

Microbiome/microbiota and allergies

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Abstract Allergies are characterized by a hypersensitive immune reaction to originally harmless antigens. In recent decades, the incidence of allergic diseases has markedly increased, especially in developed countries. The increase in the frequency of allergic diseases is thought to be primarily due to environmental changes related to a westernized lifestyle, which affects the commensal microbes in the human body. The human gut is the largest organ colonized by bacteria and contains more than 1000 bacterial species, called the “gut microbiota.” The recent development of sequencing technology has enabled researchers to genetically investigate and clarify the diversity of all species of commensal microbes. The collective genomes of commensal microbes are together called the “microbiome.” Although the detailed mechanisms remain unclear, it has been proposed that the microbiota/microbiome, especially that in the gut, impacts the systemic immunity and metabolism, thus affecting the development of various immunological diseases, including allergies. In this review, we summarize the recent findings regarding the importance of the microbiome/microbiota in the development of allergic diseases and also the results of interventional studies using probiotics or prebiotics to prevent allergies.

Keywords Microbiome · Microbiota · Dysbiosis · Allergy · Probiotics · Prebiotics

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Introduction

Allergies are characterized by a hypersensitive immune reaction to originally harmless antigens. In recent decades, the incidence of allergic diseases, such as asthma, allergic rhinitis, atopic dermatitis, and food allergies, has markedly increased, especially in developed countries, and the burden of allergic diseases, not only on patients, but also on family members and society, is rising. Although both genetic and environmental factors influence the etiology of allergic diseases, the increase in the frequency of allergic diseases is thought to be primarily due to environmental changes related to a westernized lifestyle.

Factors associated with a Western lifestyle affect commensal microbes in the human body. The human gut is the largest organ colonized by bacteria ($\sim 10^{14}$) and contains more than 1000 bacterial species [1], called the “gut microbiota.” The recent development of sequencing technology has enabled researchers to genetically investigate and clarify the diversity of all species of commensal microbes [2]. The collective genomes of commensal microbes are together called the “microbiome.” Recent studies have documented the characteristics of the microbiome in the gut and skin, as well as the lungs, which have historically been considered to be sterile. Although the detailed mechanisms remain unclear, it has been proposed that the microbiota/microbiome, especially that in the gut, impacts systemic immunity and metabolism [3], thus affecting the development of various immunological diseases, including allergies. In this review, we summarize recent findings regarding the importance of the microbiome/microbiota in the development of allergic diseases and the results of interventional studies using probiotics or prebiotics to prevent allergies.

New concept of the hygiene hypothesis

The prevalence of allergic diseases has increased significantly in recent years. Although genomewide association studies have identified genetic risk factors for the development of allergic diseases, such as genetic variations at the IL1RL1 loci or IL13 loci [4], the recent rise in the rate of allergic diseases cannot be explained only by genetic factors, as such factors do not change that rapidly.

The “hygiene hypothesis,” which has been proposed to explain the increase in allergic diseases in Western countries, argues that infection and/or exposure to microbial components, such as that encountered in farm work [5], in early childhood inhibits the development of allergic diseases [6]. This hypothesis is based on the concept of the Th1/Th2 model, as microbial components, such as LPS, are strong activators of the induction of Th1 immunity. In utero acquired immunity in the fetus is skewed to Th2 immunity in order to prevent a Th1-type immune response to the mother leading to miscarriage. In nonatopic infants, the capacity to produce Th1 cytokine interferon γ in response to allergens increases after birth, while the Th2 response to allergens is rapidly suppressed during the first year of life. In contrast, atopic infants exhibit continued fetal allergen-specific Th2 responses with a decreased ability to produce interferon γ during infancy [7]. This model, however, is now considered to be insufficient to explain the hygiene hypothesis. In fact, there are conflicting reports as to whether treatment with the *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) vaccine, a strong activator of Th1 immunity, in early life inhibits the development of asthma [8]. In inner city communities, domestic endotoxin exposure is positively associated with wheezing in children 2 years of age [9]. Moreover, the Th1/Th2 model does not explain the recent increase in the prevalence of autoimmune diseases, most of which are Th1-mediated, in association with the decrease in the rate of infectious diseases [10].

The counter-regulatory model [11] is another explanation for the hygiene hypothesis in which a decrease in regulatory immune responses, such as IL-10 production, to inflammatory processes in both Th1 and Th2 immunity results in an increased incidence of both allergies and autoimmune diseases. Recently, it has been shown that a healthy microbiota plays a critical role in the maturation of the regulatory immune system, and changes in human ecology in recent decades, including improvements in sanitation leading to cleaner water, social changes leading to smaller families, the increased use of preterm antibiotics, the lower rate of breast-feeding, and the widespread application of antibiotics, have been documented to affect the composition of gut microbiota. The “microbiota hypothesis” is explained by the counter-regulatory model and states that the disruption of mucosal regulatory immune responses caused by the perturbation of microbiota due to Western lifestyle factors leads to the development of both

Th1- and Th2-mediated diseases. Consequently, this hypothesis is considered an alternative interpretation of the evidence supporting the hygiene hypothesis.

Establishment of microbiome/microbiota in healthy children

It was previously thought that the fetus remains under sterile conditions, with the colonization of microbes starting after birth. However, recent reports have demonstrated the existence of bacteria in umbilical cord blood and the placenta in healthy neonates, suggesting the prenatal transfer of microbiota from mother to child in utero [12–14]. Microbes are present in the amniotic fluid [15] and thus in the meconium, even in Cesarean-born infants [16]. As the microbial DNA present in amniotic fluid and the placenta is associated with changes in the TLR-related gene expression in the fetal intestines [14], the immune system of the fetus may be influenced by intrauterine exposure to microbes.

During delivery, microbes colonize the infant from the maternal vaginal microbiota and colonic microbiota, the largest sources of the initial gut microbiota in the newborn. Cesarean-born infants are deprived of this natural exposure and consequently exhibit an increased risk of atopy and allergic diseases due to immature microbiota-derived development of the immune system [17].

The composition and temporal pattern of the gut microbiota vary widely from infant to infant in the first year of life, after which the idiosyncratic microbiome in each infant converges toward a profile characteristic of the gut microbiota observed in adults [18]. Early selective culture-based assessments have shown that the initial gut microbiota contains facultative anaerobic bacteria, mainly *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Enterobacteriaceae* species. Due to the initial abundance of oxygen in the neonatal gut, the growth of anaerobes is prevented. However, *Bifidobacterium*, *Bacteroides*, and *Clostridium* species have been isolated in the first week of life, after facultative anaerobes begin to consume oxygen [19].

Recently, breast milk has been proposed to be a major source of bacterial diversity in the neonatal gut. Breast-fed infants generally exhibit greater diversity of *Bifidobacterium* species than formula-fed infants [20, 21]. Moreover, the diversity of the gut microbiota increases during lactation [22]. *Weissella*, *Leuconostoc*, *Staphylococcus*, *Streptococcus*, and *Lactococcus* are predominant in the colostrum, whereas oral cavity microbes, such as *Veillonella*, *Leptotrichia*, and *Prevotella*, are prevalent in mature milk samples.

Colonization of microbes on the skin also begins shortly after birth. Staphylococci and streptococci dominate early colonization on the skin; however, with age, the levels of *Staphylococcus* and *Streptococcus* decrease, while the levels

of less frequent genera increase. In adults, *Propionibacterium* species dominate sebaceous areas, while *Staphylococcus* and *Corynebacterium* species dominate moist areas [23].

The process underlying the establishment of the lung microbiome is unclear, compared to that observed in the gut or skin, due to difficulties in obtaining lower airway samples, such as bronchial lavage fluid, from infants. The composition of the lung microbiota in infants has been studied only in patients with cystic fibrosis, and the lung microbiome is thought to develop in accordance with the gut microbiome during infancy [24].

Impact of the microbiome/microbiota on allergic diseases

Epidemiological studies have shown that infants who develop allergies display an altered commensal population in the gut, called dysbiosis, early in life [25, 26]. A birth cohort study in the Netherlands was recently conducted to evaluate early life exposure to antibiotics and the subsequent development of allergic sensitization in infancy (KOALA study [27, 28]). Antibiotic intake was evaluated with respect to both maternal antibiotic use during breast-feeding and infant oral medication. Consequently, antibiotic exposure via breast-feeding was not found to be associated with sensitization at 2 years of age. On the other hand, the presence of a specific bacterium, *Clostridium difficile*, in the feces of 1-month-old infants was identified as being significantly associated with a higher risk of allergic sensitization at 2 years of age [26], as also observed in mice [29]. These findings indicate that a healthy gut microbiome/microbiota plays a protective role in the development of allergies. Interestingly, both germ-free [30, 31] and antibiotic-treated [32] mice, as well as mice monocolonized with *Escherichia coli* or lactobacilli, exhibit susceptibility to sensitization to allergens [33], thus suggesting that it is necessary to have a complex microbiota in order to prevent allergies.

Forkhead box P3 (Foxp3)+ regulatory T cells (Tregs) are a subset of T cells engaged in sustaining immunological self-tolerance and suppressing excessive immune responses deleterious to the host. These cells are generated in the thymus (tTreg) and periphery (pTreg), including the gut, and recent studies have shown that pTregs, but not tTregs, play a critical role in controlling mucosal Th2 inflammation in the gut and lungs [34]. It has been shown that certain gut bacteria [29, 35] and a symbiosis factor (polysaccharide A) from *Bacteroides fragilis* promote pTreg induction [36]. Although the detailed mechanisms remain unclear, differences in the innate immune response, such as TLR2 signaling [36], between pathogenic bacteria and commensal bacteria are thought to be important for inducing pTregs in the gut.

Another subset of T cells, the induction of which is largely altered by the gut microbiota, is Th17 cells. Th17 cells are a

subset of helper T cells that secrete IL-17, IL-17F, and IL-22. It has been clarified that the Th17-mediated neutrophil-predominant phenotype of asthma results in more severe disease that is less responsive to steroids. In the steady state, Th17 cells are abundant in gut-associated tissues, where they accumulate only in the presence of commensal microbiota, indicating the importance of microbes in the induction of Th17 cells in the gut [37]. Interestingly, the population of Th17 cells in the small intestinal lamina propria differs within the same strain (C57BL/6) between commercial vendors [38]. The authors further compared the intestinal microbiota between C57BL/6 mice obtained from different vendors and found that a segmented filamentous bacterium (SFB), which induced Th17 cells in the intestinal lamina propria, was of significantly greater relative abundance in mice with more Th17 cells [39].

Microbiota also affects other types of immune cells associated with Th2-type immune responses. Basophils are basophilic granulocytes, which represent <1 % of peripheral blood leukocytes. Although both basophils and mast cells possess the high-affinity IgE receptor, FcεRI, and the capacity to release histamine by cross-linking FcεRI, it has been recognized that basophils have distinct roles from mast cells in the pathogenesis of allergies. For example, basophils stimulate B cells to secrete IgM and undergo class switching [40]. In addition, recent studies have documented the role of basophils in initiating Th2 cell differentiation via IL-4. In mice, commensal-derived signals have been found to influence basophil development by limiting the proliferation of resident bone marrow precursor populations [32]. Consequently, the depletion or deletion of bacterial communities, as associated with an elevated serum IgE level, increases the circulating basophil population, thereby exaggerating the Th2 cell response and allergic inflammation [32].

Impact of the microbiota/microbiome on atopic eczema/dermatitis

Atopic eczema/dermatitis is an inflammatory, chronically relapsing, intensely pruritic skin disease that often occurs in families with atopic diseases (atopic dermatitis, bronchial asthma, and/or allergic rhino-conjunctivitis) [41]. Atopic eczema/dermatitis is the result of an initial process called atopic march and constitutes a strong risk factor for the development of the following allergic diseases.

In early birth cohort studies using the culture method, atopic eczema in infants has not been found to be associated with the presence of any particular gut commensal microbes [42]. However, a birth cohort study of the diversity of the gut microbiome in infants using high-throughput 16S-based molecular microbiology demonstrated that infants with IgE-associated eczema during the first 2 years of life have a lower level of diversity among the total microbiota, the bacterial

phylum *Bacteroidetes* and the genus *Bacteroides* at 1 month, but not 1 week or 12 months of age, supporting the findings of previous reports showing low diversity of the gut microbiota to be associated with an increased risk of subsequent atopic disease [43–45].

Staphylococcus aureus colonization in the skin has been shown to be associated with atopic dermatitis using culture methods [46]. The skin microbiome in patients with atopic dermatitis contains increased *Staphylococcus* species, including both *S. aureus* and *Staphylococcus epidermidis*, during flare-ups [47]. However, it remains unclear whether the shift in the skin microbiome causes the flare-up or is induced by the effects of atopic dermatitis.

Impact of the microbiota/microbiome on food allergies

A food allergy is defined as “an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food” [48]. The proteins in foods and/or gut commensal microbes are non-self antigens, although they should not be targets of the immune system. The purpose of immunological tolerance, called “oral tolerance,” is to prevent an immune reaction. One mechanism underlying the development of food allergies is thought to be the failure of oral tolerance. It has also been clarified that gut microbiota plays a critical role in oral tolerance in mouse models. Gnotobiotic mice transplanted with gut microbiota from healthy infants primarily composed of *Bifidobacterium* and *Bacteroides* exhibit less sensitization and allergic reactions to cow’s milk, compared to germ-free mice, suggesting the protective effects of these bacteria against the development of food allergies [49]. In contrast, the gut microbiome in food allergy model shows a specific gut microbiome signature characterized by increased levels of *Lachnospiraceae*, *Lactobacillaceae*, *Rikenellaceae*, and *Porphyromonadaceae* [50]. This disease-associated microbiome is capable of transmitting susceptibility to food allergies and is restorable by enforced tolerance via the infusion of allergen-specific Tregs [50]. To our knowledge, there is only one human study comparing the gut microbiome between infants with food allergies and healthy infants. The authors found that infants with food allergies have a specific gut microbiome signature [51].

Impact of the microbiota/microbiome on asthma

Asthma is a chronic lung disease characterized by airway hyperresponsiveness that causes recurrent episodes of wheezing, breathlessness, chest tightness, and coughing. Significant evidence suggests that the Th2 cytokines, IL-4, IL-5, and IL-13, play an important role in inducing the pathophysiological features of asthma.

The lungs were previously considered to be sterile according to culture-based assessments. However, recently developed culture-independent techniques have demonstrated this to be not accurate. Asthmatics have a higher burden and greater diversity of microbiome in the lungs than healthy individuals [52]. In detail, compared to healthy individuals, both asthmatic adults and children exhibit increased levels of *Proteobacteria*, particularly *Haemophilus* species, in the lung microbiome. In contrast, *Bacteroides*, especially the *Prevotella* species, are more frequent in healthy individuals than either asthmatic adults or children [53]. However, the association between disruption of the lung microbiota and asthma is a chicken-and-egg problem, and further studies focusing on the effects of the lung microbiota are needed.

Studies using mouse models have clarified that allergic airway inflammation is regulated by the gut microbiota. In an ovalbumin-induced allergic airway inflammation model, germ-free mice show a significantly higher degree of airway hyperresponsiveness and Th2-mediated lung inflammation [54].

Effects of probiotics and prebiotics on allergic diseases

Probiotics are defined as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.” Although this definition excludes beneficial microorganisms originally not found in food, probiotics can now be found in food as well as nutritional supplements, drugs, and medical foods [55]. Most of the probiotics used today belong to the genera *Lactobacillus* and *Bifidobacterium* [56], and the effects of these probiotics are attributed to their ability to reverse increased intestinal permeability and an unbalanced gut microbiota, improve the intestine’s immunologic barrier function, and decrease the generation of proinflammatory cytokines associated with local and systemic allergic inflammation [57]. Following the publication of a randomized placebo-controlled trial in Finland showing the preventive effects of *Lactobacillus* GG on the development of infantile eczema [58], a number of analyses of the efficacy of probiotics in preventing allergic diseases, especially eczema, were conducted. However, evidence confirming the efficacy of this treatment is lacking due to study limitations, such as differences in the initial supplementation period, duration of treatment, strain, length of follow-up period, and host factors [59]. Although several meta-analyses have shown the administration of probiotics both prenatally and postnatally to be effective in preventing eczema [60–62], a meta-analysis demonstrated that this effect was only significant for lactobacilli, not a mixture of various bacterial strains [63]. In addition, the use of probiotic treatment to prevent the development of wheezing in early childhood and/or the onset of asthma failed to decrease the incidence of these diseases [64–66]. Moreover,

there are few studies of the preventive effects of probiotics in cases of allergic rhinitis [67] or food allergies [68]. Consequently, the latest Cochrane review concluded that further research is needed before the routine use of prebiotics can be recommended to prevent allergies [69].

Prebiotics are defined as “a selectively fermented ingredient that allows for specific changes, both in the composition and/or activity in the gastrointestinal microbiota, that confers benefits upon host well-being and health” [70]. Human milk contains prebiotics, and the presence of these substances in the gut enhances the proliferation of certain probiotic bacteria, particularly bifidobacteria species. Commercially available prebiotics include fructooligosaccharides, inulin, galactooligosaccharides, and soybean oligosaccharides [71]. Several randomized controlled trials have reported that prebiotics have an effect in preventing eczema [72–76]. However, it remains uncertain whether prebiotic supplements have a preventive effect on the development of other allergic disorders.

Synbiotics refers to a combination of probiotics and prebiotics. Previous studies have found that treatment with synbiotics significantly changes the gut microbiota [77, 78], although it does not result in positive preventive effects against the development of infantile eczema [66, 79].

Do the benefits of probiotics require live organisms [80]? Several reports have demonstrated that heat-killed lactobacilli strains improve the Th1/Th2 balance and inhibit IgE production in mice [81–83], thus suggesting that dead probiotics continue to have the capacity to modify immunological responses. Hence, further studies regarding the application of dead probiotics to prevent allergic diseases are needed.

Future perspectives

Various studies have revealed that the microbiome/microbiota plays an important role in the pathogenesis of allergic diseases. However, it remains unclear how differences in age, region, dietary intake, or other environmental factors, such as allergen exposure or infection, affect this role. Rapid and low-cost sequencing of the microbiome would allow researchers to analyze the impact of the microbiome under specific conditions. In addition, further studies clarifying the detailed mechanisms of immunological modulation induced by the microbiome and probiotics/prebiotics may help to develop novel therapeutic targets to prevent or cure allergic diseases.

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Conflict of interest None.

References

1. Noverr MC, Huffnagle GB (2005) The ‘microflora hypothesis’ of allergic diseases. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* 35(12):1511–1520. doi:10.1111/j.1365-2222.2005.02379.x
2. Human Microbiome Project C (2012) Structure, function and diversity of the healthy human microbiome. *Nature* 486(7402):207–214. doi:10.1038/nature11234
3. Hooper LV, Littman DR, Macpherson AJ (2012) Interactions between the microbiota and the immune system. *Science* 336(6086):1268–1273. doi:10.1126/science.1223490
4. Tamari M, Tanaka S, Hirota T (2013) Genome-wide association studies of allergic diseases. *Allergol Int Off J Jpn Soc Allergol* 62(1):21–28. doi:10.2332/allergolint.13-RAI-0539
5. Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrlander C, Heederik D, Piarroux R, von Mutius E, Group GTS (2011) Exposure to environmental microorganisms and childhood asthma. *N Engl J Med* 364(8):701–709. doi:10.1056/NEJMoa1007302
6. Strachan DP (1989) Hay fever, hygiene, and household size. *BMJ* 299(6710):1259–1260
7. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG (1999) Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 353(9148):196–200. doi:10.1016/S0140-6736(98)05104-6
8. Linehan MF, Nurmatov U, Frank TL, Niven RM, Baxter DN, Sheikh A (2014) Does BCG vaccination protect against childhood asthma? Final results from the Manchester Community Asthma Study retrospective cohort study and updated systematic review and meta-analysis. *J Allergy Clin Immunol* 133(3):688–695. doi:10.1016/j.jaci.2013.08.007, e614
9. Perzanowski MS, Miller RL, Thorne PS, Barr RG, Divjan A, Sheares BJ, Garfinkel RS, Perera FP, Goldstein IF, Chew GL (2006) Endotoxin in inner-city homes: associations with wheeze and eczema in early childhood. *J Allergy Clin Immunol* 117(5):1082–1089. doi:10.1016/j.jaci.2005.12.1348
10. Bach JF (2002) The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 347(12):911–920. doi:10.1056/NEJMr020100
11. Wills-Karp M, Santeliz J, Karp CL (2001) The germless theory of allergic disease: revisiting the hygiene hypothesis. *Nat Rev Immunol* 1(1):69–75. doi:10.1038/35095579
12. Satokari R, Gronroos T, Laitinen K, Salminen S, Isolauri E (2009) Bifidobacterium and Lactobacillus DNA in the human placenta. *Lett Appl Microbiol* 48(1):8–12. doi:10.1111/j.1472-765X.2008.02475.x
13. Jimenez E, Fernandez L, Marin ML, Martin R, Odriozola JM, Nueno-Palop C, Narbad A, Olivares M, Xaus J, Rodriguez JM (2005) Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. *Curr Microbiol* 51(4):270–274. doi:10.1007/s00284-005-0020-3
14. Rautava S, Collado MC, Salminen S, Isolauri E (2012) Probiotics modulate host-microbe interaction in the placenta and fetal gut: a randomized, double-blind, placebo-controlled trial. *Neonatology* 102(3):178–184. doi:10.1159/000339182
15. DiGiulio DB, Romero R, Amogan HP, Kusanovic JP, Bik EM, Gotsch F, Kim CJ, Erez O, Edwin S, Relman DA (2008) Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. *PLoS One* 3(8):e3056. doi:10.1371/journal.pone.0003056
16. Jimenez E, Marin ML, Martin R, Odriozola JM, Olivares M, Xaus J, Fernandez L, Rodriguez JM (2008) Is meconium from healthy newborns actually sterile? *Res Microbiol* 159(3):187–193. doi:10.1016/j.resmic.2007.12.007
17. Bager P, Melbye M, Rostgaard K, Benn CS, Westergaard T (2003) Mode of delivery and risk of allergic rhinitis and asthma. *J Allergy Clin Immunol* 111(1):51–56

18. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO (2007) Development of the human infant intestinal microbiota. *PLoS Biol* 5(7):e177. doi:10.1371/journal.pbio.0050177
19. Jost T, Lacroix C, Braegger CP, Chassard C (2012) New insights in gut microbiota establishment in healthy breast fed neonates. *PLoS One* 7(8):e44595. doi:10.1371/journal.pone.0044595
20. Favier CF, de Vos WM, Akkermans AD (2003) Development of bacterial and bifidobacterial communities in feces of newborn babies. *Anaerobe* 9(5):219–229. doi:10.1016/j.anaerobe.2003.07.001
21. Roger LC, Costabile A, Holland DT, Hoyles L, McCartney AL (2010) Examination of faecal Bifidobacterium populations in breast- and formula-fed infants during the first 18 months of life. *Microbiology* 156(Pt 11):3329–3341. doi:10.1099/mic.0.043224-0
22. Cabrera-Rubio R, Collado MC, Laitinen K, Salminen S, Isolauri E, Mira A (2012) The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. *Am J Clin Nutr* 96(3):544–551. doi:10.3945/ajcn.112.037382
23. Chen YE, Tsao H (2013) The skin microbiome: current perspectives and future challenges. *J Am Acad Dermatol* 69(1):143–155. doi:10.1016/j.jaad.2013.01.016
24. Madan JC, Koestler DC, Stanton BA, Davidson L, Moulton LA, Housman ML, Moore JH, Guill MF, Morrison HG, Sogin ML, Hampton TH, Karagas MR, Palumbo PE, Foster JA, Hibberd PL, O'Toole GA (2012) Serial analysis of the gut and respiratory microbiome in cystic fibrosis in infancy: interaction between intestinal and respiratory tracts and impact of nutritional exposures. *mBio* 3(4). doi:10.1128/mBio.00251-12
25. Kalliomaki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E (2001) Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 107(1):129–134. doi:10.1067/mai.2001.111237
26. van Nimwegen FA, Penders J, Stobberingh EE, Postma DS, Koppelman GH, Kerkhof M, Reijmerink NE, Dompeling E, van den Brandt PA, Ferreira I, Mommers M, Thijs C (2011) Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. *J Allergy Clin Immunol* 128(5):948–955. doi:10.1016/j.jaci.2011.07.027, e941–943
27. Kummeling I, Stelma FF, Dagnelie PC, Snijders BE, Penders J, Huber M, van Ree R, van den Brandt PA, Thijs C (2007) Early life exposure to antibiotics and the subsequent development of eczema, wheeze, and allergic sensitization in the first 2 years of life: the KOALA Birth Cohort Study. *Pediatrics* 119(1):e225–e231. doi:10.1542/peds.2006-0896
28. Penders J, Thijs C, van den Brandt PA, Kummeling I, Snijders B, Stelma F, Adams H, van Ree R, Stobberingh EE (2007) Gut microbiota composition and development of atopic manifestations in infancy: the KOALA Birth Cohort Study. *Gut* 56(5):661–667. doi:10.1136/gut.2006.100164
29. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y, Taniguchi T, Takeda K, Hori S, Ivanov II, Umesaki Y, Itoh K, Honda K (2011) Induction of colonic regulatory T cells by indigenous Clostridium species. *Science* 331(6015):337–341. doi:10.1126/science.1198469
30. Bashir ME, Louie S, Shi HN, Nagler-Anderson C (2004) Toll-like receptor 4 signaling by intestinal microbes influences susceptibility to food allergy. *J Immunol* 172(11):6978–6987
31. Rodriguez B, Priault B, Bibiloni R, Nicolis I, Mercenier A, Butel MJ, Waligora-Dupriet AJ (2011) Germ-free status and altered caecal subdominant microbiota are associated with a high susceptibility to cow's milk allergy in mice. *FEMS Microbiol Ecol* 76(1):133–144. doi:10.1111/j.1574-6941.2010.01035.x
32. Hill DA, Siracusa MC, Abt MC, Kim BS, Kobuley D, Kubo M, Kambayashi T, Larosa DF, Renner ED, Orange JS, Bushman FD, Artis D (2012) Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. *Nat Med* 18(4):538–546. doi:10.1038/nm.2657
33. Rask C, Evertsson S, Telemo E, Wold AE (2005) A full flora, but not monocolonization by Escherichia coli or lactobacilli, supports tolerogenic processing of a fed antigen. *Scand J Immunol* 61(6): 529–535. doi:10.1111/j.1365-3083.2005.01598.x
34. Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y, Umetsu DT, Rudensky AY (2012) Extrathymically generated regulatory T cells control mucosal TH2 inflammation. *Nature* 482(7385): 395–399. doi:10.1038/nature10772
35. Round JL, Mazmanian SK (2010) Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci U S A* 107(27):12204–12209. doi:10.1073/pnas.0909122107
36. Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, Mazmanian SK (2011) The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* 332(6032):974–977. doi:10.1126/science.1206095
37. Esplugues E, Huber S, Gagliani N, Hauser AE, Town T, Wan YY, O'Connor W Jr, Rongvaux A, Van Rooijen N, Haberman AM, Iwakura Y, Kuchroo VK, Kolts JK, Bluestone JA, Herold KC, Flavell RA (2011) Control of TH17 cells occurs in the small intestine. *Nature* 475(7357):514–518. doi:10.1038/nature10228
38. Ivanov II, Frutos Rde L, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, Finlay BB, Littman DR (2008) Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* 4(4):337–349. doi:10.1016/j.chom.2008.09.009
39. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139(3): 485–498. doi:10.1016/j.cell.2009.09.033
40. Chen K, Xu W, Wilson M, He B, Miller NW, Bengten E, Edholm ES, Santini PA, Rath P, Chiu A, Cattalini M, Litzman J, Bussell JB, Huang B, Meini A, Riesbeck K, Cunningham-Rundles C, Plebani A, Cerutti A (2009) Immunoglobulin D enhances immune surveillance by activating antimicrobial, proinflammatory and B cell-stimulating programs in basophils. *Nat Immunol* 10(8):889–898. doi:10.1038/ni.1748
41. Ring J, Przybilla B, Ruzicka T (2006) Handbook of atopic eczema. Springer, Berlin
42. Adlerberth I, Strachan DP, Matricardi PM, Ahme S, Orfei L, Aberg N, Perkin MR, Tripodi S, Hesselmar B, Saalman R, Coates AR, Bonanno CL, Panetta V, Wold AE (2007) Gut microbiota and development of atopic eczema in 3 European birth cohorts. *J Allergy Clin Immunol* 120(2):343–350. doi:10.1016/j.jaci.2007.05.018
43. Bisgaard H, Li N, Bonnelykke K, Chawes BL, Skov T, Paludan-Muller G, Stokholm J, Smith B, Krogfelt KA (2011) Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age. *J Allergy Clin Immunol* 128(3):646–652. doi:10.1016/j.jaci.2011.04.060, e641–645
44. Wang M, Karlsson C, Olsson C, Adlerberth I, Wold AE, Strachan DP, Matricardi PM, Aberg N, Perkin MR, Tripodi S, Coates AR, Hesselmar B, Saalman R, Molin G, Ahme S (2008) Reduced diversity in the early fecal microbiota of infants with atopic eczema. *J Allergy Clin Immunol* 121(1):129–134. doi:10.1016/j.jaci.2007.09.011
45. Forno E, Onderdonk AB, McCracken J, Litonjua AA, Laskey D, Delaney ML, Dubois AM, Gold DR, Ryan LM, Weiss ST, Celedon JC (2008) Diversity of the gut microbiota and eczema in early life. *Clin Mol Allergy CMA* 6:11. doi:10.1186/1476-7961-6-11
46. Leyden JJ, Marples RR, Kligman AM (1974) Staphylococcus aureus in the lesions of atopic dermatitis. *Br J Dermatol* 90(5):525–530
47. Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, Nomicos E, Polley EC, Komarow HD, Murray PR, Turner ML, Segre JA (2012) Temporal shifts in the skin microbiome associated

- with disease flares and treatment in children with atopic dermatitis. *Genome Res* 22(5):850–859. doi:[10.1101/gr.131029.111](https://doi.org/10.1101/gr.131029.111)
48. Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, Plaut M, Cooper SF, Fenton MJ, Arshad SH, Bahna SL, Beck LA, Byrd-Bredbenner C, Camargo CA Jr, Eichenfield L, Furuta GT, Hanifin JM, Jones C, Kraft M, Levy BD, Lieberman P, Luccioli S, McCall KM, Schneider LC, Simon RA, Simons FE, Teach SJ, Yawn BP, Schwaninger JM (2010) Guidelines for the diagnosis and management of food allergy in the United States: Summary of the NIAID-sponsored expert panel report. *J Allergy Clin Immunol* 126(6):1105–1118. doi:[10.1016/j.jaci.2010.10.008](https://doi.org/10.1016/j.jaci.2010.10.008)
 49. Rodriguez B, Prioult G, Hacini-Rachinel F, Moine D, Bruttin A, Ngom-Bru C, Labellie C, Nicolis I, Berger B, Mercenier A, Butel MJ, Waligora-Dupriet AJ (2012) Infant gut microbiota is protective against cow's milk allergy in mice despite immature ileal T-cell response. *FEMS Microbiol Ecol* 79(1):192–202. doi:[10.1111/j.1574-6941.2011.01207.x](https://doi.org/10.1111/j.1574-6941.2011.01207.x)
 50. Noval Rivas M, Burton OT, Wise P, Zhang YQ, Hobson SA, Garcia Lloret M, Chehoud C, Kuczynski J, DeSantis T, Warrington J, Hyde ER, Petrosino JF, Gerber GK, Bry L, Oettgen HC, Mazmanian SK, Chatila TA (2013) A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. *J Allergy Clin Immunol* 131(1):201–212. doi:[10.1016/j.jaci.2012.10.026](https://doi.org/10.1016/j.jaci.2012.10.026)
 51. Ling Z, Li Z, Liu X, Cheng Y, Luo Y, Tong X, Yuan L, Wang Y, Sun J, Li L, Xiang C (2014) Altered fecal microbiota composition associated with food allergy in infants. *Appl Environ Microbiol* 80(8):2546–2554. doi:[10.1128/AEM.00003-14](https://doi.org/10.1128/AEM.00003-14)
 52. Huang YJ, Nelson CE, Brodie EL, Desantis TZ, Baek MS, Liu J, Woyke T, Allgaier M, Bristow J, Wiener-Kronish JP, Sutherland ER, King TS, Icitovic N, Martin RJ, Calhoun WJ, Castro M, Denlinger LC, Dimango E, Kraft M, Peters SP, Wasserman SI, Wechsler ME, Boushey HA, Lynch SV, National Heart, Lung, and Blood Institute's Asthma Clinical Research Network (2011) Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. *J Allergy Clin Immunol* 127(2):372–381. doi:[10.1016/j.jaci.2010.10.048](https://doi.org/10.1016/j.jaci.2010.10.048), e371–373
 53. Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, Davies J, Ervine A, Poulter L, Pachter L, Moffatt ME, Cookson WO (2010) Disordered microbial communities in asthmatic airways. *PLoS One* 5(1):e8578. doi:[10.1371/journal.pone.0008578](https://doi.org/10.1371/journal.pone.0008578)
 54. Herbst T, Sichelstiel A, Schar C, Yadava K, Burki K, Cahenzli J, McCoy K, Marsland BJ, Harris NL (2011) Dysregulation of allergic airway inflammation in the absence of microbial colonization. *Am J Respir Crit Care Med* 184(2):198–205. doi:[10.1164/rccm.201010-1574OC](https://doi.org/10.1164/rccm.201010-1574OC)
 55. Szajewska H (2013) Understanding the role of probiotics and prebiotics in preventing allergic disease: evidence and methodological issues. *Immunotherapy* 5(8):869–878. doi:[10.2217/imt.13.85](https://doi.org/10.2217/imt.13.85)
 56. Dongarra ML, Rizzello V, Muccio L, Fries W, Cascio A, Bonaccorsi I, Ferlazzo G (2013) Mucosal immunology and probiotics. *Curr Allergy Asthma Rep* 13(1):19–26. doi:[10.1007/s11882-012-0313-0](https://doi.org/10.1007/s11882-012-0313-0)
 57. Isolauri E, Rautava S, Salminen S (2012) Probiotics in the development and treatment of allergic disease. *Gastroenterol Clin N Am* 41(4):747–762. doi:[10.1016/j.gtc.2012.08.007](https://doi.org/10.1016/j.gtc.2012.08.007)
 58. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E (2001) Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 357(9262):1076–1079. doi:[10.1016/S0140-6736\(00\)04259-8](https://doi.org/10.1016/S0140-6736(00)04259-8)
 59. Kim HJ, Kim HY, Lee SY, Seo JH, Lee E, Hong SJ (2013) Clinical efficacy and mechanism of probiotics in allergic diseases. *Korean J Pediatr* 56(9):369–376. doi:[10.3345/kjp.2013.56.9.369](https://doi.org/10.3345/kjp.2013.56.9.369)
 60. Lee J, Seto D, Bielory L (2008) Meta-analysis of clinical trials of probiotics for prevention and treatment of pediatric atopic dermatitis. *J Allergy Clin Immunol* 121(1):116–121. doi:[10.1016/j.jaci.2007.10.043](https://doi.org/10.1016/j.jaci.2007.10.043), e111
 61. Betsi GI, Papadavid E, Falagas ME (2008) Probiotics for the treatment or prevention of atopic dermatitis: a review of the evidence from randomized controlled trials. *Am J Clin Dermatol* 9(2):93–103
 62. Pelucchi C, Chatenoud L, Turati F, Galeone C, Moja L, Bach JF, La Vecchia C (2012) Probiotics supplementation during pregnancy or infancy for the prevention of atopic dermatitis: a meta-analysis. *Epidemiology* 23(3):402–414. doi:[10.1097/EDE.0b013e31824d5da2](https://doi.org/10.1097/EDE.0b013e31824d5da2)
 63. Doege K, Grajecki D, Zyriax BC, Detinkina E, Zu Eulenburg C, Buhling KJ (2012) Impact of maternal supplementation with probiotics during pregnancy on atopic eczema in childhood—a meta-analysis. *Br J Nutr* 107(1):1–6. doi:[10.1017/S0007114511003400](https://doi.org/10.1017/S0007114511003400)
 64. Abrahamsson TR, Jakobsson T, Bottcher MF, Fredrikson M, Jenmalm MC, Bjorksten B, Oldaeus G (2007) Probiotics in prevention of IgE-associated eczema: a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 119(5):1174–1180. doi:[10.1016/j.jaci.2007.01.007](https://doi.org/10.1016/j.jaci.2007.01.007)
 65. Taylor AL, Dunstan JA, Prescott SL (2007) Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. *J Allergy Clin Immunol* 119(1):184–191. doi:[10.1016/j.jaci.2006.08.036](https://doi.org/10.1016/j.jaci.2006.08.036)
 66. Kuitunen M, Kukkonen K, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, Haahtela T, Savilahti E (2009) Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. *J Allergy Clin Immunol* 123(2):335–341. doi:[10.1016/j.jaci.2008.11.019](https://doi.org/10.1016/j.jaci.2008.11.019)
 67. Rose MA, Stieglitz F, Koksai A, Schubert R, Schulze J, Zielen S (2010) Efficacy of probiotic *Lactobacillus GG* on allergic sensitization and asthma in infants at risk. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* 40(9):1398–1405. doi:[10.1111/j.1365-2222.2010.03560.x](https://doi.org/10.1111/j.1365-2222.2010.03560.x)
 68. Hol J, van Leer EH, Elink Schuurman BE, de Ruiter LF, Samsom JN, Hop W, Neijens HJ, de Jongste JC, Nieuwenhuis EE (2008) The acquisition of tolerance toward cow's milk through probiotic supplementation: a randomized, controlled trial. *J Allergy Clin Immunol* 121(6):1448–1454. doi:[10.1016/j.jaci.2008.03.018](https://doi.org/10.1016/j.jaci.2008.03.018)
 69. Osborn DA, Sinn JK (2013) Prebiotics in infants for prevention of allergy. *Cochrane Database Syst Rev* 3, CD006474. doi:[10.1002/14651858.CD006474.pub3](https://doi.org/10.1002/14651858.CD006474.pub3)
 70. Roberfroid M (2007) Prebiotics: the concept revisited. *J Nutr* 137(3 Suppl 2):830S–837S
 71. Thomas DW, Greer FR (2010) Probiotics and prebiotics in pediatrics. *Pediatrics* 126(6):1217–1231. doi:[10.1542/peds.2010-2548](https://doi.org/10.1542/peds.2010-2548)
 72. Arslanoglu S, Moro GE, Schmitt J, Tandoi L, Rizzardi S, Boehm G (2008) Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. *J Nutr* 138(6):1091–1095
 73. Moro G, Arslanoglu S, Stahl B, Jelinek J, Wahn U, Boehm G (2006) A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch Dis Child* 91(10):814–819. doi:[10.1136/adc.2006.098251](https://doi.org/10.1136/adc.2006.098251)
 74. Ziegler E, Vanderhoof JA, Petschow B, Mitmesser SH, Stolz SI, Harris CL, Berseth CL (2007) Term infants fed formula supplemented with selected blends of prebiotics grow normally and have soft stools similar to those reported for breast-fed infants. *J Pediatr Gastroenterol Nutr* 44(3):359–364. doi:[10.1097/MPG.0b013e31802fca8c](https://doi.org/10.1097/MPG.0b013e31802fca8c)
 75. Gruber C, van Stuijvenberg M, Mosca F, Moro G, Chirico G, Braegger CP, Riedler J, Boehm G, Wahn U (2010) Reduced occurrence of early atopic dermatitis because of immunoactive prebiotics among low-atopy-risk infants. *J Allergy Clin Immunol* 126(4):791–797. doi:[10.1016/j.jaci.2010.07.022](https://doi.org/10.1016/j.jaci.2010.07.022)
 76. Niele N, van Zwol A, Westerbeek EA, Lafeber HN, van Elburg RM (2013) Effect of non-human neutral and acidic oligosaccharides on

- allergic and infectious diseases in preterm infants. *Eur J Pediatr* 172(3):317–323. doi:[10.1007/s00431-012-1886-2](https://doi.org/10.1007/s00431-012-1886-2)
77. Kukkonen K, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, Kuitunen M (2007) Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 119(1):192–198. doi:[10.1016/j.jaci.2006.09.009](https://doi.org/10.1016/j.jaci.2006.09.009)
 78. van der Aa LB, Heymans HS, van Aalderen WM, Sillevs Smitt JH, Knol J, Ben Amor K, Goossens DA, Sprickelman AB (2010) Effect of a new synbiotic mixture on atopic dermatitis in infants: a randomized-controlled trial. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* 40(5):795–804. doi:[10.1111/j.1365-2222.2010.03465.x](https://doi.org/10.1111/j.1365-2222.2010.03465.x)
 79. van der Aa LB, Lutter R, Heymans HS, Smids BS, Dekker T, van Aalderen WM, Sillevs Smitt JH, Knippels LM, Garssen J, Nauta AJ, Sprickelman AB (2012) No detectable beneficial systemic immunomodulatory effects of a specific synbiotic mixture in infants with atopic dermatitis. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* 42(4):531–539. doi:[10.1111/j.1365-2222.2011.03890.x](https://doi.org/10.1111/j.1365-2222.2011.03890.x)
 80. Adams CA (2010) The probiotic paradox: live and dead cells are biological response modifiers. *Nutr Res Rev* 23(1):37–46. doi:[10.1017/S0954422410000090](https://doi.org/10.1017/S0954422410000090)
 81. Matsuzaki T, Yamazaki R, Hashimoto S, Yokokura T (1998) The effect of oral feeding of *Lactobacillus casei* strain Shirota on immunoglobulin E production in mice. *J Dairy Sci* 81(1):48–53. doi:[10.3168/jds.S0022-0302\(98\)75549-3](https://doi.org/10.3168/jds.S0022-0302(98)75549-3)
 82. Segawa S, Nakakita Y, Takata Y, Wakita Y, Kaneko T, Kaneda H, Watari J, Yasui H (2008) Effect of oral administration of heat-killed *Lactobacillus brevis* SBC8803 on total and ovalbumin-specific immunoglobulin E production through the improvement of Th1/Th2 balance. *Int J Food Microbiol* 121(1):1–10. doi:[10.1016/j.ijfoodmicro.2007.10.004](https://doi.org/10.1016/j.ijfoodmicro.2007.10.004)
 83. Sashihara T, Sueki N, Ikegami S (2006) An analysis of the effectiveness of heat-killed lactic acid bacteria in alleviating allergic diseases. *J Dairy Sci* 89(8):2846–2855. doi:[10.3168/jds.S0022-0302\(06\)72557-7](https://doi.org/10.3168/jds.S0022-0302(06)72557-7)