excellent review on the role of IgA in structuring the gut microbiome.
52. Hill DA, Siracusa MC, Abt MC, *et al.* Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. *Nat Med* 2012; 18:538–546.

53. Smith PM, Howitt MR, Panikov N, *et al.* The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 2013; 341:569–573.
 54. Furusawa Y, Obata Y, Fukuda S, *et al.* Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 2013; 504:446–450.
 55. Arpaia N, Campbell C, Fan X, *et al.* Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 2013; 504:451–455.
 56. Trompette A, Gollwitzer ES, Yadava K, *et al.* Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med* 2014; 20:159–166.
 57. Awasthi S, Wilken R, Patel F, *et al.* Dietary supplementation with *Bifidobacterium longum* subsp. *infantis* (*B. infantis*) in healthy breastfed infants: study protocol for a randomised controlled trial. *Trials* 2016; 17:340.
 58. Berni Canani R, Nocerino R, Terrin G, *et al.* Effect of *Lactobacillus* GG on tolerance acquisition in infants with cow's milk allergy: a randomized trial. *J Allergy Clin Immunol* 2012; 129:580–582; 2 e1-5.
 59. Hol J, van Leer EH, Elink Schuurman BE, *et al.* The acquisition of tolerance toward cow's milk through probiotic supplementation: a randomized, controlled trial. *J Allergy Clin Immunol* 2008; 121:1448–1454.
 60. Morisset M, Aubert-Jacquin C, Soulaines P, *et al.* A nonhydrolyzed, fermented milk formula reduces digestive and respiratory events in infants at high risk of allergy. *Eur J Clin Nutr* 2011; 65:175–183.
 61. Zhang GQ, Hu HJ, Liu CY, *et al.* Probiotics for prevention of atopy and food hypersensitivity in early childhood: A PRISMA-compliant systematic review and meta-analysis of randomized controlled trials. *Medicine (Baltimore)* 2016; 95:e2562.
- This meta-analysis suggests that timing and duration of therapy with probiotics play a critical role in their capacity to prevent atopy and food hypersensitivity.
62. Tang ML, Ponsonby AL, Orsini F, *et al.* Administration of a probiotic with peanut oral immunotherapy: a randomized trial. *J Allergy Clin Immunol* 2015; 135:737–744; e8.
 63. Vickery BP, Scurlock AM, Kulis M, *et al.* Sustained unresponsiveness to peanut in subjects who have completed peanut oral immunotherapy. *J Allergy Clin Immunol* 2014; 133:468–475.