



Early life microbial exposures and allergy risks: opportunities for prevention

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Abstract | Allergies, including asthma, food allergy and atopic dermatitis, are increasing in prevalence, particularly in westernized countries. Although a detailed mechanistic explanation for this increase is lacking, recent evidence indicates that, in addition to genetic predisposition, lifestyle changes owing to modernization have an important role. Such changes include increased rates of birth by caesarean delivery, increased early use of antibiotics, a westernized diet and the associated development of obesity, and changes in indoor and outdoor lifestyle and activity patterns. Most of these factors directly and indirectly impact the formation of a diverse microbiota, which includes bacterial, viral and fungal components; the microbiota has a leading role in shaping (early) immune responses. This default programme is markedly disturbed under the influence of environmental and lifestyle risk factors. Here, we review the most important allergy risk factors associated with changes in our exposure to the microbial world and the application of this knowledge to allergy prevention strategies.

Allergies, including atopic dermatitis (also known as eczema), allergic rhinoconjunctivitis, allergic asthma and food allergy, are among the most prevalent chronic inflammatory disorders worldwide. They clinically manifest themselves at the interface of the body and the environment and are due to an immune response to usually harmless environmental antigens, termed ‘allergens’. The progression from atopic dermatitis in early infancy to other allergic disorders later in childhood has been described as the ‘atopic march’. However, the transition from cutaneous to respiratory manifestations, together with the manifestation of food allergy, occurs to a variable degree and extent^{1–5}. The development of allergy is the result of complex gene–environment interactions with the implementation of epigenetic changes in any of the several susceptibility genes^{6–9}. The dramatic increase in allergic diseases over the past 50 years is strongly related to worldwide changes in lifestyle conditions, many of which affect our exposure to a diverse microbial environment. Early-life exposure to a biodiverse environment is crucial for proper development and education of the immune system. In this Review, we provide an overview of the allergy risk factors that are linked to changes in exposure to microorganisms and highlight opportunities for allergy prevention.

Building a healthy microbiota

Bacterial microbiota. Extensive bacterial communities are found in the gastrointestinal tract, skin, oral cavity, nasal passages and urogenital tract, with the bacterial

microbiota of the gut being the best studied. Bacterial colonization begins a few hours after birth and becomes established through the first month of life, largely dependent on the nutritional sources for the baby. Breastfed babies show a dominance of *Bifidobacterium*, *Lactobacillus* and *Veillonella* species, which shifts to a dominance of *Bacteroides* and Clostridiales species following the introduction of solid foods. Although there is a clear dominance of certain strains owing to nutritional sources, the microbiota during the first few years in life is characterized by a large degree of interindividual and intraindividual heterogeneity on the taxon level. Overall, this period of life is characterized by an increasing diversity and richness of the microbiota over time. However, the first few years of life are also a highly vulnerable period of microbiota development, with events such as infections, fevers and episodes of antibiotic treatment having an immediate direct or indirect impact on the microbiota, and some of these changes persist over time. Beyond a certain age (usually defined as 3–4 years), the microbiota reaches a stable composition that persists into adulthood.

It is now well established that the early-life microbiota has an important role in the development of tolerogenic immune functions, preventing undesirable inflammatory responses against self antigens and non-self antigens (for example, allergens). Prominent examples of tolerogenic organisms include *Bacteroides fragilis*, which produces polysaccharide A, which

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Short-chain fatty acids (SCFAs). Subgroup of fatty acids with only two to six carbon atoms. Important representatives are butyrate, acetate and propionate.

Dysbiosis

A condition in which there is a qualitative and quantitative imbalance of bacterial communities that constitute the microbiota, which could represent a predisposition factor for several diseases. Dysbiosis refers mostly to the intestinal microbiota, but it can also occur at other body sites and in other habitats.

induces regulatory T cell (T_{reg} cell) development via Toll-like receptor 2 (TLR2)^{10,11}; *Clostridium* clusters IV and XIVa, which induce T_{reg} cells via transforming growth factor- β (TGF β)^{12,13}; and other, unclassified commensals that induce colonic T_{reg} cells that express retinoic acid receptor-related orphan receptor- γ t (ROR γ t) or induce T_{reg} cells from naive CD4⁺ T cells via TLR- and MyD88-dependent pathways^{14–16}. Furthermore, nutrients also have an important role in promoting this microbiota–immune axis^{17–21}. A prominent example is the fermentation of starch by some members of the Firmicutes resulting in short-chain fatty acids (SCFAs) such as butyrate. Butyrate acts as a histone deacetylase inhibitor that favours the expression of forkhead box protein P3 (FOXP3) and, therefore, enhances the suppressive functions of induced T_{reg} cells. *Faecalibacterium*, *Lactobacillus* and *Veillonella* are genera of Firmicutes^{22–24}.

Many patients with allergy and atopy have an altered microbiota²⁵, as determined predominately by assessing the stool microbiota^{26–28}. Dysbiosis has been reported also in the skin microbiota in individuals with atopic dermatitis^{29,30}, in the microbiota of the upper and lower respiratory tracts in individuals with asthma³¹ and in the gastrointestinal tract in individuals with food allergies^{32,33}. Dysbiosis can be detected even before the onset of clinical signs and symptoms, and longitudinal studies suggest that there is a cause-and-effect relationship between disturbance of microbial colonization at mucosal and cutaneous surfaces and the development of chronic inflammatory responses.

Virome. The human virome comprises viruses that infect the host (eukaryotic virome), virus-derived genetic elements in host chromosomes that can change host gene expression, express proteins or even generate infectious virus (prophages, endogenous retroviruses and endogenous viral elements), and viruses that infect members of the bacterial microbiota (most importantly bacteriophages)³⁴. Moreover, all adult humans are chronically infected with viruses that may be common pathogens, rare pathogens or viruses of unknown pathogenicity³⁵.

Diverse bacteriophages and sporadically eukaryotic viruses have been identified in the infant gut³⁶. Several pathogenic eukaryotic viruses (such as HIV, cytomegalovirus, human papillomavirus, varicella zoster virus and rubella virus) may be transmitted to the offspring in utero or during vaginal birth. Furthermore, eukaryotic viruses (such as respiratory syncytial virus (RSV), Epstein–Barr virus and herpes simplex virus) have been detected in amniotic fluid without evident clinical implications, although the in utero virome has not been fully characterized. Environmental factors such as diet, living conditions, water quality, hygiene and socio-economic status possibly all influence the composition of the gut virome. Close contact can also influence virome sharing, as shown among twin infants.

Similarly to the gut bacterial microbiota, the gut virome is dynamic in infancy and stable in adult life. Bacteriophage diversity is high among infants and low among adults, while interindividual virome variation remains high throughout life³⁷. The early infant gut virome comprises a highly abundant and diverse

bacteriophage community. From birth to 2 years of age, the eukaryotic virome and the bacterial microbiota expand, but this is accompanied by a contraction and shift in the bacteriophage virome composition towards a *Microviridae*-dominated community³⁶. Enteric eukaryotic viruses (such as members of the *Picornaviridae*, *Adenoviridae* and *Reoviridae*) are sporadically identified among infants rather than persisting during early development³⁶.

Mycobiome. The human mycobiome is less diverse and of lower abundance than the bacterial microbiota. Infants are colonized by fungi shortly after birth through caregivers and the environment, primarily by members of the genera *Cladosporium*, *Cryptococcus*, *Saccharomyces*, *Candida* and *Malassezia*³⁸. The last three are also dominant in the stools of healthy adults. Fungal taxa in humans show lower diversity than in the environment, and associations with specific anatomical sites show selection and adaptation of fungi³⁹. For example, skin fungi differ depending on age and sex, and children have more fungi and of higher diversity than adults, who are dominated by *Malassezia*⁴⁰. Such changes may be associated with differences in sebum composition and sebaceous gland activation⁴¹. *Malassezia* dominates most skin sites and produces an aryl hydrocarbon receptor ligand, which supports epithelial cells and offers UV protection^{42–44}.

Factors affecting the microbiota

Allergy risk starts in utero and continues after birth, with important factors such as type of delivery and antibiotic use, which impact microbial colonization of the infant's intestinal and respiratory tracts. The effects of such factors may be cumulative and dose dependent and may influence immune homeostasis and the risk of asthma and allergy development long term. Indeed, the early-life microbiota are critical for proper immunological development and beneficial for overall health status^{42,44}.

Caesarean delivery. Over the past few decades, there has been a considerable increase in births by caesarean delivery in both developed and developing countries, raising the question of whether this has contributed to the increase in allergies. Although many studies on the impact of the delivery mode on a child's health have been published, the evidence is still being debated. An increased risk of the development of food allergy has been reported for children born by caesarean delivery compared with those born by vaginal delivery^{45,46}, with the strongest evidence for cow's milk allergy^{45–47}. A meta-analysis of 23 relevant studies revealed an approximately 20% increase in the subsequent risk of asthma among children born by caesarean delivery⁴⁸. This association between caesarean delivery and asthma was supported by some recent studies^{49–53} but not others. The association between caesarean delivery and atopic dermatitis is less clear⁵⁴.

There might be several underlying mechanisms for caesarean delivery as a risk factor for allergy. A likely mechanism is the direct impact on early phases of microbiota development in the neonate. However, as

many babies born by caesarean delivery receive perinatal antibiotic prophylaxis, this might also contribute to the risk. Furthermore, the absence of perinatal stress factors associated with a vaginal delivery (for example, early profound immune activation with neonatal leukocytosis and other changes) might also play a role.

The delivery mode shapes the early microbial pattern after birth⁵⁵ (FIG. 1). The vaginal microbiota in normal pregnant women shows a relatively high abundance of particularly four lactobacillus phylotypes (*Limosilactobacillus vaginalis*, *Lactobacillus jensenii*, *Lactobacillus crispatus* and *Lactobacillus gasseri*) and a lower abundance of 22 other phylotypes compared with that in non-pregnant women⁵⁶. This signature shows relatively high stability throughout pregnancy and particularly when oestrogen concentrations are high⁵⁷. Oestrogen favours maturation of the vaginal epithelium, and this results in an accumulation of glycogen, which is metabolized to lactic acid by lactobacilli⁵⁸, indicating an important functional relationship between the vaginal epithelium and resident microbiota. Vaginally delivered infants acquire bacterial communities (on skin, oral and nasopharyngeal surfaces) that strongly resemble their mother's vaginal microbiota (dominated by *Lactobacillus*, *Prevotella* and/or *Sneathia*), whereas infants born by caesarean delivery harbour bacterial communities that resemble more the mother's cutaneous surface, which is dominated by *Staphylococcus* and *Propionibacterium*⁵⁵. Over the next few months, the oral microbiota undergoes

marked reorganization: *Propionibacterium* or *Lactobacillus* largely composed the neonatal oral microbiota on delivery, whereas by 6 weeks of age, *Streptococcus* was dominant in most oral samples⁵⁹.

Another microbial risk factor for allergy and asthma is the colonization of children with *Clostridioides difficile* at 1 month of age, which is associated with wheezing (asthma) and atopic dermatitis later in life. *C. difficile* colonization was linked to caesarean delivery and the place of delivery (home vaginal birth versus hospital vaginal birth)⁶⁰.

In children whose mother and/or father has atopy and allergy, caesarean delivery is associated with the highest risk of development of various allergic phenotypes later in life^{45,61}. This observation is an important example for the relevance of gene–environment interaction contributing to the individual allergy risk profile. High levels of type 2 inflammation in atopic mothers have a strong impact on fetal immune programming. A disturbed neonatal programming may be further accelerated and aggravated by caesarean delivery-derived signals⁶². This was shown for atopic dermatitis in a Korean cohort in which development of the disease is impacted by the combination of a genetic risk factor (a certain maternal *IL13* polymorphism) and the environmental factor (the mode of delivery). Caesarean delivery has a strong impact on neonatal immune programming, as indicated by decreased pro-inflammatory cytokine responses following TLR1 or TLR2 stimulation and reduced tumour necrosis factor

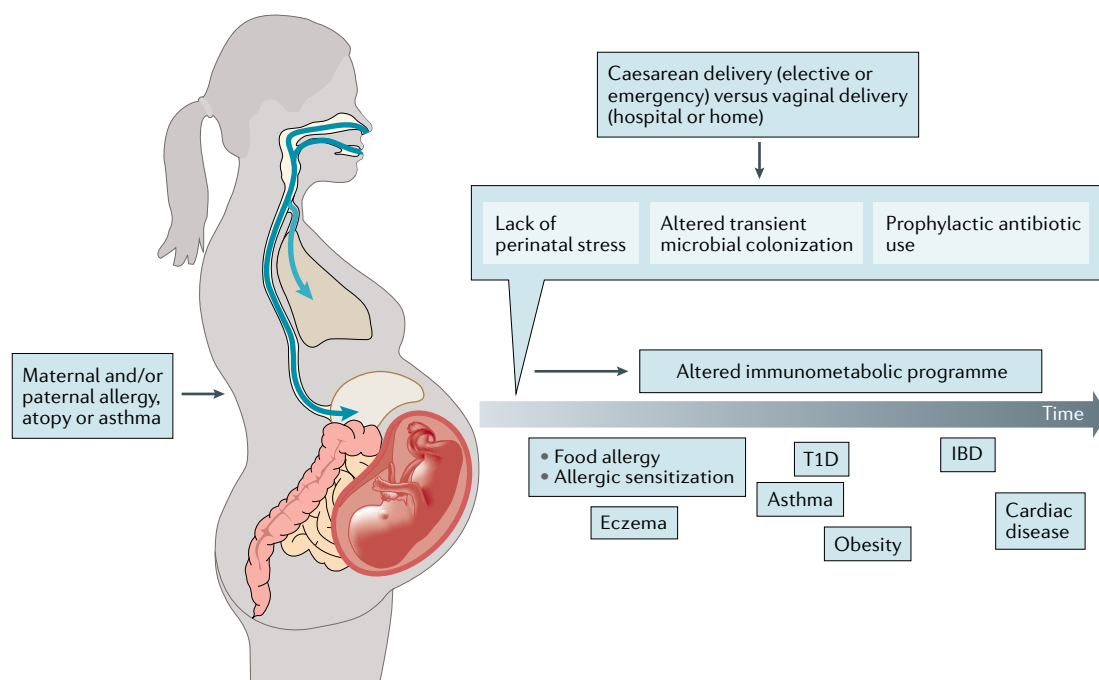


Fig. 1 | Caesarean delivery is an important risk factor for the development of chronic immune and metabolic disease. Birth by caesarean delivery as opposed to vaginal delivery has been identified as an important risk factor for the development of allergic and other inflammatory and metabolic diseases later in life. This has been attributed to altered transient microbial colonization in the infant gut. Other factors, such as the lack of perinatal stress and the use of antibiotic prophylaxis, may also contribute to this effect of caesarean delivery. Altered immunometabolic programming has been observed. Babies born by caesarean delivery show a different innate–adaptive immune profile and have a higher risk of chronic inflammatory and metabolic disease. A close association between caesarean delivery and disease outcomes later in life has been shown for food allergy, allergic sensitization, atopic dermatitis, asthma and obesity, as well as type 1 diabetes (T1D), inflammatory bowel disease (IBD) and cardiac disease.

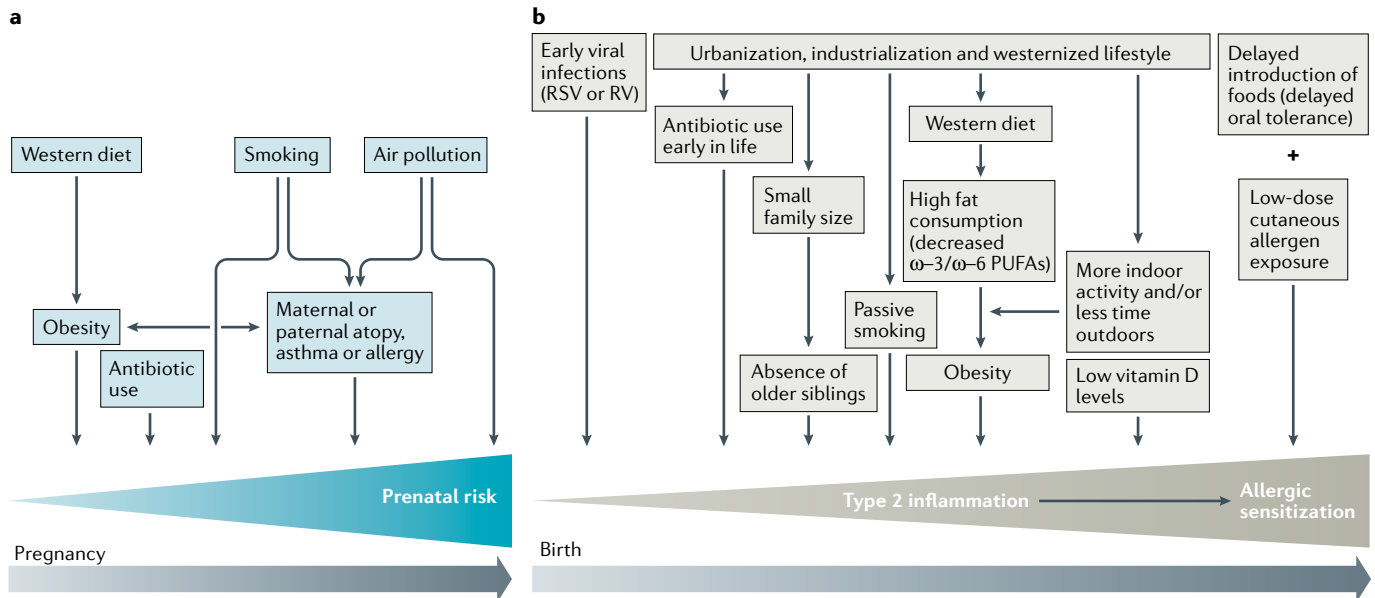


Fig. 2 | Prenatal and postnatal risk factors for the development of allergy and asthma. **a** | One of the most important prenatal risk factors is the presence of maternal and/or paternal atopy, asthma and allergy. Other lifestyle factors closely associated with a westernized lifestyle may contribute to this risk in a direct or an indirect manner. A bidirectional association exists between obesity and asthma. **b** | Viral infections in early life, particularly with respiratory syncytial virus (RSV) and rhinovirus (RV), are important risk factors for the development of asthma. Many other

risk factors share the common relationship and association with urbanization, industrialization and a westernized lifestyle. These include antibiotic use early in life, exposure to exhaled tobacco smoke, a Western diet with high fat consumption and obesity, a reduced pattern of indoor and outdoor activities and low vitamin D levels. Furthermore, delayed introduction of foods (delayed development of tolerance) together with a low-dose cutaneous allergen exposure triggers allergic sensitizations. PUFAs, polyunsaturated fatty acids.

(TNF) and IL-6 levels at birth, followed by a higher abundance of bacterial colonization in the nasopharyngeal space and association with childhood wheezing⁶³. These and other data serve as an example for the strong regulatory loop between the mode of delivery, microbial colonization and neonatal immune programming, which contribute synergistically to allergy risk.

A pilot study was recently performed in which infants born by caesarean delivery were exposed to maternal vaginal fluid collected 1 hour before delivery. Follow-up analysis of neonatal microbiota development showed that gut, oral and skin surfaces were enriched in vaginal bacteria that are otherwise under-represented in babies born by caesarean delivery (such as *Actinobacteria*, *Bacteroidetes* and *Bifidobacterium*)⁶⁴. Although the long-term effects on immune metabolic functions of newborns and on health outcomes later in life still need to be investigated, these data demonstrate that the early colonization pattern is susceptible to manipulation.

Antibiotic use. As antibiotic use has substantially increased both during pregnancy (FIG. 2a) and in the first few years of life (FIG. 2b), there is increasing interest in studying the potential relationship between antibiotic use in this critical period and the impact on health outcomes later in life^{65,66}. A positive association between antibiotic use and disease development has been reported for childhood obesity^{67,68}, type 2 diabetes⁶⁹, asthma^{24,70,71} and (childhood) inflammatory bowel disease^{72–74}. Antibiotic use in the first 6 months and the first 2 years of life was associated with the development of asthma at 3 years of age and 7.5 years of age, respectively. Similar results

were obtained for the development of infant cow's milk allergy⁷⁵. Such an association was also found for maternal antibiotic exposures before and during pregnancy⁷⁶. With an increasing number of antibiotic cycles, the risk of asthma development increased. The strongest relationship has been reported for broad-spectrum antibiotics (cephalosporins)^{76,77}, the particular use of which has increased over recent decades.

Antibiotic treatments influence microbial diversity and composition in a qualitative and quantitative manner^{78,79}. Subtherapeutic antibiotic treatment has been used to disrupt the microbiota in young mice. This results not only in altered microbiota composition but also in increased fat mass, altered metabolic hormones and hepatic metabolism⁷⁸. Low-dose penicillin given to mice from birth induces metabolic changes and changes in ileal expression of immunity-related genes and also has long-term effects on metabolism and enhances susceptibility to high-fat diet-induced obesity. Transfer of microbial communities recovered after antibiotic cessation into germ-free mice transferred the growth promotion phenotype^{65,80}.

However, it cannot be ruled out that associations between antibiotic use and asthma could be caused by various forms of bias such as consulting behaviour, misclassification bias, recall bias and confounding by indication^{81,82}. For example, a confounding factor could be the increased number of respiratory infections observed in children at higher risk of developing asthma or with established asthma. Antibiotics not only reduce the abundance of important protolerogenic gut bacteria, including *Lactobacillus* and segmented filamentous

Human milk oligosaccharides

(HMOs). Complex sugar molecules that are present in human breast milk in relatively high concentrations; their composition shows high interindividual and intraindividual variability.

Group 2 innate lymphoid cells

(ILC2s). Derived from lymphoid progenitors, these cells lack B and T cell receptors and produce type 2 cytokines such as IL-4, IL-5 and IL-9

Inflammasome

Cytosolic multiprotein oligomer of the innate immune system responsible for the activation of the inflammatory responses.

Asthma endotypes

Forms of asthma with distinct mechanistic pathways that have therapeutic and prognostic implications.

Neutrophil extracellular traps

Networks of extracellular fibres, produced by neutrophils, which bind to pathogens and thus allow neutrophils to kill them with minimal host damage. The extracellular fibril matrix is composed of decondensed chromatin.

Plasmacytoid dendritic cell

Derived from bone marrow haematopoietic stem cells, this type of immune cell circulates in the blood and in peripheral lymphoid organs and is known to secrete large quantities of type I interferon following viral infection.

bacteria as well as certain clostridia^{65,83,84}, they also increase the abundance of gastrointestinal fungi⁸⁵ and impair antiviral immunity in a direct or an indirect manner⁸⁶, which is also confirmed in humans⁸⁷.

Breastfeeding. Longer duration of breastfeeding is associated with a reduced risk of childhood asthma, atopic dermatitis and rhinitis⁸⁸. Exclusively breastfed infants have greater microbial diversity of their intestinal microbiota, which influences immune system maturation and may protect from allergies, although the results are mixed⁸⁹. In a recent large multicentre study, breastfeeding was the most significant factor associated with early-life microbiota composition and diversity^{90,91}. Breastfeeding shapes the early-life gut microbiota directly by transfer of the human milk microbiota (which includes lactobacilli and bifidobacteria) and indirectly by exposure to factors such as human milk oligosaccharides (HMOs), secretory IgA, glycomacropptides, tryptophan metabolites, lipids, components of the innate immune system (such as soluble TLR2, soluble TLR4 and their co-receptors) and antimicrobial proteins and peptides, which collectively influence and direct microbial growth and metabolism. The microbiome of newborns and young infants is enriched in microbial genes that are necessary for the degradation of HMOs⁹².

HMOs are complex short-chain sugars, and more than 200 different oligosaccharides have been identified to date⁹³, the composition and diversity of which differ among mothers. This interindividual heterogeneity has been attributed to the differential effect of breast milk from various mothers in terms of maturation of the intestinal mucosal immune system⁹⁴. HMOs also affect epithelial barrier functions by acting as soluble decoy receptors that block the attachment of bacteria and other microorganisms to epithelial cell surface sugars. It has been proposed that this helps to prevent infectious diseases in the gut and also in the respiratory and urinary tracts⁹⁴. Low concentrations of the HMO lacto-*N*-fucopentose III are associated with increased likelihood of infants developing cow's milk allergy⁹⁵.

Breastfeeding is also responsible for the transmission of eukaryotic viruses (such as members of the families *Herpesviridae*, *Poxviridae*, *Mimiviridae* and *Iridoviridae*) as well as bacteriophages (such as members of the families *Myoviridae*, *Siphoviridae* and *Podoviridae*) between mother and child. Bacteriophages represent the majority (95%) of the viruses in human milk⁹⁶. Eukaryotic viruses in human milk may have a direct effect on the infant's health, whereas bacteriophages contribute to shaping the infant gut microbiota. Furthermore, regurgitation by infants following breastfeeding may also seed the upper respiratory tract with microorganisms. Nevertheless, available virome data are mostly cross sectional including small numbers of individuals and without considering mother-child pairings.

Rekima et al.⁹⁷ demonstrated that mice nursed by mothers exposed to house dust mite Der p allergen (versus exposure to phosphate-buffered saline) show increased permeability, IL-33 levels, activation of group 2 innate lymphoid cells (ILC2s) and differentiation of T helper 2 (T_H2) cells in the gut mucosa. This resulted

in inhibition of the induction of antigen-specific T_{reg} cells and the prevention of food allergy by ovalbumin exposure through breast milk. This work highlighted the importance of the presence of a respiratory allergen in breast milk as a risk factor for food allergy due to interference with oral induction. Validation in a human setting would have implications for manipulation of the levels of Der p (and/or its protease activity) as a means of food allergy protection.

Viral infection. Viral infections have also been closely linked to the development of asthma. Lower respiratory tract infections, particularly with RSV or rhinovirus, in early life may induce the development of wheezing and asthma later in life^{35,98}. Allergen sensitization seems to precede rhinovirus-induced wheezing⁹⁹. Recent studies suggest that the host response to febrile infections and associated activation of the inflammasome may be critical for asthma promotion¹⁰⁰. Indeed, certain asthma endotypes are associated with neutrophilic airway inflammation based on the axis of inflammasome activation, IL-1 β and IL-18 production, T helper 17 (T_H17) cell activation, and IL-8 and IL-6 overproduction. Eighty per cent of the cells that infiltrate the airways of children with RSV-associated bronchiolitis are neutrophils. During early lung development, neutrophils may induce injury to the airways with long-term consequences for pulmonary architecture and may predispose to the development of asthma. Indeed, an excessive neutrophil response leads to mast cell recruitment via IL-9. Lung-resident mast cells, in turn, induce airway hyper-reactivity, thus predisposing to asthma. In addition, excessive neutrophils may lead to lung injury via the release of proteases, reactive oxygen species and neutrophil extracellular traps, ultimately causing chronic pulmonary impairment¹⁰¹, a process that can be triggered by experimental rhinovirus infection. This concept opens up new clinical implications that require further study.

Allergens and viral respiratory tract infections may interact in several ways, including via defective epithelial barrier function. Viral respiratory tract infections are associated with disrupted tight junctions, impaired innate immunity and suppressed antioxidant properties, which may lead to exaggerated sensitivity to infections and allergens¹⁰². Furthermore, early RSV exposure in mice suppressed T_{reg} cell development and enhanced susceptibility to allergy¹⁰³. Particular attention has been paid to the fact that cells or tissues from individuals with asthma respond suboptimally to viral infections in terms of interferon production. Deficient interferon responses in asthma have been attributed to either single or multiple gene defects, microRNAs or epigenetic determinants^{104,105}. Furthermore, T_H2 cell responses, such as IgE crosslinking and type 2 cytokine production, suppress the production of interferons by bronchial epithelial cells, and in turn plasmacytoid dendritic cell (pDC)-derived type I and type III interferons suppress type 2 pathways in T_H2 cells and ILC2s¹⁰⁴ (FIG. 3). If this holds true, current anti-T_H2 biologics (antibodies to IgE, IL-5 and IL-13, IL-5 receptor antagonist, IL-4 receptor antagonist and small molecules such as chemoattractant

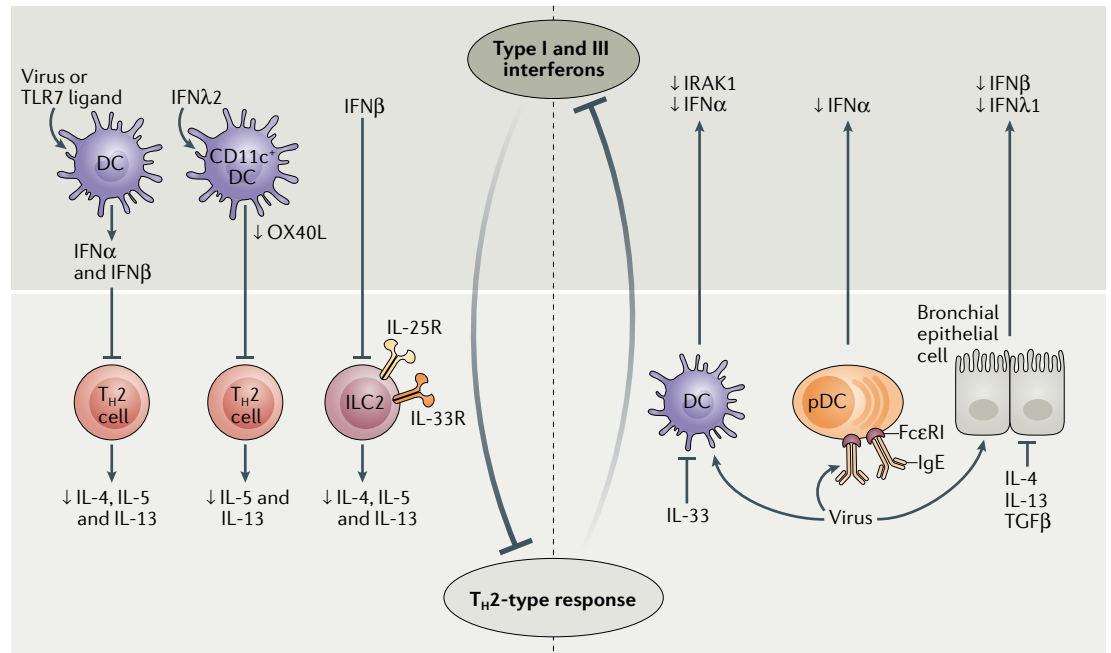


Fig. 3 | Reciprocal negative regulation of type I and III interferons and T_H2 -type responses in patients with asthma. Type I and III interferons suppress T_H2 -type responses. In dendritic cells (DCs), viruses or Toll-like receptor 7 (TLR7) agonists induce the production of type I interferons, which suppress the release of IL-4, IL-5 and IL-13 from T_H2 cells. Treatment of group 2 innate lymphoid cells (ILC2s), which are normally activated by the pro- T_H2 cytokines IL-25 and IL-33, with interferon- β (IFN β) leads to reduced production of IL-4, IL-5 and IL-13. The type III interferon IFN λ 2 (also known as IL-28A) indirectly reduces the expression of OX40 ligand (OX40L) and the release of IL-5 and IL-13 from T_H2 cells by modulating the effect of CD11c $^+$ DCs on these cells. Conversely, T_H2 cytokines can suppress the innate interferon response on viral infection. Crosslinking of IgE bound on plasmacytoid DCs (pDCs) by viral particles reduces the release of IFN α . Similarly, IL-33 prevents DCs from expressing IL-1 receptor-associated kinase 1 (IRAK1) and IFN α during viral infection. In this case, T_H2 cytokines also lead to lower production of IFN β and IFN λ 1 (also known as IL-29) by bronchial epithelial cells. IL-25R, IL-25 receptor; IL-33R, IL-33 receptor; TGF β , transforming growth factor- β .

receptor-homologous molecule expressed on T_H2 cells (CRTH2) and DNazymes) would not only attenuate T_H2 -type inflammation but would also restore antiviral responses. Indeed, omalizumab (anti-IgE) treatment of inner city children with asthma resulted in augmentation of pDC-derived type I interferon responses and attenuation of pDC Fc ϵ R1 α expression following ex vivo stimulation with rhinovirus or influenza virus in the presence of IgE crosslinking¹⁰⁶. These findings were associated with a lower asthma exacerbation rate during the outcome period.

Allergic mechanisms

Linked to altered microbiota. There is now accumulating evidence that dysbiosis precedes the development of allergic manifestations. Studies of birth cohorts revealed the absence of certain bacterial genera from the infants' gut microbiota between 1 and 3 months of age, and this was associated with increased risk of atopy, recurrent wheeze or asthma development later in life^{24,107}. Such alterations were associated with reduction of the levels of or absence of anti-inflammatory polyunsaturated fatty acids¹⁰⁷. Furthermore, sterilized faecal water from high-risk neonates promoted the expansion of IL-4-producing CD4 $^+$ T cells at the expense of FOXP3 $^+$ T_{reg} cells ex vivo¹⁰⁸. On the basis of recent findings, dysbiosis may start even earlier, as

meconium of at-risk babies exhibits delayed gut microbial diversification and an altered microbiota-derived metabolome, characterized mainly by depletion of anti-inflammatory faecal lipids¹⁰⁹. This dysbiosis is strongly associated with parental disease, indicating that maternal health status during pregnancy may influence vertical transmission of microorganisms affecting microbiota development early in life. This is not restricted to asthma alone, as changes in the composition of the intestinal microbiota can also increase susceptibility or resistance to food allergy through a microbially responsive ROR γ $^+$ FOXP3 $^+$ T_{reg} cell subset, which is known to be crucial for the maintenance of tolerance to food¹¹⁰.

Experimental evidence for a link between allergy development and the microbiota comes from mouse models and particularly from experiments with germ-free mice. Germ-free mice are reprogrammed with regard to their adaptive immune response profile. In particular, they show a default bias towards T_H2 cell development^{13,111}. Germ-free mice can be reconstituted with defined microbial strains; and it has been shown that Clostridia species and other species, which have been associated with allergy-protective effects, trigger the development of allergy protection through the induction of T_{reg} cells, IgA production and other immunological effects^{84,112,113}.

Recently, it was shown that germ-free mice colonized with commensals from healthy infants, but not those colonized with commensals from infants allergic to cow's milk, were protected against anaphylactic responses to cow's milk allergens. Further delineation identified the Clostridia member *Anaerostipes caccae* as protecting against allergic responses to food in this model¹¹⁴. In another model of food allergy, it was shown that colonization with a Clostridiales consortium, or with a Bacteroidales consortium consisting of seven species, suppressed food allergy in the mouse model¹¹⁵. Further functional experiments identified activation of the MyD88–ROR γ t pathway by commensals leading to the development of T_{reg} cells¹¹⁵. These important experiments close the gap towards understanding the functional relevance of dysbiosis observed in patients with food allergy. However, more experiments are needed to firmly establish the cause-and-effect relationship between the microbiota and other clinical phenotypes of allergy and asthma.

The diet, microbiota and subsequent metabolic and immunological functions are intimately connected. One important example is the role of microbially derived SCFAs in immune function¹¹⁶. It was recently shown that children raised in an 'asthma-protective' environment, known as the PASTURE cohort, had a diet early in life that resulted in high butyrate levels in the faeces. These children were followed up for 6 years and were shown to have significantly reduced atopic sensitization, asthma and food allergy compared with controls¹¹⁷. Butyrate's protective role has been observed in children with allergies¹¹⁸, and an enrichment of butyrate-producing taxa (Clostridia and Firmicutes) has been reported in children with faster resolution of cow's milk allergy¹¹⁹. SCFAs have been shown to decrease IL-12 and IL-6 secretion by DCs and promote the development of T_{reg} cells. Butyrate may also influence ILC2 proliferation and function via inhibition of histone deacetylases, and this pathway has been suggested as a potential therapeutic target for asthma¹²⁰. Indeed, in vivo reconstitution of germ-free mice with specific butyrate-producing Clostridia species attenuated ILC2-dependent airway hyper-responsiveness¹²¹. Important molecular mechanisms include an effect on the epigenetic cell programme at the level of both DNA methylation and histone acetylation. These effects are mediated through G protein-coupled receptor 43, which has been identified as an important SCFA receptor^{12,122,123}.

Linked to environmental microorganisms. With regard to allergy and asthma protection, there are several unique living conditions that have been used to investigate the cause-and-effect relationship between microbial biodiversity and immune programming at cellular and molecular levels. These living conditions include traditional agriculture and farming methods (for example, in the central European alpine region), anthroposophic lifestyle (associated with consumption of unprocessed foods, low antibiotic use and high outdoor activity levels), the presence of pets, particularly dogs and cats, in the living environment, birth order and the early use of day care facilities.

An inverse relationship between microbial exposure and the probability of asthma development has been elegantly demonstrated in the central European traditional farming environment¹²⁴. This study showed the importance of microbial diversity in promoting this beneficial effect, and further investigations have identified the bacterial species involved. With use of either conventional mouse models of experimental asthma and allergic sensitization, or cell culture experiments, it was shown that allergy-protective bacteria promote the development of immune responses that counteract T_H2-type immunity and inflammation through various means. Among them are *Staphylococcus sciuri*¹²⁵, *Acinetobacter lwoffii*¹²⁶ and *Lactococcus lactis*^{127,128}. Some of these strains induce DC activation with or without the production of IL-12, others have a broader anti-inflammatory activity with regard to the production of not only T_H2-type inflammation but also T_H1-type and T_H17-type inflammation, and others are more selective in terms of preventing experimental asthma. To exert these allergy- and asthma-protective effects, recognition by and signalling through pattern recognition receptors are required¹²⁹ (FIG. 4).

The Gram-negative bacterium *A. lwoffii* can serve as a model microorganism to study allergy and asthma protection and was the subject of a study conducted in Karelian populations in Finland and Russia¹³⁰. The Karelian population is unique as it has a genetically homogeneous population living in two different countries. The Russian Karelian population lives in traditional rural conditions, whereas the Finnish Karelian population lives in westernized rural conditions and shows increased susceptibility to allergic diseases and less abundance and diversity of *Acinetobacter* genus. This bacterium may also be associated with the skin microbiota, environmental exposome and development of atopy or atopic dermatitis¹³⁰. Mimicking the repeated and continuous microbial exposure of the traditional farming environment through repeated intranasal administration of *A. lwoffii* to pregnant mice protected against the development of experimental asthma in the progeny, thus having an impact across generations¹²⁶. Specifically, *A. lwoffii* administration induced lung and serum pro-inflammatory cytokines and lung TLR mRNA expression in the mother. Surprisingly, however, TLR expression in placental tissue was broadly suppressed. Among the many pro-inflammatory cytokines, only IL-6 remained at an elevated level over long periods, although at a relatively low level. In contrast to other pro-inflammatory cytokines, there is no adaptation to the IL-6 responses even after more than ten consecutive applications of *A. lwoffii*. This contrasts with what is observed during lipopolysaccharide tolerance, which occurs after chronic and continuous lipopolysaccharide stimulation, resulting in reduced pro-inflammatory cytokine production, particularly by innate immune cells. The molecular basis for these selected changes involves epigenetic modifications and chromatin remodelling resulting in altered gene transcription¹³¹. *A. lwoffii* has a highly specific effect on the epigenetic programme of CD4⁺ T cells (but not CD8⁺ T cells or natural killer T cells), modifying the *Ifng* promoter to increase the transcription and translation of interferon- γ ¹³² (A. Alhamwe, unpublished observations).

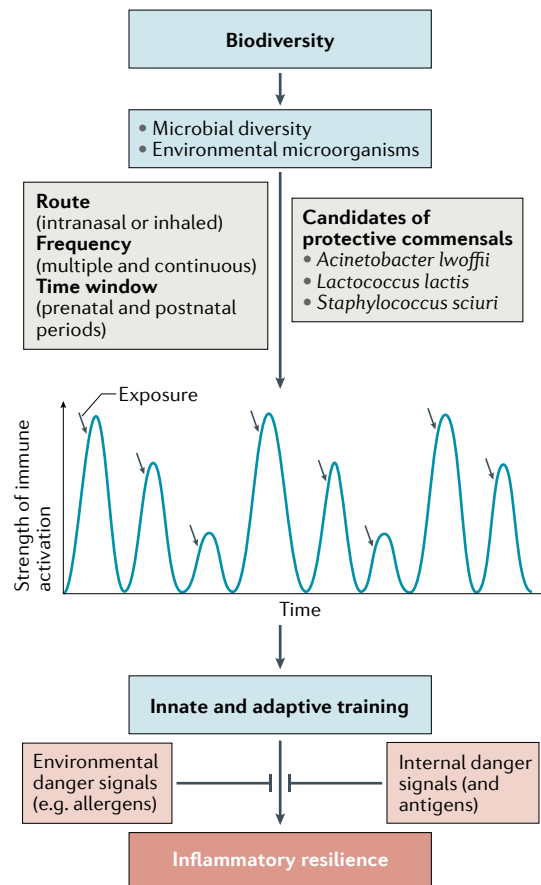


Fig. 4 | **The concept of inflammatory resilience.**

Biodiversity is closely linked to microbial diversity and exposure to environmental microorganisms. It is now well demonstrated that repeated, continuous exposure to allergy-protective microorganisms, particularly during the critical time window of prenatal and postnatal periods, results in a form of innate and adaptive immune memory or immune training. This immune training enables the individual to resist danger signals from the environment, and microbial communities mount an appropriate tolerogenic immune response. This is the hypothesis of inflammatory resilience. Conversely, a lack of biodiversity results in weak innate and adaptive immune training and, following exposure to danger signals, inappropriate overzealous (chronic) inflammatory responses and/or diseases develop.

Many of these allergy-protective microorganisms are constituents of a biodiverse environment. The biodiversity hypothesis states that contact with the natural environment enriches the human microbiota, promotes immune balance and protects from pathological chronic inflammation, including allergy and autoimmunity¹³³. We propose that this inflammatory resilience is due to the repeated nature of these activation signals derived from microbial encounter (FIG. 4). As indicated by many observational longitudinal and cross-sectional studies, a particularly vulnerable window of opportunity occurs early in life when immunological tolerance is developing. This window of opportunity overlaps with the window of microbiota development and extends from early in utero developmental periods to early childhood. As an

important consequence, if an immune system that has developed an appropriate level of resilience encounters environmental allergens, food antigens or autoantigens, the system is preprogrammed to prevent the development of pathological chronic inflammatory disease. Conversely, in the absence of such repeated microbial exposure originating from the biodiverse exposome, inflammatory resilience does not occur, and in this situation allergens or autoantigens trigger an unwanted immune response, leading to chronic inflammation, allergies, asthma and/or autoimmunity.

The immune response can show either a relatively rapid adaptation to environmental microorganisms or a lack of adaptation for others. This adaptation is characterized by a low-grade inflammatory response originating at the local mucosal site of entry (for example, the airways) and spreading systemically, thus serving as an additional strategy to promote resilience against unwanted pathological inflammatory responses. This concept of inflammatory resilience goes hand in hand with building a tolerogenic immune system.

Another relevant example supporting the role of bacterial species in allergy is the ancient bacterium *Helicobacter pylori*, the presence of which in the gastrointestinal tract has been substantially reduced over the past few decades. Experimental data show that trans-maternal exposure to *H. pylori* reduces allergic airway inflammation in F1 and F2 offspring. This protection against allergy required T_{reg} cells and was associated with robust shifts in the gastrointestinal microbiota¹³⁴. This is in contrast to older data showing that *H. pylori* increases epithelial permeability to food antigens in human gastric biopsy samples¹³⁵.

Linked to the virome. Through long-term infections, viruses may contribute to the host phenotype, and the presence of some viruses may be beneficial through immunomodulation. Virus-mediated immunomodulation impacts antiviral defence and susceptibility to various disorders, including allergies and secondary infections, which may in turn impact allergy development (see the section entitled Viral infection). On the one hand, the presence of a virus may enhance the magnitude of an immune response or decrease the threshold for immune activation. On the other hand, a virus may suppress an immune response or diminish its magnitude, or may alter the nature of a response to a subsequent antigenic exposure, favouring a more potent T_H1 -type¹³⁶ or T_H2 -type¹³⁷ response and/or with sustained type I interferon levels¹³⁸. In this context, impaired type I interferon responses to rhinoviruses have been associated with asthma exacerbation¹³⁹, whereas intranasal infection of mice with gammaherpesvirus protected them from allergic asthma by alteration of alveolar macrophage subsets¹⁴⁰, favouring the infiltration of lungs with regulatory monocytes. These monocyte-derived macrophages inhibited the DC-mediated T_H2 -type response to house dust mite antigen and therefore allergic asthma. Furthermore, viral immunomodulation is spatially and temporally dynamic and impacts T cell and B cell receptor repertoires.

The anelloviruses are representative members of the virome and have been linked to eosinophilic activation

among individuals with asthma. Anelloviruses represent about 70% of the total viruses described in blood and in most tissues and organs¹⁴¹. The anellovirus torque teno virus (TTV) may modulate host defence in several ways: first, TTV stimulates immune defences through CpG-mediated TLR9 activation and induction of pro-inflammatory mediators (such as exhaled nitric oxide and secreted eosinophil cationic protein); second, TTV inhibits host immune responses by interference with interferon signalling and T cell depletion³⁵. High TTV loads correlate with a decrease in circulating CD4⁺ T cells, an increase in B cells and increased activity of eosinophils¹⁴². Furthermore, a positive association between nasal TTV loads and high levels of eosinophilic cationic protein among children with asthma with moderately to severely compromised spirometric indices has been reported¹⁴². This suggests that TTV (alone or synergistically with other viruses) plays a role in lung impairment, perhaps mediated through induction of inflammation systemically or locally¹⁴³. Enhanced inflammation may, in turn, be caused by immune complex formation on TTV replication in blood or following allergen exposure in sensitized individuals¹⁴⁴.

Linked to altered mycobiome. Immune responses to fungi involve innate immune pathways as well as cellular immunity, which regulates tolerance and immunosurveillance¹⁴⁵. Overzealous immune responses may lead to fungal allergy, possibly due to perturbations of immunoregulatory mechanisms. Changes in the gut mycobiome have been associated with allergic airway disease^{85,146,147}. For example, the presence of *Candida albicans* in the gut affects T_H17-type pathways, which in turn may influence *Aspergillus*-associated pathology in chronic airway disease¹⁴⁷.

In the skin, higher loads of *Malassezia* species or an impaired immune response against them have been related to atopic dermatitis⁴⁴. *Malassezia sympodialis*, *Malassezia slooffiae* and *Malassezia dermatis* have been consistently associated with atopic dermatitis and its exacerbations, although non-*Malassezia* fungi have also been described in this context. Patients with this disorder produce IgE to *Malassezia* species and *Malassezia*-derived vesicles have been associated with a well-defined consistent set of RNA sequences, which were not influenced by elevated pH levels, a hallmark of individuals with atopic dermatitis¹⁴⁸. Moreover, *Malassezia* may induce cutaneous T_H17 cell-induced inflammation with enhanced *Malassezia*-specific CCR6⁺ memory T_H17 cells among patients with atopic dermatitis¹⁴⁹.

There are also differences between the healthy lung mycobiome and that observed in chronic inflammatory respiratory diseases such as asthma and chronic obstructive pulmonary disease¹⁵⁰. In these disorders, lungs exhibit lower fungal diversity and poor lung function potentially owing to fungal overgrowth or loss of diversity¹⁵¹. In asthma particularly, higher loads of certain fungi (for example, *Psathyrella*, *Malassezia*, *Termitomyces* and *Grifola*) have been described^{152,153}. Children with severe asthma have a higher abundance

of *Rhodospiridium*, *Pneumocystis*, *Leucosporidium* and *Rhodotorula* than children without asthma¹⁵⁴. Bronchiectasis is associated with an unfavourable allergic sensitization and immune response to *Aspergillus*¹⁵⁵. In both asthma and cystic fibrosis, an allergic response to *Aspergillus* may be induced such as in the case of allergic bronchopulmonary aspergillosis¹⁵⁶.

Microbial manipulation for allergy prevention

A detailed understanding of early-life events that impact allergy risk is particularly important for establishing primary prevention strategies (that is, blocking allergen sensitization) and secondary prevention strategies (that is, blocking of development of clinical symptoms following sensitization)¹⁵⁷.

As described, there is substantial clinical and experimental evidence that the microbiota plays a pivotal role in training early-life immune responses towards tolerance. However, it remains unclear whether the microbiota can be changed permanently following bacterial therapy. Currently, a few small phase I clinical trials for the treatment of peanut allergy are under way to explore the possibility of fetal microbial transfer, as recently summarized by others¹¹⁰. Results from these and other studies will provide further insight into whether manipulation of the microbiota will be a realistic option for the prevention and/or treatment of (food) allergies.

Enrichment of the environment for key bacteria, parenting practices, including cleaning the pacifier in the mother's mouth¹⁵⁸, and consumption of fermented foods¹⁵⁹, as well as washing dishes by hand instead of using a dishwasher¹⁶⁰, have been discussed as alternative approaches to prevent allergy. However, positive effects need to be confirmed through additional randomized controlled studies in infants and children. Furthermore, community-level interventions aimed at increasing green space, enhancing opportunities for young mothers and children to work in soil that is rich in microorganisms and bringing young people from urban to rural areas to experience farming are being explored as allergy prevention measures¹⁶¹. However, one must be cautious in translating findings from adult studies to infants.

Use of probiotics. Probiotics may provide directly or indirectly (for example, via correction of the intestinal microbiota) signals that allow normal immune maturation with the development of clinical and immunological tolerance. Although this is an attractive concept, the clinical evidence to support this is limited. With regard to food allergy, no positive outcome from the use of probiotics was found, even in high-risk families^{162–165} or in unselected or mixed-risk populations^{166–169}. Similarly, negative results have been obtained for asthma. By contrast, randomized trials investigating the effects of probiotic administration to pregnant women, breastfeeding mothers or infants provide evidence that probiotics could reduce the risk of atopic dermatitis in infants¹⁷⁰. These results are also reflected in international guidelines^{171–173}. The reasons for the mixed results may be the following: first, different microorganisms were used in different studies; second, it appears that different microbial strains may have different effects; and third,

timing and dosing also play an important role together with the administration schedule.

Numerous in vitro and animal in vivo studies have shown immunomodulatory effects of some probiotic strains. Among the most widely used probiotic strains are Lactobacillaceae and Bifidobacteriaceae strains. For *Lactocaseibacillus rhamnosus* GG (LGG), *Lactocaseibacillus casei*, *Limosilactobacillus reuteri*, *Lactocaseibacillus paracasei*, *Lactiplantibacillus plantarum* and *Lactobacillus acidophilus*, various degrees of increased numbers and/or functions of FOXP3⁺ T_{reg} cells with the production of IL-10 with or without TGFβ production have been observed. Some strains promote DC maturation and priming of monocyte-derived DCs^{174–180}. In mouse models of experimental asthma, LGG administration suppressed airway hyper-responsiveness and decreased T_H2-type cytokine production¹⁸¹. In children with asthma, LGG administration was reported to reduce the concentration of exhaled nitric oxide, but these results could not be replicated by others^{164,182}.

For *Bifidobacterium animalis* subsp. *lactis*, *Bifidobacterium bifidum*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium breve* and other *Bifidobacterium* species, similar results have been obtained. Induction of FOXP3⁺ T_{reg} cells, induction of IL-10 production by macrophages and DCs, and suppressive effects on TNF levels and IL-6 production have been reported^{183–185}. Further mechanisms of probiotic-mediated immunomodulation include stabilization of gut mucosal tight junctions, increased mucus secretion, enhanced gut motility and the production of amino acid by-products, including arginine and glutamine, and SCFAs that could also be responsible for some of the protolerogenic effects^{186–188}.

Use of prebiotics. Prebiotics are defined as sugars that are non-digestible for the host but serve as nutrients for selected microorganisms, which in turn have a health beneficial effect for the host. Examples include short-chain galacto-oligosaccharides, long-chain fructo-oligosaccharides and pectin. In this regard, HMOs may also serve as prebiotics for the infant's microbiota. In animal models¹⁸⁹ and also in human studies¹⁹⁰, positive effects of prebiotics on mucosal immune functions and the development of atopic dermatitis in high-risk infants have been shown, respectively^{166,168,189–191}. However, larger studies are needed to show whether supplementation of infant formula with HMOs has a beneficial effect on allergy and asthma prevention. Today only a limited number of HMOs (2'-fucosyllactose and lacto-N-tetraose) have been synthesized on a large scale so that they can be used as supplements in infant formulas.

Prevention of respiratory infections. A substantial body of evidence indicating that viruses play a central role in the induction of asthma de novo renders the prevention of respiratory infections an attractive potential measure for protection from allergy and asthma. Prevention of RSV-induced bronchiolitis with the anti-RSV monoclonal antibody palivizumab decreases the risk of RSV-associated illness, recurrent wheezing

and potentially non-atopic asthma⁹⁸. Current and candidate antivirals and vaccines for rhinovirus and RSV in clinical trials have been reviewed elsewhere¹⁹². Oral administration of immunomodulatory agents may affect migratory T and B cell populations, which mature in the gut-associated lymphoid tissues and develop homing receptors that preferentially move to mucosal (including respiratory) sites. Such cell subsets may act by controlling bystander inflammation as a result of local lung antiviral responses. Other mechanisms of protection could also be involved, such as priming of endogenous inflammasome functions in the lung, resulting in boosting of local adaptive antiviral immunity¹⁹³. Immunomodulation could be offered by live probiotics or bacterial extracts¹⁹⁴ or inhibition of pathogenic bacteria that synergize with viruses and add to illness severity via, for example, antibacterial vaccines or targeted antimicrobial therapy. In the latter case, benefits must be balanced with potential detrimental effects on commensal bacteria and selection of antimicrobial-resistant organisms.

Early food introduction. Older UK and US recommendations suggested avoidance of peanuts and tree nuts during pregnancy for prevention of nut allergy in offspring^{195,196}. However, no benefits were identified and thus the recommendations were reversed^{197,198}. Indeed, data from the Danish National Birth Cohort showed that maternal consumption of peanuts and tree nuts was inversely associated with asthma in children at 18 months of age¹⁹⁸. The Learning Early About Peanut Allergy (LEAP) study revolutionized current dogma by showing a low incidence of peanut allergy (81% relative reduction at the age of 5 years) in high-risk infants who were fed peanuts before 1 year of age (4–11 months) on a regular basis¹⁹⁹. Considering that peanut allergy currently affects 1–3% of children in westernized countries, the findings of this first randomized controlled trial to study the introduction or avoidance of dietary protein are highly relevant. However, it is difficult to generalize the results from a trial involving high-risk infants, which screened patients for the presence of peanut allergy, before universal peanut introduction to the general population. A wide-scale screening programme is logistically demanding, while the dose and timing of introduction also remain open issues. Peanut introduction in restricted time windows along with mechanistic studies looking into immunomodulation as a function of the age at introduction is warranted. Results from the follow-up LEAP-On study demonstrate that among children with early peanut introduction, a 12-month period of peanut avoidance was associated not with a transient desensitization but rather with sustained unresponsiveness to peanuts²⁰⁰. A significant decline in peanut-specific IgE levels was observed in the initial peanut-consuming groups when they were assessed at the age of 6 years following 1 year of avoidance (LEAP-On). These findings provided important insights for allergy prevention and relevant future studies. They demonstrated that food-specific IgE production starts early in infancy and may take a long time to switch off, potentially due to the presence of long-lived IgE-producing memory B and

plasma cells. They showed that mean peanut-specific IgE values were similar between exposure and avoidance groups, but extreme value analysis showed that high levels of peanut-specific IgE were increasingly observed over time in the group that eventually had allergy. However, this is a minority of study participants compared with those who remained tolerant, and thus their data would be diluted²⁰¹.

Five trials have examined the impact of early introduction of egg on the development of egg allergy. Four trials selected high-risk infants with a family history of allergy and/or a personal history of atopic dermatitis²⁰², and one study was population based but excluded infants who were sensitized to egg²⁰³. All five trials showed a trend for protection but only one reached statistical significance in the intention-to-treat analyses²⁰⁴ and one in per-protocol analysis²⁰⁵. On the other hand, the Enquiring About Tolerance (EAT) study did not show any efficacy of early introduction of allergenic foods in an intention-to-treat analysis but demonstrated that it is a safe approach that does not affect breastfeeding rates²⁰⁶. However, consumption of 2 g of peanut or egg white protein per week was associated with a significantly lower prevalence of the associated allergies than less consumption²⁰⁶. Multiple allergen oral tolerance induction is a significant unmet need, which requires further study.

Along the same lines, increased diversity of food introduced in the first year of life seems to have a protective effect with regard to allergic diseases via the induction of immunological tolerance²⁰⁷. Dietary diversity may reduce allergic disease by enhancing microbiota diversity²⁰⁸, which in turn improves intestinal wall integrity and regulation of the immune system, although this remains to be confirmed. Similarly, food production and

cooking methods as well as previously described 'healthy diets' (for example, a Mediterranean-style diet) are also discussed in the scientific community but evidence regarding any allergy-protective effect is so far limited and often controversial²⁰⁷.

Use of hydrolysed formulas. Hydrolysed cow's milk formulas have been developed in which protein modification is performed through a variety of physical and chemical processes, including ultraheating and enzymatic cleavage with trypsin and chymotrypsin. The degree of protein modification differs among formulas and is referred to as partial or extensive hydrolysis. Such formulas are derived from either whey or casein proteins. Universal agreement about the definition of such extensively or partially hydrolysed formulas has not been reached so far^{209–212}. The concept behind this approach is the balance between allergenicity and anti-genicity. Partially and extensively hydrolysed food formula contains a marked reduction of allergenic epitopes. However, the antigenicity and, therefore, the capacity to mount immunological tolerance is still retained under these conditions²¹³. This concept is supported by animal model systems, but clinical trials have shown contradictory results. A meta-analysis²¹⁴, together with other studies²¹⁵, suggests that the clinical observations and effects are hydrolysate specific. For prevention of food allergy, two systematic reviews and several randomized trials found beneficial effects for extensively hydrolysed whey or casein formula^{171,216}. However, there were also mixed findings regarding the development of allergic diseases, and recent findings do not support the use of hydrolysed formula to prevent allergic diseases in high-risk infants^{214,215}. Partial hydrolysates with proven efficacy are recommended in some guidelines for the prevention of cow's milk allergy and allergic diseases in at-risk infants and may therefore be considered as a protein source in starter infant formulas.

Box 1 | Aims and outcomes of the Finnish Allergy Programme

The Finnish Allergy Programme (2008–2018) involved several institutions and funding bodies, including the European Research Council, the European Commission's Seventh Framework Programme, the Ministry of Social Welfare and Health, the Academy of Finland and Helsinki University Hospital^{221,222}. It aimed to reduce allergy burden primarily by promoting the development of tolerance via healthy lifestyle attitudes. Primary preventive measures (blockade of initial immunological sensitization), secondary preventive measures (blockade of the development of clinical manifestations of allergic disease) and tertiary preventive measures (attenuation of symptoms and the avoidance of exacerbations and new allergic disorders), along with support and improvement of diagnostics, were implemented to attenuate allergy-associated morbidity and costs. All proposed measures were communicated to thousands of health-care professionals across the country through multidisciplinary educational meetings, websites and guidelines for child welfare clinics. These practical guidelines discuss the role of diet, probiotics, airborne allergens and smoking in the prevention of allergy. Furthermore, diagnostic and treatment modalities for common allergic disorders such as atopic dermatitis, food allergy and asthma were presented. Specific considerations regarding day care and counselling of children with clinical allergies were also briefly discussed. The mid-term results of the Finnish campaign demonstrated a decline in the burden, including disability and costs of allergies and asthma. Allergy diets in day care settings were reduced by 40% aimed at improving tolerance, whereas 30 hospitals and other centres were educated, audited and certified to improve allergy diagnostics. Importantly, occupational allergies, emergency visits and asthma-associated hospital days were reduced by 40%, 46% and 67%, respectively. Finally, there are already indications for a decrease in the prevalence of major allergic disorders, although it is too early to confirm this effect.

Conclusions

On the basis of our current understanding of healthy immune development and risk factors for allergy, there seems to be a vulnerable period in life during which allergy prevention could be most effective. This window of opportunity can be defined as the prenatal and early postnatal environment. In line with recent evidence, we suggest that modulation of a single risk factor alone would not be sufficient to substantially improve the course of health status of a young child²¹⁷. Mathematical modelling of the contribution of risk factors for asthma development indicates that a considerable proportion of (childhood) asthma is attributable to a set of modifiable environmental risk factors^{76,218}. This in turn leads to an extended list of preventive measures, which could be implemented on the population level worldwide. The grand challenge is now to develop public health strategies to implement allergy prevention programmes, such as the one adopted by Finland^{219,220} (BOX 1). This requires concerted actions involving all stakeholders on the national and supranational levels.

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1. Hill, D. A. & Spergel, J. M. The atopic march: critical evidence and clinical relevance. *Ann. Allergy Asthma Immunol.* **120**, 131–137 (2018).
2. Belgrave, D. C. M. et al. Developmental profiles of eczema, wheeze, and rhinitis: two population-based birth cohort studies. *PLoS Med.* **11**, e1001748 (2014).
3. Saunes, M. et al. Early eczema and the risk of childhood asthma: a prospective, population-based study. *BMC Pediatr.* **12**, 168 (2012).
4. Kobyletzki, L. B. von et al. Eczema in early childhood is strongly associated with the development of asthma and rhinitis in a prospective cohort. *BMC Dermatol.* **12**, 11 (2012).
5. Alduraywish, S. A. et al. The march from early life food sensitization to allergic disease: a systematic review and meta-analyses of birth cohort studies. *Allergy* **71**, 77–89 (2016).
6. Brough, H. A. et al. Peanut allergy. Effect of environmental peanut exposure in children with filaggrin loss-of-function mutations. *J. Allergy Clin. Immunol.* **134**, 867–875.e1 (2014).
7. Bonnelykke, K. et al. Meta-analysis of genome-wide association studies identifies ten loci influencing allergic sensitization. *Nat. Genet.* **45**, 902–906 (2013).
8. Torgerson, T. R. et al. Severe food allergy as a variant of IPEX syndrome caused by a deletion in a noncoding region of the FOXP3 gene. *Gastroenterology* **132**, 1705–1717 (2007).
9. Ober, C. & Yao, T.-C. The genetics of asthma and allergic disease: a 21st century perspective. *Immunol. Rev.* **242**, 10–30 (2011).
10. Round, J. L. & Mazmanian, S. K. Inducible Foxp3⁺ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc. Natl Acad. Sci. USA* **107**, 12204–12209 (2010).
11. Dasgupta, S., Erturk-Hasdemir, D., Ochoa-Reparaz, J., Reinecker, H.-C. & Kasper, D. L. Plasmacytoid dendritic cells mediate anti-inflammatory responses to a gut commensal molecule via both innate and adaptive mechanisms. *Cell Host Microbe* **15**, 413–423 (2014).
12. Atarashi, K. et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* **500**, 232–236 (2013).
13. Atarashi, K. et al. Induction of colonic regulatory T cells by indigenous Clostridium species. *Science* **331**, 337–341 (2011).
14. Chng, K. R. et al. Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare. *Nat. Microbiol.* **1**, 16106 (2016).
15. Meylan, P. et al. Skin colonization by *Staphylococcus aureus* precedes the clinical diagnosis of atopic dermatitis in infancy. *J. Invest. Dermatol.* **137**, 2497–2504 (2017).
16. Tauber, M. et al. *Staphylococcus aureus* density on lesional and nonlesional skin is strongly associated with disease severity in atopic dermatitis. *J. Allergy Clin. Immunol.* **137**, 1272–1274.e3 (2016).
17. McKenzie, C., Tan, J., Macia, L. & Mackay, C. R. The nutrition-gut microbiome-physiology axis and allergic diseases. *Immunol. Rev.* **278**, 277–295 (2017).
18. Wang, S. et al. MyD88 adaptor-dependent microbial sensing by regulatory T cells promotes mucosal tolerance and enforces commensalism. *Immunity* **43**, 289–303 (2015).
19. Round, J. L. et al. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* **332**, 974–977 (2011).
20. Noval Rivas, M. et al. Regulatory T cell reprogramming toward a Th2-cell-like lineage impairs oral tolerance and promotes food allergy. *Immunity* **42**, 512–523 (2015).
21. Hill, D. A. et al. Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation. *Nat. Med.* **18**, 538–546 (2012).
22. Tamburini, S., Shen, N., Wu, H. C. & Clemente, J. C. The microbiome in early life: implications for health outcomes. *Nat. Med.* **22**, 713–722 (2016).
23. Kemter, A. M. & Nagler, C. R. Influences on allergic mechanisms through gut, lung, and skin microbiome exposures. *J. Clin. Invest.* **130**, 1483–1492 (2019).
24. Arrieta, M.-C. et al. Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Sci. Transl. Med.* **7**, 307ra152 (2015).
25. Huang, Y. J. et al. The microbiome in allergic disease: current understanding and future opportunities-2017 PRACTALL document of the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergy and Clinical Immunology. *J. Allergy Clin. Immunol.* **139**, 1099–1110 (2017).
26. Berni Canani, R. et al. Gut microbiota composition and butyrate production in children affected by non-IgE-mediated cow's milk allergy. *Sci. Rep.* **8**, 12500 (2018).
27. Wang, M. et al. Reduced diversity in the early fecal microbiota of infants with atopic eczema. *J. Allergy Clin. Immunol.* **121**, 129–134 (2008).
28. Abrahamsson, T. R. et al. Low diversity of the gut microbiota in infants with atopic eczema. *J. Allergy Clin. Immunol.* **129**, 434–440 (2012).
29. Byrd, A. L., Belkaid, Y. & Segre, J. A. The human skin microbiome. *Nat. Rev. Microbiol.* **16**, 143–155 (2018).
30. Paller, A. S. et al. The microbiome in patients with atopic dermatitis. *J. Allergy Clin. Immunol.* **143**, 26–35 (2019).
31. Huang, Y. J. & Boushey, H. A. The microbiome in asthma. *J. Allergy Clin. Immunol.* **135**, 25–30 (2015).
32. Nowak-Węgrzyn, A., Szajewska, H. & Lack, G. Food allergy and the gut. *Nat. Rev. Gastroenterol. Hepatol.* **14**, 241–257 (2017).
33. Sampson, H. A. et al. Mechanisms of food allergy. *J. Allergy Clin. Immunol.* **141**, 11–19 (2018).
34. Cadwell, K. The virome in host health and disease. *Immunity* **42**, 805–813 (2015).
35. Freer, G. et al. The virome and its major component, anellovirus, a convoluted system molding human immune defenses and possibly affecting the development of asthma and respiratory diseases in childhood. *Front. Microbiol.* **9**, 686 (2018).
36. Lim, E. S. et al. Early life dynamics of the human gut virome and bacterial microbiome in infants. *Nat. Med.* **21**, 1228–1234 (2015).
37. Lim, E. S., Wang, D. & Holtz, L. R. The bacterial microbiome and virome milestones of infant development. *Trends Microbiol.* **24**, 801–810 (2016).
38. Stewart, C. J. et al. Bacterial and fungal viability in the preterm gut: NEC and sepsis. *Arch. Dis. Child. Fetal Neonatal Ed.* **98**, F298–F303 (2013).
39. Findley, K. et al. Topographic diversity of fungal and bacterial communities in human skin. *Nature* **498**, 367–370 (2013).
40. Limon, J. J., Skalski, J. H. & Underhill, D. M. Commensal fungi in health and disease. *Cell Host Microbe* **22**, 156–165 (2017).
41. Jo, J.-H. et al. Diverse human skin fungal communities in children converge in adulthood. *J. Invest. Dermatol.* **136**, 2356–2363 (2016).
42. Kong, H. H. & Morris, A. The emerging importance and challenges of the human mycobiome. *Virulence* **8**, 310–312 (2017).
43. Gensollen, T., Iyer, S. S., Kasper, D. L. & Blumberg, R. S. How colonization by microbiota in early life shapes the immune system. *Science* **352**, 539–544 (2016).
44. Gomez de Agüero, M. et al. The maternal microbiota drives early postnatal innate immune development. *Science* **351**, 1296–1302 (2016).
45. Eggesbø, M., Botten, G., Stigum, H., Nafstad, P. & Magnus, P. Is delivery by caesarean section a risk factor for food allergy? *J. Allergy Clin. Immunol.* **112**, 420–426 (2003).
46. Koplin, J. J. et al. Do factors known to alter infant microbial exposures alter the risk of food allergy and eczema in a population-based infant study? *J. Allergy Clin. Immunol.* **129**, AB231 (2012).
47. Laubereau, B. et al. Caesarean section and gastrointestinal symptoms, atopic dermatitis, and sensitization during the first year of life. *Arch. Dis. Child.* **89**, 993–997 (2004).
48. Thavagnanam, S., Fleming, J., Bromley, A., Shields, M. D. & Cardwell, C. R. A meta-analysis of the association between caesarean section and childhood asthma. *Clin. Exp. Allergy* **38**, 629–633 (2008).
49. Kristensen, K. & Henriksen, L. Caesarean section and disease associated with immune function. *J. Allergy Clin. Immunol.* **137**, 587–590 (2016).
50. Tollånes, M. C., Moster, D., Daltveit, A. K. & Irgens, L. M. Caesarean section and risk of severe childhood asthma: a population-based cohort study. *J. Pediatr.* **153**, 112–116 (2008).
51. Cho, C. E. & Norman, M. Caesarean section and development of the immune system in the offspring. *Am. J. Obstet. Gynecol.* **208**, 249–254 (2013).
52. Bager, P., Wohlfahrt, J. & Westergaard, T. Caesarean delivery and risk of atopy and allergic disease: meta-analyses. *Clin. Exp. Allergy* **38**, 634–642 (2008).
53. Roduit, C. et al. Asthma at 8 years of age in children born by caesarean section. *Thorax* **64**, 107–113 (2009).
54. Pyrhönen, K., Näyhä, S., Hiltunen, L. & Läärä, E. Caesarean section and allergic manifestations: insufficient evidence of association found in population-based study of children aged 1 to 4 years. *Acta Paediatr.* **102**, 982–989 (2013).
55. Dominguez-Bello, M. G. et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc. Natl Acad. Sci. USA* **107**, 11971–11975 (2010).
56. Romero, R. et al. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome* **2**, 4 (2014).
57. Gajer, P. et al. Temporal dynamics of the human vaginal microbiota. *Sci. Transl. Med.* **4**, 132ra52 (2012).
58. Miller, L. et al. Depomedroxyprogesterone-induced hypoestrogenism and changes in vaginal flora and epithelium. *Obstet. Gynecol.* **96**, 431–439 (2000).
59. Chu, D. M. et al. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nat. Med.* **23**, 314–326 (2017).
60. Penders, J. et al. Establishment of the intestinal microbiota and its role for atopic dermatitis in early childhood. *J. Allergy Clin. Immunol.* **132**, 601–607.e8 (2013).
61. Pistiner, M., Gold, D. R., Abdulkerim, H., Hoffman, E. & Celedón, J. C. Birth by caesarean section, allergic rhinitis, and allergic sensitization among children with a parental history of atopy. *J. Allergy Clin. Immunol.* **122**, 274–279 (2008).
62. Lee, S.-Y. et al. Additive effect between IL-13 polymorphism and caesarean section delivery/prenatal antibiotics use on atopic dermatitis. A birth cohort study (COCO). *PLoS ONE* **9**, e96603 (2014).
63. Liao, S.-L. et al. Caesarean section is associated with reduced perinatal cytokine response, increased risk of bacterial colonization in the airway, and infantile wheezing. *Sci. Rep.* **7**, 9053 (2017).
64. Dominguez-Bello, M. G. et al. Partial restoration of the microbiota of caesarean-born infants via vaginal microbial transfer. *Nat. Med.* **22**, 250–253 (2016).
65. Cox, L. M. et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell* **158**, 705–721 (2014).
66. Stokholm, J. et al. Prevalence and predictors of antibiotic administration during pregnancy and birth. *PLoS ONE* **8**, e82932 (2013).
67. Bailey, L. C. et al. Association of antibiotics in infancy with early childhood obesity. *JAMA Pediatr.* **168**, 1063–1069 (2014).
68. Saari, A., Virta, L. J., Sankilampi, U., Dunkel, L. & Saxen, H. Antibiotic exposure in infancy and risk of being overweight in the first 24 months of life. *Pediatrics* **135**, 617–626 (2015).
69. Mikkelsen, K. H., Knop, F. K., Frost, M., Hallas, J. & Pottegård, A. Use of antibiotics and risk of type 2 diabetes: a population-based case-control study. *J. Clin. Endocrinol. Metab.* **100**, 3633–3640 (2015).
70. Risnes, K. R., Belanger, K., Murk, W. & Bracken, M. B. Antibiotic exposure by 6 months and asthma and allergy at 6 years: findings in a cohort of 1,401 US children. *Am. J. Epidemiol.* **173**, 310–318 (2011).
71. Hoskin-Parr, L., Teyhan, A., Blocker, A. & Henderson, A. J. W. Antibiotic exposure in the first two years of life and development of asthma and other allergic diseases by 7.5 yr: a dose-dependent relationship. *Pediatr. Allergy Immunol.* **24**, 762–771 (2013).
72. Hviid, A., Svanström, H. & Frisch, M. Antibiotic use and inflammatory bowel diseases in childhood. *Gut* **60**, 49–54 (2011).
73. Shaw, S. Y., Blanchard, J. F. & Bernstein, C. N. Association between the use of antibiotics and new diagnoses of Crohn's disease and ulcerative colitis. *Am. J. Gastroenterol.* **106**, 2133–2142 (2011).

74. Kronman, M. P., Zautis, T. E., Haynes, K., Feng, R. & Coffin, S. E. Antibiotic exposure and IBD development among children: a population-based cohort study. *Pediatrics* **130**, e794–e803 (2012).
75. Metsälä, J. et al. Mother's and offspring's use of antibiotics and infant allergy to cow's milk. *Epidemiology* **24**, 303–309 (2013).
76. Wu, P. et al. Relative importance and additive effects of maternal and infant risk factors on childhood asthma. *PLoS ONE* **11**, e0151705 (2016).
77. Kozyskyj, A. L., Ernst, P. & Becker, A. B. Increased risk of childhood asthma from antibiotic use in early life. *Chest* **131**, 1753–1759 (2007).
78. Cho, I. et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature* **488**, 621–626 (2012).
79. Pérez-Cobas, A. E. et al. Gut microbiota disturbance during antibiotic therapy: a multi-omic approach. *Gut* **62**, 1591–1601 (2013).
80. Schuller, A. F. et al. Intergenerational transfer of antibiotic-perturbed microbiota enhances colitis in susceptible mice. *Nat. Microbiol.* **3**, 234–242 (2018).
This article shows that antibiotic exposure shapes the maternal gut microbiota and this effect extends to their offspring, with both ecological and long-term disease consequences.
81. Murk, W., Risnes, K. R. & Bracken, M. B. Prenatal or early-life exposure to antibiotics and risk of childhood asthma: a systematic review. *Pediatrics* **127**, 1125–1138 (2011).
82. Heintze, K. & Petersen, K.-U. The case of drug causation of childhood asthma: antibiotics and paracetamol. *Eur. J. Clin. Pharmacol.* **69**, 1197–1209 (2013).
83. Ivanov, I. I. et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* **139**, 485–498 (2009).
84. Stefa, A. T. et al. Commensal bacteria protect against food allergen sensitization. *Proc. Natl Acad. Sci. USA* **111**, 13145–13150 (2014).
85. Noverr, M. C., Falkowski, N. R., McDonald, R. A., McKenzie, A. N. & Huffnagle, G. B. Development of allergic airway disease in mice following antibiotic therapy and fungal microbiota increase: role of host genetics, antigen, and interleukin-13. *Infect. Immun.* **73**, 30–38 (2005).
86. Gonzalez-Perez, G. et al. Maternal antibiotic treatment impacts development of the neonatal intestinal microbiome and antiviral immunity. *J. Immunol.* **196**, 3768–3779 (2016).
87. Semic-Jusufagic, A. et al. Assessing the association of early life antibiotic prescription with asthma exacerbations, impaired antiviral immunity, and genetic variants in 17q21: a population-based birth cohort study. *Lancet Respir. Med.* **2**, 621–630 (2014).
88. Lodge, C. J. et al. Breastfeeding and asthma and allergies: a systematic review and meta-analysis. *Acta Paediatr.* **104**, 38–53 (2015).
89. Heinrich, J. Modulation of allergy risk by breast feeding. *Curr. Opin. Clin. Nutr. Metab. Care* **20**, 217–221 (2017).
90. Stewart, C. J. et al. Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature* **562**, 583–588 (2018).
This is an extensive characterization of the microbiota in early life in a large multicentre population and provides the background for future mechanistic studies.
91. van den Elsen, L. W. J., Garssen, J., Burcelin, R. & Verhasselt, V. Shaping the gut microbiota by breastfeeding: the gateway to allergy prevention? *Front. Pediatr.* **7**, 47 (2019).
92. Bäckhed, F. et al. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe* **17**, 690–703 (2015).
This article shows that the gut microbiota of infants born by caesarean delivery has less resemblance to their mother's microbiota than vaginally delivered infants and describes breastfeeding cessation as being required for maturation into an adult-like microbiota.
93. Alderete, T. L. et al. Associations between human milk oligosaccharides and infant body composition in the first 6 mo of life. *Am. J. Clin. Nutr.* **102**, 1381–1388 (2015).
94. Bode, L. et al. Overcoming the limited availability of human milk oligosaccharides: Challenges and opportunities for research and application. *Nutr. Rev.* **74**, 635–644 (2016).
95. Seppo, A. E., Autran, C. A., Bode, L. & Järvinen, K. M. Human milk oligosaccharides and development of cow's milk allergy in infants. *J. Allergy Clin. Immunol.* **139**, 708–711.e5 (2017).
96. Pannaraj, P. S. et al. Shared and distinct features of human milk and infant stool viromes. *Front. Microbiol.* **9**, 1162 (2018).
97. Rekima, A. et al. A role for early oral exposure to house dust mite allergens through breast milk in IgE-mediated food allergy susceptibility. *J. Allergy Clin. Immunol.* **145**, 1416–1429.e11 (2020).
98. Jartti, T. & Gern, J. E. Role of viral infections in the development and exacerbation of asthma in children. *J. Allergy Clin. Immunol.* **140**, 895–906 (2017).
99. Jackson, D. J. et al. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. *Am. J. Respir. Crit. Care Med.* **185**, 281–285 (2012).
100. Kusel, M. M. H. et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J. Allergy Clin. Immunol.* **119**, 1105–1110 (2007).
101. Geerdink, R. J., Pillay, J., Meyard, L. & Bont, L. Neutrophils in respiratory syncytial virus infection. A target for asthma prevention. *J. Allergy Clin. Immunol.* **136**, 838–847 (2015).
102. Gavala, M. L., Bashir, H. & Gern, J. E. Virus/allergen interactions in asthma. *Curr. Allergy Asthma Rep.* **13**, 298–307 (2013).
103. Krishnamoorthy, N. et al. Early infection with respiratory syncytial virus impairs regulatory T cell function and increases susceptibility to allergic asthma. *Nat. Med.* **18**, 1525–1530 (2012).
104. Edwards, M. R. et al. Viral infections in allergy and immunology. How allergic inflammation influences viral infections and illness. *J. Allergy Clin. Immunol.* **140**, 909–920 (2017).
Edwards et al. discuss evidence for a reciprocal inverse correlation between innate interferons and Th2 cell mediators and how biologics targeting Th2 cell mediators contribute in this regard.
105. Rupani, H. et al. Toll-like receptor 7 is reduced in severe asthma and linked to an altered MicroRNA profile. *Am. J. Respir. Crit. Care Med.* **194**, 26–37 (2016).
106. Gill, M. A. et al. Enhanced plasmacytoid dendritic cell antiviral responses after omalizumab. *J. Allergy Clin. Immunol.* **141**, 1735–1743.e9 (2018).
107. Fujimura, K. E. et al. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. *Nat. Med.* **22**, 1187–1191 (2016).
108. Durack, J. et al. Delayed gut microbiota development in high-risk for asthma infants is temporarily modifiable by Lactobacillus supplementation. *Nat. Commun.* **9**, 707 (2018).
This study shows that early-life gut microbial development is distinct but plastic in infants at high risk of asthma.
109. Milani, C. et al. The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. *Microbiol. Mol. Biol. Rev.* **81**, e00036-17 (2017).
110. Bunyavanich, S. & Berin, M. C. Food allergy and the microbiome. Current understandings and future directions. *J. Allergy Clin. Immunol.* **144**, 1468–1477 (2019).
111. Russell, S. L. et al. Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. *EMBO Rep.* **13**, 440–447 (2012).
112. Geuking, M. B. et al. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity* **34**, 794–806 (2011).
113. Smith, P. M. et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* **341**, 569–573 (2013).
114. Feehley, T. et al. Healthy infants harbor intestinal bacteria that protect against food allergy. *Nat. Med.* **25**, 448–453 (2019).
115. Abdel-Gadir, A. et al. Microbiota therapy acts via a regulatory T cell MyD88/RORγt pathway to suppress food allergy. *Nat. Med.* **25**, 1164–1174 (2019).
Using a model of food allergy, these authors show that microbiota therapy can suppress food allergy via innate signalling pathways.
116. Tan, J. et al. The role of short-chain fatty acids in health and disease. *Adv. Immunol.* **121**, 91–119 (2014).
117. Roduit, C. et al. High levels of butyrate and propionate in early life are associated with protection against atopy. *Allergy* **74**, 799–809 (2019).
118. Sandin, A., Bråbäck, L., Norin, E. & Björkstén, B. Faecal short chain fatty acid pattern and allergy in early childhood. *Acta Paediatr.* **98**, 823–827 (2009).
119. Bunyavanich, S. et al. Early-life gut microbiome composition and milk allergy resolution. *J. Allergy Clin. Immunol.* **138**, 1122–1130 (2016).
120. Thio, C. L.-P., Chi, P.-Y., Lai, A. C.-Y. & Chang, Y.-J. Regulation of type 2 innate lymphoid cell-dependent airway hyperactivity by butyrate. *J. Allergy Clin. Immunol.* **142**, 1867–1883.e12 (2018).
121. Lewis, G. et al. Dietary fiber-induced microbial short chain fatty acids suppress ILC2-dependent airway inflammation. *Front. Immunol.* **10**, 2051 (2019).
122. Furusawa, Y. et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* **504**, 446–450 (2013).
This article reports a mechanistic link between dietary fibre, commensal microorganisms, production of SCFAs and differentiation of Treg cells.
123. Trompette, A. et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat. Med.* **20**, 159–166 (2014).
In this study, the effects of dietary fibre on microbiota-mediated effector responses in the lung (allergic airway diseases) and haematopoiesis are described.
124. Ege, M. J. et al. Exposure to environmental microorganisms and childhood asthma. *N. Engl. J. Med.* **364**, 701–709 (2011).
This is a cross-sectional study on the hygiene hypothesis: environmental microorganisms and asthma prevention.
125. Hagner, S. et al. Farm-derived Gram-positive bacterium *Staphylococcus sciuri* W620 prevents asthma phenotype in HDM- and OVA-exposed mice. *Allergy* **68**, 322–329 (2013).
126. Conrad, M. L. et al. Maternal TLR signaling is required for prenatal asthma protection by the nonpathogenic microbe *Acinetobacter lwoffii* F78. *J. Exp. Med.* **206**, 2869–2877 (2009).
127. Debarry, J. et al. *Acinetobacter lwoffii* and *Lactococcus lactis* strains isolated from farm cowsheds possess strong allergy-protective properties. *J. Allergy Clin. Immunol.* **119**, 1514–1521 (2007).
128. Stein, K. et al. Endosomal recognition of *Lactococcus lactis* G121 and its RNA by dendritic cells is key to its allergy-protective effects. *J. Allergy Clin. Immunol.* **139**, 667–678.e5 (2017).
129. Renz, H., Brandtzaeg, P. & Horne, M. The impact of perinatal immune development on mucosal homeostasis and chronic inflammation. *Nat. Rev. Immunol.* **12**, 9–23 (2011).
130. Ruokolainen, L. et al. Significant disparities in allergy prevalence and microbiota between the young people in Finnish and Russian Karelia. *Clin. Exp. Allergy* **47**, 665–674 (2017).
131. Seeley, J. J. & Ghosh, S. Molecular mechanisms of innate memory and tolerance to LPS. *J. Leukoc. Biol.* **101**, 107–119 (2017).
132. Brand, S. et al. Epigenetic regulation in murine offspring as a novel mechanism for transmaternal asthma protection induced by microbes. *J. Allergy Clin. Immunol.* **128**, 618–625.e7 (2011).
133. Haahela, T. A biodiversity hypothesis. *Allergy* **74**, 1445–1456 (2019).
134. Kyburz, A. et al. Transmaternal *Helicobacter pylori* exposure reduces allergic airway inflammation in offspring through regulatory T cells. *J. Allergy Clin. Immunol.* **143**, 1496–1512.e11 (2019).
135. Matysiak-Budnik, T. et al. *Helicobacter pylori* increases the epithelial permeability to a food antigen in human gastric biopsies. *Am. J. Gastroenterol.* **99**, 225–232 (2004).
136. Skevaki, C. et al. Influenza-derived peptides cross-react with allergens and provide asthma protection. *J. Allergy Clin. Immunol.* **142**, 804–814 (2018).
This is the first demonstration of virus-induced T cell-mediated heterologous immune responses to allergens with implications for asthma protection.
137. Culley, F. J., Pennycook, A. M. J., Tregoning, J. S., Hussell, T. & Openshaw, P. J. M. Differential chemokine expression following respiratory virus infection reflects Th1- or Th2-biased immunopathology. *J. Virol.* **80**, 4521–4527 (2006).
138. Yang, J.-Y. et al. Enteric viruses ameliorate gut inflammation via toll-like receptor 3 and toll-like receptor 7-mediated interferon-β production. *Immunity* **44**, 889–900 (2016).
139. Neil, J. A. & Cadwell, K. The intestinal virome and immunity. *J. Immunol.* **201**, 1615–1624 (2018).

140. Machiels, B. et al. A gammaherpesvirus provides protection against allergic asthma by inducing the replacement of resident alveolar macrophages with regulatory monocytes. *Nat. Immunol.* **18**, 1310–1320 (2017).
These investigators show that a murid herpesvirus may inhibit experimental asthma by replacing alveolar macrophages with regulatory monocytes and thus contributing to training of lung immune responses.
141. De Vlaminc, I. et al. Temporal response of the human virome to immunosuppression and antiviral therapy. *Cell* **155**, 1178–1187 (2013).
142. Pifferi, M. et al. Associations between nasal torquetenovirus load and spirometric indices in children with asthma. *J. Infect. Dis.* **192**, 1141–1148 (2005).
143. Maggi, F. & Bendinelli, M. Immunobiology of the Torque teno viruses and other anelloviruses. *Curr. Top. Microbiol. Immunol.* **331**, 65–90 (2009).
144. Li, L. et al. AIDS alters the commensal plasma virome. *J. Virol.* **87**, 10912–10915 (2013).
145. Tiew, P. Y. et al. The mycobiome in health and disease: emerging concepts, methodologies and challenges. *Mycopathologia* **185**, 207–231 (2020).
146. Li, X. et al. Response to fungal dysbiosis by gut-resident CX3CR1⁺ mononuclear phagocytes aggravates allergic airway disease. *Cell Host Microbe* **24**, 847–856.e4 (2018).
147. Bacher, P. et al. Human anti-fungal Th17 immunity and pathology rely on cross-reactivity against *Candida albicans*. *Cell* **176**, 1340–1355.e15 (2019).
These authors identify heterologous immunity to a single ubiquitous member of the microbiota as the culprit in human antifungal Th₁₇ cell responses, particularly in the context of airway inflammation.
148. Rayner, S. et al. Identification of small RNAs in extracellular vesicles from the commensal yeast *Malassezia sympodialis*. *Sci. Rep.* **7**, 39742 (2017).
149. Sparber, F. et al. The skin commensal yeast *Malassezia* triggers a type 17 response that coordinates anti-fungal immunity and exacerbates skin inflammation. *Cell Host Microbe* **25**, 389–403.e6 (2019).
This article highlights the importance of *Malassezia*-induced type 17 responses in driving antifungal immunity and skin inflammation.
150. Krause, R. et al. Mycobiome in the lower respiratory tract - a clinical perspective. *Front. Microbiol.* **7**, 2169 (2016).
151. Tipton, L., Ghedin, E. & Morris, A. The lung mycobiome in the next-generation sequencing era. *Virulence* **8**, 334–341 (2017).
152. Fraczek, M. G. et al. Corticosteroid treatment is associated with increased filamentous fungal burden in allergic fungal disease. *J. Allergy Clin. Immunol.* **142**, 407–414 (2018).
153. Skalski, J. H. et al. Expansion of commensal fungus *Wallemia mellicola* in the gastrointestinal mycobiota enhances the severity of allergic airway disease in mice. *PLoS Pathog.* **14**, e1007260 (2018).
154. Goldman, D. L. et al. Lower airway microbiota and mycobiota in children with severe asthma. *J. Allergy Clin. Immunol.* **141**, 808–811.e7 (2018).
155. Mac Aogáin, M. et al. Distinct “immunoeallotypes” of disease and high frequencies of sensitization in non-cystic fibrosis bronchiectasis. *Am. J. Respir. Crit. Care Med.* **199**, 842–853 (2019).
156. Richardson, M., Bowyer, P. & Sabino, R. The human lung and aspergillus: you are what you breathe in? *Med. Mycol.* **57**, S145–S154 (2019).
157. Kauth, M. & Heine, H. Allergy protection by cowshed bacteria - recent findings and future prospects. *Pediatr. Allergy Immunol.* **27**, 340–347 (2016).
158. Hesselmar, B. et al. Pacifier cleaning practices and risk of allergy development. *Pediatrics* **131**, e1829–e1837 (2013).
159. Alm, J. S., Swartz, J., Lilja, G., Scheynius, A. & Pershagen, G. Atopy in children of families with an anthroposophic lifestyle. *Lancet* **353**, 1485–1488 (1999).
160. Hesselmar, B., Hicke-Roberts, A. & Wennergren, G. Allergy in children in hand versus machine dishwashing. *Pediatrics* **135**, e590–e597 (2015).
161. Gern, J. E. Promising candidates for allergy prevention. *J. Allergy Clin. Immunol.* **136**, 23–28 (2015).
162. Morisset, M., Aubert-Jacquin, C., Soulaïnes, P., Moneret-Vautrin, D.-A. & Dupont, C. A non-hydrolyzed, fermented milk formula reduces digestive and respiratory events in infants at high risk of allergy. *Eur. J. Clin. Nutr.* **65**, 175–183 (2011).
163. Kukkonen, A. K., Savilahti, E. M., Haahela, T., Savilahti, E. & Kuitunen, M. Ovalbumin-specific immunoglobulins A and G levels at age 2 years are associated with the occurrence of atopic disorders. *Clin. Exp. Allergy* **41**, 1414–1421 (2011).
164. Kuitunen, M. et al. Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. *J. Allergy Clin. Immunol.* **123**, 335–341 (2009).
165. Marschan, E. et al. Probiotics in infancy induce protective immune profiles that are characteristic for chronic low-grade inflammation. *Clin. Exp. Allergy* **38**, 611–618 (2008).
166. Osborn, D. A. & Sinn, J. K. H. Prebiotics in infants for prevention of allergy. *Cochrane Database Syst. Rev.* <https://doi.org/10.1002/14651858.CD006474.pub3> (2013).
167. Osborn, D. A. & Sinn, J. K. Probiotics in infants for prevention of allergic disease and food hypersensitivity. *Cochrane Database Syst. Rev.* <https://doi.org/10.1002/14651858.CD006475.pub2> (2007).
168. Grüber, C. et al. Reduced occurrence of early atopic dermatitis because of immunoactive prebiotics among low-atopy-risk infants. *J. Allergy Clin. Immunol.* **126**, 791–797 (2010).
169. Prescott, S. L. et al. Early markers of allergic disease in a primary prevention study using probiotics: 2.5-year follow-up phase. *Allergy* **63**, 1481–1490 (2008).
170. Forsberg, A., West, C. E., Prescott, S. L. & Jenmalm, M. C. Pre- and probiotics for allergy prevention: time to revisit recommendations? *Clin. Exp. Allergy* **46**, 1506–1521 (2016).
171. Muraro, A. et al. EAACI food allergy and anaphylaxis guidelines. Primary prevention of food allergy. *Allergy* **69**, 590–601 (2014).
172. Braegger, C. et al. Supplementation of infant formula with probiotics and/or prebiotics: a systematic review and comment by the ESPGHAN committee on nutrition. *J. Pediatr. Gastroenterol. Nutr.* **52**, 238–250 (2011).
173. Focchi, A. et al. World Allergy Organization-McMaster University guidelines for allergic disease prevention (GLAD-P). Probiotics. *World Allergy Organ. J.* **8**, 4 (2015).
174. Niers, L. E. M. et al. Identification of strong interleukin-10 inducing lactic acid bacteria which down-regulate T helper type 2 cytokines. *Clin. Exp. Allergy* **35**, 1481–1489 (2005).
175. Niers, L. E. M. et al. Selection of probiotic bacteria for prevention of allergic diseases: immunomodulation neonatal dendritic cells. *Clin. Exp. Immunol.* **149**, 344–352 (2007).
176. Braat, H. et al. Lactobacillus rhamnosus induces peripheral hyporesponsiveness in stimulated CD4⁺ T cells via modulation of dendritic cell function. *Am. J. Clin. Nutr.* **80**, 1618–1625 (2004).
177. Rigby, R. J., Knight, S. C., Kamm, M. A. & Stagg, A. J. Production of interleukin (IL)-10 and IL-12 by murine colonic dendritic cells in response to microbial stimuli. *Clin. Exp. Immunol.* **139**, 245–256 (2005).
178. Kim, J. Y., Choi, Y. O. & Ji, G. E. Effect of oral probiotics (Bifidobacterium lactis AD011 and Lactobacillus acidophilus AD031) administration on ovalbumin-induced food allergy mouse model. *J. Microbiol. Biotechnol.* **18**, 1393–1400 (2008).
179. So, J.-S. et al. Lactobacillus casei potentiates induction of oral tolerance in experimental arthritis. *Mol. Immunol.* **46**, 172–180 (2008).
180. Hacin-Rachinel, F. et al. Oral probiotic control skin inflammation by acting on both effector and regulatory T cells. *PLoS ONE* **4**, e4903 (2009).
181. Elazab, N. et al. Probiotic administration in early life, atopy, and asthma. A meta-analysis of clinical trials. *Pediatrics* **132**, e666–e676 (2013).
182. Kalliomäki, M., Salminen, S., Poussa, T., Arvilommi, H. & Isolauri, E. Probiotics and prevention of atopic disease. 4-year follow-up of a randomised placebo-controlled trial. *Lancet* **361**, 1869–1871 (2003).
183. Karimi, K., Inman, M. D., Bienenstock, J. & Forsythe, P. Lactobacillus reuteri-induced regulatory T cells protect against an allergic airway response in mice. *Am. J. Respir. Crit. Care Med.* **179**, 186–193 (2009).
184. Hoarau, C., Lagaraine, C., Martin, L., Velge-Roussel, F. & Lebranchu, Y. Supernatant of Bifidobacterium breve induces dendritic cell maturation, activation, and survival through a Toll-like receptor 2 pathway. *J. Allergy Clin. Immunol.* **117**, 696–702 (2006).
185. Forsythe, P., Inman, M. D. & Bienenstock, J. Oral treatment with live Lactobacillus reuteri inhibits the allergic airway response in mice. *Am. J. Respir. Crit. Care Med.* **175**, 561–569 (2007).
186. Briske, D. et al. Probiotics modify tight-junction proteins in an animal model of nonalcoholic fatty liver disease. *Ther. Adv. Gastroenterol.* **9**, 463–472 (2016).
187. Goldin, B. R. & Gorbach, S. L. Clinical indications for probiotics: an overview. *Clin. Infect. Dis.* **46** (Suppl. 2), S96–S100 (2008).
188. Berni Canani, R. et al. Lactobacillus rhamnosus GG-supplemented formula expands butyrate-producing bacterial strains in food allergic infants. *ISME J.* **10**, 742–750 (2016).
189. Kerperien, J. et al. Non-digestible oligosaccharides modulate intestinal immune activation and suppress cow's milk allergic symptoms. *Pediatr. Allergy Immunol.* **25**, 747–754 (2014).
190. Moro, G. et al. A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch. Dis. Child.* **91**, 814–819 (2006).
191. Arslanoglu, S. et al. 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**, 1091–1095 (2008).
192. Edwards, M. R. et al. The potential of anti-infectives and immunomodulators as therapies for asthma and asthma exacerbations. *Allergy* **73**, 50–63 (2018).
This review article from the EAACI Anti-infectives in Asthma and Asthma Exacerbations Task Force summarizes the potential of anti-infectives and immunomodulators in asthma.
193. Ichinohe, T. et al. Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proc. Natl Acad. Sci. USA* **108**, 5354–5359 (2011).
194. Holt, P. G., Strickland, D. H. & Sly, P. D. Virus infection and allergy in the development of asthma. What is the connection? *Curr. Opin. Allergy Clin. Immunol.* **12**, 151–157 (2012).
195. DesRoches, A., Infante-Rivard, C., Paradis, L., Paradis, J. & Haddad, E. Peanut allergy: is maternal transmission of antigens during pregnancy and breastfeeding a risk factor? *J. Invest. Allergol. Clin. Immunol.* **20**, 289–294 (2010).
196. Frank, L., Marian, A., Visser, M., Weinberg, E. & Potter, P. C. Exposure to peanuts in utero and in infancy and the development of sensitization to peanut allergens in young children. *Pediatr. Allergy Immunol.* **10**, 27–32 (1999).
197. Sicherer, S. H. & Burks, A. W. Maternal and infant diets for prevention of allergic diseases. Understanding menu changes in 2008. *J. Allergy Clin. Immunol.* **122**, 29–33 (2008).
198. Maslova, E. et al. Peanut and tree nut consumption during pregnancy and allergic disease in children—should mothers decrease their intake? Longitudinal evidence from the Danish National Birth Cohort. *J. Allergy Clin. Immunol.* **130**, 724–732 (2012).
199. Du Toit, G. et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. *J. N. Engl. J. Med.* **372**, 803–813 (2015).
200. Du Toit, G. et al. Effect of avoidance on peanut allergy after early peanut consumption. *N. Engl. J. Med.* **374**, 1435–1443 (2016).
This is a landmark study on the role of avoidance of peanut allergens in the development of peanut allergy, a novel concept for oral tolerance.
201. Turcanu, V. et al. Immune mechanisms of food allergy and its prevention by early intervention. *Curr. Opin. Immunol.* **48**, 92–98 (2017).
202. Peters, R. L., Neeland, M. R. & Allen, K. J. Primary prevention of food allergy. *Curr. Allergy Asthma Rep.* **17**, 52 (2017).
203. Bellach, J. et al. Randomized placebo-controlled trial of hen's egg consumption for primary prevention in infants. *J. Allergy Clin. Immunol.* **139**, 1591–1599.e2 (2017).
204. Natsume, O. et al. Two-step egg introduction for prevention of egg allergy in high-risk infants with eczema (PETIT). A randomised, double-blind, placebo-controlled trial. *Lancet* **389**, 276–286 (2017).
205. Palmer, D. J., Sullivan, T. R., Gold, M. S., Prescott, S. L. & Makrides, M. Randomized controlled trial of early regular egg intake to prevent egg allergy. *J. Allergy Clin. Immunol.* **139**, 1600–1607.e2 (2017).
206. Perkin, M. R. et al. Randomized trial of introduction of allergenic foods in breast-fed infants. *N. Engl. J. Med.* **374**, 1733–1743 (2016).

207. Mazzocchi, A., Venter, C., Maslin, K. & Agostoni, C. The role of nutritional aspects in food allergy: prevention and management. *Nutrients* **9**, 850 (2017).
208. Claesson, M. J. et al. Gut microbiota composition correlates with diet and health in the elderly. *Nature* **488**, 178–184 (2012).
209. Høst, A. et al. Dietary products used in infants for treatment and prevention of food allergy. Joint Statement of the European Society for Paediatric Allergology and Clinical Immunology (ESPAI) Committee on Hypoallergenic Formulas and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition. *Arch. Dis. Child.* **81**, 80–84 (1999).
210. Hill, D. J., Murch, S. H., Rafferty, K., Wallis, P. & Green, C. J. The efficacy of amino acid-based formulas in relieving the symptoms of cow's milk allergy: A systematic review. *Clin. Exp. Allergy* **37**, 808–822 (2007).
211. Chafen, J. J. S. et al. Diagnosing and managing common food allergies: a systematic review. *JAMA* **303**, 1848–1856 (2010).
212. Silva, D. de et al. Primary prevention of food allergy in children and adults. *Syst. Rev. Allergy* **69**, 581–589 (2014).
213. Allen, C. W., Campbell, D. E. & Kemp, A. S. Food allergy: is strict avoidance the only answer? *Pediatr. Allergy Immunol.* **20**, 415–422 (2009).
214. Boyle, R. J. et al. Hydrolysed formula and risk of allergic or autoimmune disease: systematic review and meta-analysis. *BMJ* **352**, i974 (2016).
215. Boyle, R. J. et al. Prebiotic-supplemented partially hydrolysed cow's milk formula for the prevention of eczema in high-risk infants: a randomized controlled trial. *Allergy* **71**, 701–710 (2016).
216. Vandenplas, Y. et al. Should partial hydrolysates be used as starter infant formula? A working group consensus. *J. Pediatr. Gastroenterol. Nutr.* **62**, 22–35 (2016).
217. Wong, G. W. K. & Chow, C. M. Childhood asthma epidemiology: insights from comparative studies of rural and urban populations. *Pediatr. Pulmonol.* **43**, 107–116 (2008).
218. Abreo, A., Gebretsadik, T., Stone, C. A. & Hartert, T. V. The impact of modifiable risk factor reduction on childhood asthma development. *Clin. Transl. Med.* **7**, 15 (2018).
219. Pelkonen, A. S. et al. Allergy in children: practical recommendations of the Finnish Allergy Programme 2008–2018 for prevention, diagnosis, and treatment. *Pediatr. Allergy Immunol.* **23**, 103–116 (2012).
220. Tanno, L. K., Haahtela, T., Calderon, M. A., Cruz, A. & Demoly, P. Implementation gaps for asthma prevention and control. *Respir. Med.* **130**, 13–19 (2017).
221. Haahtela, T., Valovirta, E., Bousquet, J. & Mäkelä, M. The Finnish Allergy Programme 2008–2018 works. *Eur. Respir. J.* **49**, 1700470 (2017).
222. Burki, T. K. Asthma control, learning from Finland's success. *Lancet Respir. Med.* **7**, 207–208 (2019).

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