

Early life factors that affect allergy development

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Abstract | The incidence of allergic disease continues to rise in industrialized countries. The rapid increase in the incidence of allergic disease throughout the past half century suggests that recently altered environmental factors are driving allergy development. Accumulating evidence suggests that environmental experiences that occur during the first months of life can influence the risk of allergic sensitization. In this Review, we present the evidence relating to specific early life exposures that affect future allergy development, and discuss how these exposures may promote either tolerance or allergic sensitization.

Atopic

Describes a state of heightened immune responsiveness to foreign antigens that is mediated by the production of IgE antibodies.

Allergen

A type of antigen that elicits an inappropriate immune response to an otherwise harmless substance.

Allergy is characterized by an inappropriate immune response to one or several foreign antigens, and this response can give rise to conditions such as allergic asthma, food allergy, atopic dermatitis (eczema), allergic rhinitis (hay fever) and anaphylaxis. Individuals with an allergy typically produce high levels of IgE in response to allergen exposure (that is, an atopic response), although allergic responses that are not mediated by IgE (that is, non-atopic responses) can also occur¹. Many allergies first present in childhood, and there are several common sources of allergens (BOX 1). Often, childhood food allergies spontaneously resolve by late childhood, although sensitivity to certain allergens, such as peanuts or tree nuts, is more likely to persist into adulthood². The severity of the childhood symptoms of allergic asthma is a predictor of disease persistence into adulthood³.

Type 2 immune responses are central to the development of an allergic response. Following mucosal tissue damage and allergen exposure, epithelial cells release the cytokines thymic stromal lymphopoietin (TSLP), interleukin-25 (IL-25) and IL-33. These cytokines initiate a type 2 response by recruiting eosinophils and basophils, activating B cells and dendritic cells (DCs), and promoting the differentiation of type 2 innate lymphoid cells (ILC2s) and T helper 2 (T_H2) cells⁴. The production of IL-4, IL-5, IL-9 and IL-13 by ILC2s and T_H2 cells can stimulate several type 2 effector responses that are hallmarks of allergy: specifically, IL-4 from T_H2 cells can promote B cell isotype class-switching to IgE; IL-5 and IL-9 can promote the recruitment of eosinophils and mast cells; and IL-13 can stimulate mucus hypersecretion and airway hyper-reactivity⁵. Secreted antigen-specific IgE can bind to high-affinity Fcε receptors on basophils and mast cells, and these receptors mediate their degranulation upon subsequent antigen

exposure. Granulocyte degranulation releases inflammatory mediators that can cause airway contractility and increased vascular permeability, and promote the recruitment of additional inflammatory cells⁶. In contrast to pathways that lead to allergic sensitization, either local or systemic tolerance to potential allergens can occur. Tolerance can be achieved through lymphocyte anergy or through the suppressive actions of several regulatory populations, of which the best characterized are the following regulatory T cell (T_{reg} cell) subsets: forkhead box protein P3 (FOXP3)-expressing CD4⁺ T cells and IL-10-producing FOXP3-CD4⁺ T regulatory type 1 (T_R1) cells^{7,8}.

There is certainly a genetic component to allergy susceptibility; variants in several genes — including those encoding proteins associated with T_H2 cell differentiation — are noted as risk factors for allergy⁹. However, the rapid increase in the incidence of allergic diseases throughout the past several decades¹⁰, and the fact that second-generation migrant populations tend to acquire the atopic disease prevalence of their host countries¹¹, suggests that recently altered environmental factors are key drivers of allergy development. It is becoming increasingly clear that antigen exposures during the first few years of life are unique in their capacity to either elicit allergic sensitization or result in tolerogenic responses. Observations from international adoption and migration studies have revealed that relocation to a country in which there is a high prevalence of atopic disease during early childhood is associated with a higher risk of developing atopic disease in adulthood than is relocation later in childhood^{12,13}. This suggests the existence of a time window of vulnerability in early life, during which certain environmental factors can tip the balance towards allergic sensitization.

In this Review, we present evidence relating to post-partum early life environmental factors that can influence whether long-term tolerance or allergic

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Box 1 | Common childhood allergens: what makes an allergen an allergen?

The most common food allergies in early childhood are to peanut, milk or egg antigens. Often, a food allergy to milk or eggs will resolve by late childhood, yet allergies to peanut, tree nut and seafood antigens commonly persist into adulthood¹⁰⁵. Common inhaled allergens in children include antigens from pollen, animal dander, dust mites, cockroaches and fungi¹⁰⁶. Key characteristics of particular antigens render them pro-allergenic, and this is evident from the fact that allergies against certain antigens are very common, whereas other environmental antigens never elicit allergic sensitization. Food allergens are frequently proteins that are found at high abundance in the food source, have multiple high-affinity IgE-binding epitopes and are resistant to degradation by digestive enzymes and low pH¹⁰⁷. The use of antacids or other pharmaceuticals that raise the gastric pH has been shown to increase the allergenic potential of foods by lowering the dose of allergen required to result in allergic sensitization¹⁰⁸. Many allergens contain intramolecular disulphide bonds, and the disruption of these bonds has been demonstrated in some cases to disrupt the allergenic potential of allergens by reducing their ability to resist denaturation or affecting the processing of their antigens by antigen-presenting cells^{107,109}. The biological activity of proteins has also been noted as a risk factor for allergenicity; the house dust mite allergen Der p1 and fungal allergens such as those derived from *Alternaria* species, have protease activity^{109,110}. Protease activity is required for some allergens to elicit allergic sensitization; for example, an *Alternaria* species-specific serine protease is required for intranasal *Alternaria alternata* exposure to elicit lung interleukin-33 release, which drives T helper 2 cell-mediated inflammation⁸².

sensitization occurs. We discuss how the route and timing of first antigen exposure influences the direction of immune priming, and how co-exposure to microbial endotoxins can modulate these responses. We describe evidence that indicates a causal role of the early life microbiota in influencing allergy development later in childhood. Finally, we examine how exposure to infectious pathogens in early life can promote tolerance or exacerbate allergic disease.

Allergen exposure: tolerance or sensitization?

The route of allergen exposure. Initial antigen exposure can occur orally from breast milk or formula feeding, from aerosols, or cutaneously. Cutaneous antigen exposure has been proposed as a key route for allergic sensitization¹⁴. Exposure to antigens by the cutaneous route is more likely if the skin is already disrupted, such as through cuts or the vigorous washing of otherwise healthy skin, or during episodes of atopic dermatitis. Genetic variants that result in compromised epidermal barrier function have been strongly linked with atopic dermatitis¹⁵. Atopic dermatitis in infants often precedes the development of allergic rhinitis and atopic asthma in children — a pattern termed the ‘atopic march’ (REF. 14). Infants who present with atopic dermatitis before the age of 3 months have the highest risk of developing food allergies; the risk is lower in children who present with atopic dermatitis after 12 months of age, and adult atopic dermatitis is rarely associated with food allergy¹⁶. The use of skin creams that contain peanut oil during the first 6 months of life has been reported as a risk factor for peanut food allergy¹⁷. In addition, in a study in the United States of infants aged 3–15 months, higher levels of peanut protein in household dust correlated with increased serum levels of peanut-specific IgE¹⁸. This correlation was stronger in children who had a history of atopic dermatitis, and even stronger in those with

a history of severe atopic dermatitis¹⁸. Taken together, epidemiological evidence supports the hypothesis that exposure to environmental antigens through a disrupted skin barrier is a route for allergen sensitization.

Animal models have also provided support for the hypothesis that food allergen sensitization initially occurs through the skin (FIG. 1a). In mice, skin damage induces the epidermal production of retinoic acid early transcript 1 (RAE1; also known as mRNA export factor), a self stress-induced ligand that binds to the receptor NKG2D, which is expressed by various lymphoid and myeloid cell types¹⁹. Inducing *Rae1* expression in keratinocytes promotes $\gamma\delta$ T cell-dependent antigen-specific T_H2 cell responses to ovalbumin (OVA) placed on the skin¹⁹. The application of OVA or peanut antigen to the damaged skin of mice and subsequent oral challenge with OVA or peanut antigen induced IgE-dependent allergic symptoms including face scratching, an increase in mast cell numbers and T_H2 -type cytokine production in the jejunum, and anaphylaxis^{20–22}. Cutaneous exposure to antigen was associated with an expansion of TSLP-elicited basophils in the skin, and both TSLP and basophils were required for subsequent antigen-specific T_H2 cell responses, antigen-specific IgE production, intestinal mast cell accumulation, and anaphylaxis after oral antigen challenge^{20,21}.

Early antigen exposure through the mucosal route, in contrast to the cutaneous route, can predispose towards tolerogenic responses. Inhaled antigens rapidly reach the intestine²³, as of course do orally ingested antigens, and the intestine-draining lymph nodes are a preferential site for antigen-specific T_{reg} cell generation²⁴. CD103⁺ DCs in the intestinal lamina propria and mesenteric lymph nodes (MLNs) have a unique capacity to metabolize dietary vitamin A into retinoic acid as they express retinal dehydrogenase 2 (RALDH2), and the production of retinoic acid promotes FOXP3⁺CD4⁺ T_{reg} cell generation²⁵ (FIG. 1b). In contrast to mice in which the first exposure to OVA was epicutaneous, mice in which initial OVA exposure was oral showed tolerance to subsequent oral challenge with OVA, and did not generate OVA-specific IgE responses or display symptoms of anaphylaxis²¹. Similarly, the inhalation of OVA by 1-week-old mice was able to block the generation of OVA-specific IgE, prevent the accumulation of granulocytes in the bronchoalveolar lavage fluid (BALF) and reduce BALF IL-13 levels after sensitization and challenge with OVA as adults²⁶. Splenocytes from adult mice that have been exposed to OVA intranasally as neonates produce less IL-13 after OVA stimulation than do mice that have not been neonatally exposed to OVA, and instead they produce more interferon- γ (IFN γ)²⁶. Oral exposure to antigen in neonates via breast milk can promote tolerogenic responses to the same antigen that extend into adulthood, and this is dependent on exposure to transforming growth factor- β (TGF β) in breast milk²⁷.

In some situations, even after oral tolerance has been induced, subsequent cutaneous exposure can modify existing tolerance. When mice were given tolerance-inducing oral doses of peanut protein and then challenged with a footpad injection of peanut protein,

Airway hyper-reactivity

A typical characteristic of patients with asthma in which the airways constrict in response to a variety of inhaled stimuli. This can be measured in people and in laboratory mice by changes in airway resistance that are induced by inhaled methacholine.

Allergic sensitization

The process of generating antigen-specific IgE antibodies against an allergen.

Lymphocyte anergy

An unresponsive state that can be induced in B cells or T cells that have been chronically stimulated, or when their antigen receptor is stimulated in the absence of co-stimulatory signals.

Regulatory T cell

(T_{reg} cell). A specialized type of CD4⁺ T cell that suppresses the activity of other immune cells.

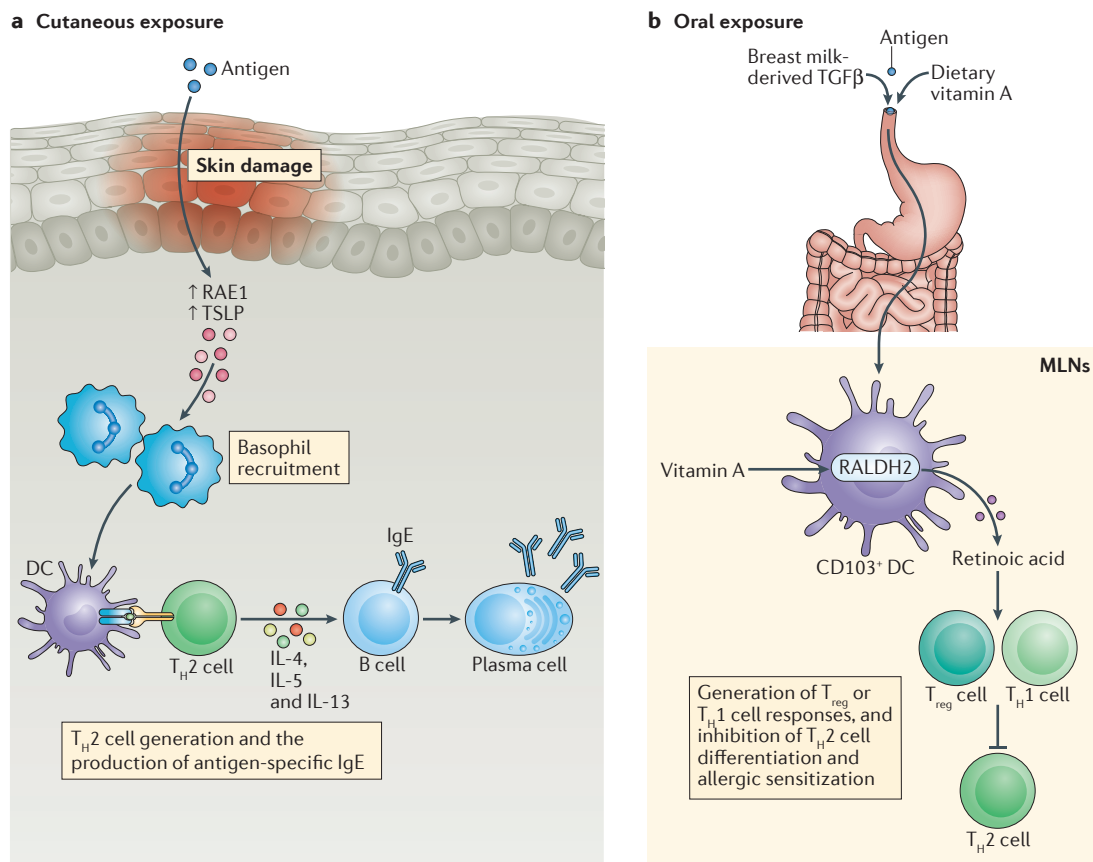


Figure 1 | The route of antigen exposure influences immune priming. a | Cutaneous antigen exposure elicits T helper 2 (T_H2) cell-biased responses, which lead to allergic sensitization. The release of retinoic acid early transcript 1 (RAE1) and thymic stromal lymphopoietin (TSLP) following skin damage results in the recruitment of basophils, which promote T_H2 cell differentiation — probably through an effect on dendritic cells (DCs) — leading to the release of T_H2 -type cytokines, which stimulate the production of antigen-specific IgE^{19–22}. **b** | Mesenteric lymph nodes (MLNs) that drain the intestine are a privileged site for the induction of tolerogenic responses to orally ingested antigen²⁵. CD103⁺ DCs in the MLNs express retinal dehydrogenase 2 (RALDH2), which enables the conversion of dietary vitamin A into retinoic acid. In turn, retinoic acid promotes the differentiation of regulatory T (T_{reg}) cells, maintains the stability of T_H1 cells, and blocks the induction of T_H2 cell responses and allergic sensitization^{25,28,111}. In neonates, breast milk-derived transforming growth factor-β (TGFβ) has been shown to be required to generate tolerogenic responses to antigen that is transferred in breast milk²⁷. IL, interleukin.

they did not show peanut protein-specific T cell proliferation, IL-4 production and footpad swelling, whereas these responses were seen in mice that had not been orally tolerized²². However, mice that were cutaneously exposed to peanut protein after being fed tolerance-inducing oral peanut protein were less protected from subsequent footpad challenge with peanut protein, although they still showed greater protection than did those mice that had never been orally tolerized to peanut protein²². Hence, the route of initial antigen exposure is crucial in determining whether tolerance or allergic sensitization occurs, and the route of subsequent antigen exposures might modify this response to some extent.

The timing of allergen exposure. In a mouse model of oral tolerance, the ability to generate a tolerogenic response to OVA was not fully efficient until 3 weeks after birth owing to lower circulating levels of retinol and fewer MLN CD103⁺ DCs showing RALDH2 activity in the first week of life²⁸. Providing the lactating mothers

of 1-week-old mice with a vitamin A-enriched diet was sufficient to increase the circulating levels of retinol and the frequency of MLN CD103⁺ DCs displaying RALDH2 activity in 1-week-old mice, and crucially, this diet increased the capacity for oral tolerance generation to the levels seen in 3-week-old mice²⁸. After oral exposure to antigen, vitamin A-supplemented mice had a higher frequency of antigen-specific IFNγ-producing CD4⁺ T cells than did mice that did not receive vitamin A supplementation, which suggests that the induction of tolerance in neonatal mice can result from a response that is biased towards type 1 cytokine-producing cells rather than type 2 cytokine-producing cells²⁸. Certainly, blocking T_H2 cell differentiation in neonates has been shown to protect against the generation of T_H2 cell-driven allergic inflammation later in life²⁹. Suppressing T_H2 cell differentiation by administering a signal transducer and activator of transcription 6 (STAT6)-inhibitory peptide to 1-week-old mice suppressed the subsequent induction of T_H2 -driven allergic airway inflammation

Signal transducer and activator of transcription 6 (STAT6). A transcription factor that is required for the differentiation of T helper 2 cells.

in adult mice²⁹. Animal experiments have been valuable for elucidating the immunological pathways that are necessary for tolerance induction early in life, but how this time period translates into the human setting has not yet been established.

Throughout the past several decades, there has been a drastic shift in guidelines recommending the timing of introduction to potential allergens³⁰. Previous guidelines suggested that pregnant women and infants under 3 years of age should avoid potentially allergenic foods such as peanuts, other nuts and shellfish. However, these guidelines have now been reversed. In fact, rather than reducing the rates of allergy, allergen avoidance during the first 6 months of life has been shown to increase the likelihood of allergic sensitization. In a recent study in the United Kingdom, infants who had been exclusively breastfed for their first 3 months of life were either introduced to potentially allergenic foods (peanut, cooked egg, cow's milk, sesame, white fish and wheat) at 3 months of age, or they were exclusively breastfed until 6 months, after which allergenic foods were introduced at the parents' discretion³¹. Those children for whom allergenic foods were introduced early had a significantly lower rate of both peanut and egg allergy at 3 years of age than did those for whom the introduction was delayed³¹. Similarly, in another study based in the United Kingdom, delaying peanut introduction until 5 years of age resulted in significantly increased rates of peanut allergy at the ages of 5 and 6 years compared with children who had been given peanuts regularly from when they were 4–11 months old^{32,33}. A recent study in Italy reported a lower incidence of atopic dermatitis in infants who were introduced to solid foods at 4 or 5 months of age than in those who were exclusively breastfed for the first 6 months of life³⁴.

Together, these studies clearly suggest that delaying exposure to potential allergens is detrimental. However, generating tolerogenic responses to potential allergens probably depends on the immune context in which the potential allergen is recognized, and thus the early life living environment can also influence the capacity for tolerance. Supporting this, alongside a protective effect of early life exposure to cockroach, mouse and cat allergens against wheeze symptoms at 3 years of age, a birth cohort study carried out in the United States noted that exposure to high levels of bacterial content in household dust throughout the first year of life was associated with protection against atopy and wheeze symptoms³⁵.

Co-exposure to endotoxins. It has long been noted that the early life living environment is associated with the risk of allergic disease development. For example, a higher number of siblings in the childhood family home and early life farm-living are both associated with protection against allergies^{36,37}. Farm living³⁸, as well as dog ownership in the urban environment³⁹, are both associated with greater exposure to a diverse range of bacterial microorganisms. At school age (5–13 years), children of farmers show higher expression of the genes encoding Toll-like receptor 2 (TLR2) and CD14 — which is a co-receptor for lipopolysaccharide (LPS)

and other bacterial cell wall microorganism-associated molecular patterns (MAMPs) — on peripheral blood cells than do children whose parents are not farmers^{40,41}. This parallels the upregulation of these receptors that is seen on human blood cells in response to *in vitro* LPS stimulation. Polymorphisms in *CD14*, *TLR2* and *TLR4* have been associated with atopy, which supports the association between the immune detection of microbial products and allergy development⁴². However, growing up in a farming environment is not always associated with protection against atopy; farming practices and consequential microbial exposures vary widely between farms^{43,44}. In a study of children (aged 5–13 years) living in rural areas in Europe, specific exposures — including the presence of pigs, drinking farm milk, regular stays in animal sheds and involvement in haying — were associated with protection against atopic asthma⁴³.

Further work has provided causal evidence that differential microbial exposure on farms affects allergy development. In a comparative study of two agricultural populations in the United States that share similar lifestyles but use distinct farming practices — namely, Amish individuals, who live on traditional single-family dairy farms, and Hutterite individuals, who live on highly industrialized communal farms — household endotoxin levels were associated with allergy prevalence⁴⁴. Median endotoxin levels in house dust from the homes of Amish individuals were 6.8 times higher than those in house dust from the homes of Hutterite individuals, and these higher endotoxin levels were associated with a fourfold-to-sixfold lower prevalence of asthma and allergic sensitization in children (aged 7–14 years). Importantly, when house dust extract from the homes of Amish individuals and those of Hutterite individuals was administered intranasally to mice at the same time as intraperitoneal sensitization to OVA, those mice that received the dust from the homes of Amish individuals showed reductions in airway hyper-reactivity, eosinophil numbers in the BALF and levels of circulating OVA-specific IgE after subsequent intranasal challenge with OVA⁴⁴. The protective effects of the dust extract from the homes of Amish individuals were lost in mice that lacked expression of myeloid differentiation primary response protein 88 (MYD88) and TIR domain-containing adaptor protein inducing IFN β (TRIF; also known as TICAM2)⁴⁴, which suggests that the innate sensing of MAMPs during antigen exposure is responsible for protection against allergic sensitization.

Although much work to date has focused on the total level of endotoxin exposure, further work is necessary to determine the microbial source or sources of endotoxins that can confer protection against allergic sensitization. In a cohort of infants in the United States who were at a high risk of developing asthma, specific exposure to bacteria belonging to the Prevotellaceae, Lachnospiraceae and Ruminococcaceae families in household dust during the first year of life was associated with protection against recurrent wheeze and/or atopy at 3 years of age³⁵. Endotoxins from different bacteria have differing effects on immune activation, and thus focusing on the total levels of endotoxin exposure

Microorganism-associated molecular patterns (MAMPs). Highly conserved patterns that are found within microbial molecules such as components of bacteria cell walls and are recognized by host innate immune cells through pattern recognition receptors.

during early life without examining the source of the endotoxin is likely to be a reductionist approach. For example, LPS from a strain of *Escherichia coli*, but not from *Bacteroides dorei*, elicited potent TLR4-dependent nuclear factor- κ B (NF- κ B) activity, and IL-10, tumour necrosis factor (TNF), IL-1 β and IL-6 production in reporter cell lines and human peripheral blood mononuclear cells (PBMCs)⁴⁵. Even within the *Bacteroides* genus, stimulation with LPS from different species resulted in marked variation in the levels of inflammatory cytokines released⁴⁵. Furthermore, the exposure of mice to *E. coli* LPS, but not *B. dorei* LPS, resulted in endotoxin tolerance to the TLR2 agonist zymosan, which indicates that exposure to LPS from different sources can have long-term effects on cellular responses⁴⁵. Thus, along with the total level of endotoxin sensed, the microbial source of endotoxin exposure is probably a determining factor in whether endotoxin exposure can protect against allergic sensitization.

Microbiota composition and function

Microbial exposures in the early life living environment also have a substantial effect on the composition of microorganisms that colonize the human body. Exposing mice to house dust from homes with dogs significantly altered their caecal microbiota composition, and this was associated with protection against sensitization to OVA or cockroach allergen⁴⁶. Throughout the past 50 years, during which developed countries have experienced a substantial rise in allergy incidence, several factors have contributed to modifying the composition and reducing the diversity of the microbiota. The use of antibiotics, sanitation standards, birth method, feeding method, dietary habits and urban versus farm living all affect the microbiota composition⁴⁷, and several studies have found associations between these factors and allergy development⁴⁸. These observations led to the proposal of the microbiota hypothesis of allergic disease, which suggests that an absence of crucial species within the microbiota results in an incomplete or altered maturation of the mammalian immune system, thus driving a heightened sensitivity to allergens⁴⁹.

Recent studies have examined how the composition and diversity of the early life microbiota affect allergy development later in childhood. In independent Scandinavian studies, low intestinal microbiota diversity in the first month of life was associated with allergic sensitization⁵⁰ and asthma⁵¹ in children aged 6–7 years. In a cohort of children from the United Kingdom, colonization by particular *Bifidobacterium* species in newborns as young as 1-week-old was indicative of later allergy risk⁵². Whereas *Bifidobacterium breve* colonization was associated with a reduced risk of atopic dermatitis in the first year of life, *Bifidobacterium catenulatum* colonization was associated with a higher risk of atopic dermatitis⁵². Two large independent birth cohort studies, one in Canada⁵³ and one in the United States⁵⁴, have recently suggested that the composition of the early life microbiota correlates with the development of atopy and asthma beyond the first year of life.

In a study of Canadian infants, the abundance of four bacterial genera in the faeces of 3-month-old infants was predictive of multisensitized atopy and wheeze in the first year of life, and the asthma predicative index at 3 years old⁵³. A low abundance of the *Faecalibacterium*, *Lachnospira*, *Veillonella* and *Rothia* (FLVR) genera at 3 months of age was associated with a higher risk of allergy and asthma development. Importantly, to confirm that the absence of these bacteria was a driving factor, rather than an effect of atopy development, breeding pairs of germ-free mice were colonized with faeces from a 3-month-old infant who had a positive asthma diagnosis by 3 years of age; the faeces were either supplemented with or lacked species from FLVR genera. The gnotobiotic offspring of these mice were sensitized to OVA by intraperitoneal injection and were then challenged with OVA intranasally. Supplementation with species from the FLVR genera protected mice from OVA-induced airway inflammation, as indicated by reduced lung histopathology scores, reduced lymphocyte and neutrophil numbers in the BALF, and reduced levels of IFN γ , TNF, IL-17A and IL-6 in the lungs⁵³. This suggests that the absence of colonization by particular microbial species early in life can be a driving factor for allergic airway disease⁵³. Similarly, in a longitudinal study of babies from the United States, a particular faecal microbiota composition at 1 month of age was predictive of a higher risk of multi-sensitized atopy at 2 years of age and asthma at 4 years of age⁵⁴. Infants with a lower abundance of species within the *Bifidobacterium*, *Lactobacillus*, *Akkermansia* and *Faecalibacterium* genera at 1-month-old had a higher rate of asthma at the age of 4 than did those with a higher abundance of these species. Alongside differing bacterial microbiota abundances, colonization by fungal taxa was associated with asthma: the group of infants who showed a higher rate of asthma development at 4 years of age had been colonized with higher levels of *Candida* spp. and *Rhodotorula* spp., and lower levels of *Malassezia* spp., at 1 month of age than had the group of infants who showed a lower rate of asthma development at 4 years of age. The authors suggested that the long-term immunological consequences of particular early life microbiota profiles may be exerted through the production of distinct metabolites. They reported that the early life faecal metabolomes of the group of children who showed a higher rate of asthma at 4 years old were distinct from those of the group with lower rates of asthma at this age. Furthermore, when filter-sterilized faecal contents (containing microbial ligands and metabolites) from high-asthma-risk infants were cultured with adult human DCs before their co-culture with human peripheral T cells, the resulting proportion of IL-4-producing CD4⁺ T cells was higher than it was after the exposure of DCs to faecal contents from infants in the lower-risk group⁵⁴.

Early life microbiota depletion in animal models. Both germ-free mice and neonatally antibiotic-treated mice are more sensitive to several models of allergy induction — including models of food allergy⁵⁵, allergic

Germ-free mice

Mice that are not colonized by any microorganisms and so do not have a microbiota.

Gnotobiotic

Colonized with a known microbiota composition.

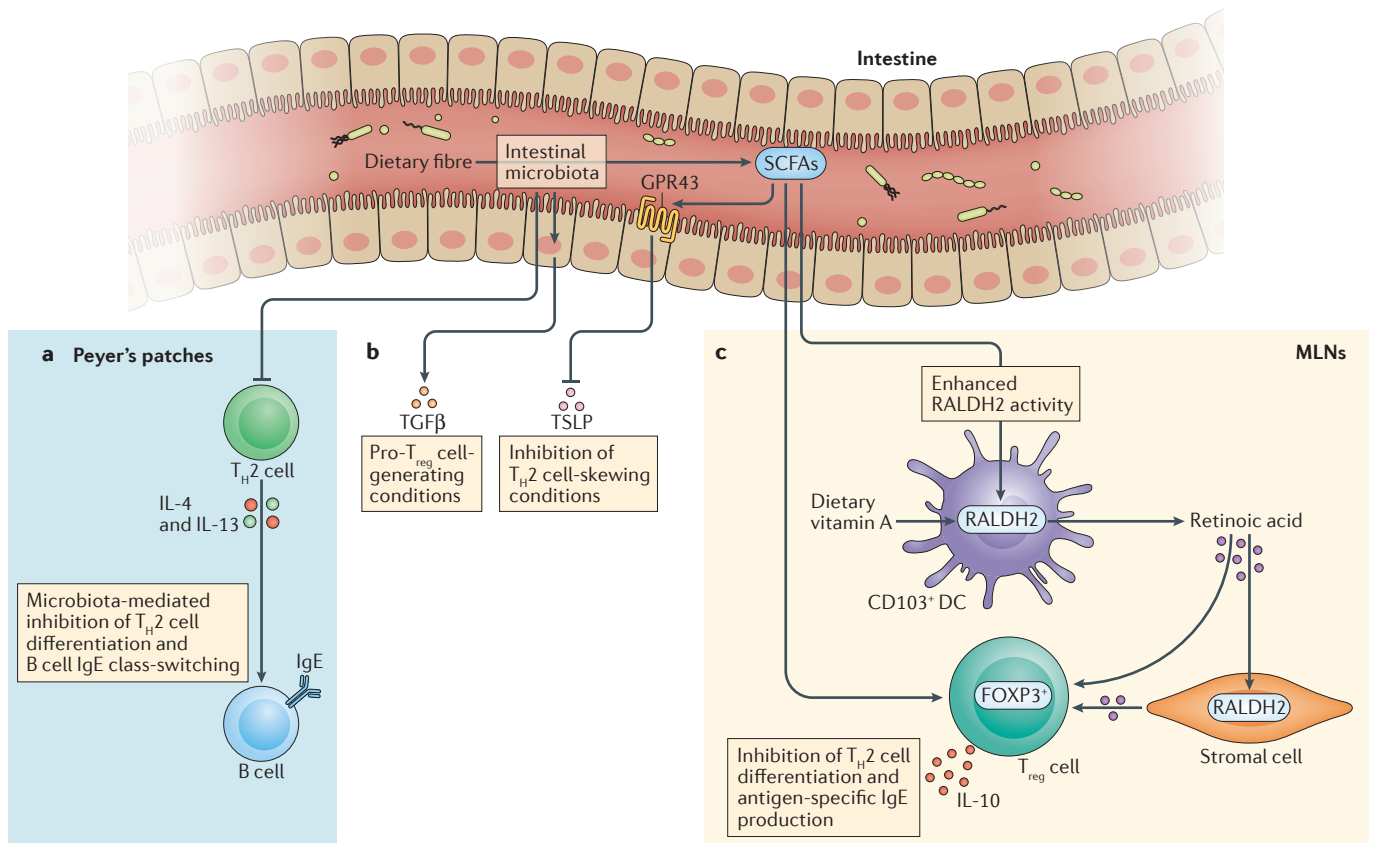


Figure 2 | A diverse microbiota can inhibit allergic sensitization. a | A diverse microbiota can control circulating IgE levels by limiting the levels of T helper 2 (T_H2)-type cytokines such as interleukin-4 (IL-4) and IL-13 in the Peyer's patches before adulthood⁵⁹ or by stimulating myeloid differentiation primary response protein 88 (MYD88) signalling in B cells (not shown)⁵⁶. **b** | The microbiota can influence cytokine production by intestinal epithelial cells, thus altering the cytokine milieu of the underlying lamina propria. A selection of *Clostridia* spp. can stimulate the production of transforming growth factor β 1 (TGF β 1) by epithelial cells, and this results in the induction of regulatory T (T_{reg}) cells^{64,69}. Short-chain fatty acids (SCFAs) released by the microbiota during the fermentation of dietary fibre act at least in part through G protein-coupled receptor 43 (GPR43; also known as free fatty acid receptor 2), which is expressed on epithelial cells, to inhibit expression of the gene that encodes the T_H2 cell-skewing cytokine thymic stromal lymphopoietin (TSLP)⁷⁰. **c** | SCFAs can boost the frequency of T_{reg} cells and increase their IL-10 production^{66–68}, thus inhibiting pathways that lead to allergic sensitization^{70,71}. SCFA exposure increases the activity of retinal dehydrogenase 2 (RALDH2) in CD103⁺ dendritic cells (DCs) in the mesenteric lymph nodes (MLNs); RALDH2 mediates the conversion of dietary vitamin A to retinoic acid, which stimulates T_{reg} cell generation⁷⁰. The microbiota imparts T_{reg} cell-inducing properties on MLN stromal cells²⁴, possibly through the induction of RALDH2 in stromal cells by SCFAs (not shown). Retinoic acid can also promote the expression of RALDH2 in both DCs and stromal cells¹¹². FOXP3, forkhead box protein P3.

airway inflammation^{56–58} and oral anaphylaxis⁵⁹ — than are specific pathogen-free mice (SPF mice) and non-antibiotic-treated mice, respectively. The exposure of mice to vancomycin *in utero* and for a period until weaning is sufficient to result in heightened adult susceptibility to a model of OVA-induced allergic inflammation^{57,60}. Mice exposed neonatally to antibiotics have elevated adult steady-state serum IgE levels compared with mice that were not treated with antibiotics^{57,60}, and similarly, germ-free mice exhibit elevated IgE levels compared with SPF mice^{56,59}. Microbiota colonization within a week post-weaning was necessary to limit adult IgE levels in previously germ-free mice, and colonization with multiple members of altered Schaedler flora species was required to limit adult IgE levels⁵⁹. This suggests the existence of a time window in early life during which a

diverse microbiota is required to inhibit lifelong IgE levels. A diverse microbiota may limit B cell class-switching to IgE by directly stimulating TLRs on B cells⁵⁶ and/or by reducing the production of IgE class-switch-promoting IL-4 production in Peyer's patches⁵⁹ (FIG. 2a).

In mice, the microbiota has been shown to contribute to intestinal barrier function, and this may protect against sensitization to dietary allergens by stimulating retinoic acid receptor-related orphan receptor- γ t (ROR γ t)-expressing ILCs and T cells in the lamina propria to produce IL-22 (REF. 55), by inducing mucosal IgA production⁶¹ and by modulating colonic mucus structure⁶². A 'leaky' intestine in the absence of adequate microbial colonization could result in increased antigen contact with and uptake by lamina propria DCs, which, in the absence of concurrent TLR stimulation,

Specific pathogen-free mice (SPF mice). Mice that are free from colonization by particular pathogens but that have a microbiota.

could promote allergic sensitization to dietary antigens. Intestinal microbial colonization in mice also contributes to a tolerogenic intestinal environment by inducing the generation and suppressive capacity of T_{reg} cells^{63–68}. An absence of key T_{reg} cell-inducing bacterial species during the neonatal period in mice can result in exaggerated allergic inflammation later in life^{57,60}. Within the microbiota, particular bacterial species are potent inducers of extrathymic T_{reg} cells. Some *Clostridia* species can induce FOXP3⁺CD4⁺ T_{reg} cells in mice by stimulating intestinal epithelial cells to produce TGFβ1 (REFS 64, 69) (FIG. 2b). Other bacterial species can induce peripheral T_{reg} cell differentiation and inhibit T_H2 cell differentiation by producing metabolites, particularly the short-chain fatty acids (SCFAs) acetate, butyrate and propionate, during dietary fibre fermentation^{66–68}. SCFAs can increase RALDH2 activity in MLN CD103⁺ DCs⁷⁰, boost IL-10 production by T_{reg} cells^{67,68}, inhibit intestinal epithelial cell expression of *Tslp*⁷⁰, reduce the activation state of DCs and impair the production of T_H2-type cytokines by CD4⁺ T cells⁷¹ (FIG. 2b,c). A high dietary fibre intake, which leads to increased microbiota-driven SCFA release, can inhibit allergic inflammation in mouse models of food allergy and allergic airway inflammation^{70,71}. Signals from the microbiota and metabolites can imprint long-lasting tolerogenic properties on the stromal cells of intestine-draining lymph nodes. Stromal cells from the MLNs of germ-free mice are unable to support the generation of T_{reg} cells, in contrast to MLN stromal cells from SPF mice, which are able to promote the generation of T_{reg} cells even 20 weeks after transplantation²⁴. The ability of MLN stromal cells to promote T_{reg} cell generation is not inhibited by antibiotic-mediated depletion of the microbiota in adulthood, which suggests that the tolerogenic properties of MLN stromal cells are stable throughout life, provided that they are exposed to microbiota signals in early life²⁴. Taken together, a diverse early life intestinal microbiota can inhibit the pathways that lead to allergic sensitization by multiple mechanisms.

Microbiota colonization beyond the intestine. The skin also harbours microbial communities, and these can regulate components of the complement system and local IL-1 and IL-17A production⁷². Whether the composition of the skin microbiota is an initial driving factor in allergy development or can simply aggravate existing atopy is not clear. Skin colonization or infection by *Staphylococcus aureus* has been associated with flares of atopic dermatitis in humans⁷³. δ-toxin from *S. aureus* can induce potent mast cell degranulation, and *S. aureus* recovered from patients with atopic dermatitis was found to produce high levels of δ-toxin⁷⁴. In a mouse model, skin colonization with *S. aureus* promoted IgE production, IL-4 production and dermatitis, whereas this effect was lost when mice were skin-colonized with a *S. aureus* mutant deficient in δ-toxin⁷⁴. Successful treatments for atopic dermatitis have been associated with an increase in skin microbiota diversity in patients, which suggests that the restoration of skin microbiota diversity could precede improvements in clinical disease symptoms⁷⁵.

Given the strong association between atopic dermatitis and the subsequent development of allergic asthma or food allergy, it will be worth investigating whether skin microbial communities during the neonatal period can be a driving factor for atopic dermatitis development.

The timing of lung colonization by microorganisms, as well as the timing of intestinal colonization, may also influence whether tolerance or allergic sensitization to foreign antigen exposure occurs. Increasing levels of bacteria are detectable in the lungs of neonatal mice throughout the first 2 weeks of life⁷⁶. SPF mice, but not germ-free mice, have a peak in programmed cell death protein 1 ligand 1 (PDL1) expression on lung CD11b⁺ DCs at 8 days following birth, and this correlates with a peak in the frequency of lung FOXP3⁺CD4⁺ T_{reg} cells⁷⁶. Before this time point, 3-day-old mice produced more T_H2-type cytokines and had a higher frequency of eosinophils in the BALF in response to house dust mite (HDM) stimulation than did 15-day-old mice or adult mice. Blockade of PDL1 during the first 2 weeks of life resulted in exaggerated responses to HDM being maintained into adulthood⁷⁶. These findings, together with the observation that adult germ-free mice show exaggerated responses to HDM compared with adult SPF mice⁷⁷, suggest that microbial colonization early in life can boost the frequency of lung T_{reg} cells and promote tolerance to foreign aero-antigens through a mechanism involving PDL1-expressing lung CD11b⁺ DCs. It remains to be determined whether microbial colonization of the lung specifically, as opposed to intestinal colonization, is required for the induction of PDL1-expressing CD11b⁺ DCs.

Within 5 minutes of birth, bacteria are present in the oral cavity and nasopharynx of human neonates⁷⁸. A recent Australian study reported six compositionally distinct profiles of bacterial microorganisms in the nasopharynx of infants during the first year of life, each dominated by species from one of the following genera: *Moraxella*, *Corynebacterium*, *Alloiococcus*, *Staphylococcus*, *Haemophilus* or *Streptococcus*⁷⁹. Specific nasopharynx microbiota states were associated with differential risks of developing childhood asthma in the future, as well as with the frequency and severity of acute respiratory infections⁷⁹. This suggests that particular microbiota compositional states could alter the risk of allergic sensitization by promoting colonization by pathogenic bacterial or viral species that have been associated with driving or exacerbating allergic sensitization.

Early life infections

Early life infections that exacerbate allergic disease.

Airway fungal infections have been linked with exacerbations of existing allergic asthma in adults and children, and fungal skin infections have been associated with worsened symptoms of atopic dermatitis⁸⁰. Fungal proteins can cause airway damage, thus increasing the allergenic capacity of bystander proteins, and can themselves be potent allergens⁸¹. In a mouse model, intranasal exposure to *Alternaria alternata* extract resulted in the release of IL-33 into the BALF within 1 hour, and increased the recruitment of eosinophils,

ILC2s, macrophages, neutrophils and lymphocytes to the lungs in mice that had previously been sensitized to HDM antigen⁸². *A. alternata*-induced IL-33 production has been associated with an increased resistance of mice to steroid therapy⁸³. In a study of children with severe therapy-resistant asthma, those with fungal-specific IgE responses in a skin-prick test had higher levels of BALF IL-33 than did children who had no evidence of fungal sensitization⁸³. Hence, exposure to fungal allergens seems to exacerbate inflammation responses to previously sensitized allergens by boosting T_H2 cell responses.

Airway viral infections have also been associated with exacerbated symptoms of established allergic asthma, and this has been attributed to lung epithelial cell damage that results in the increased recruitment and activation of lymphocytes, neutrophils and eosinophils⁸⁴. Severe early life viral infections — particularly with respiratory syncytial virus (RSV) and human rhinovirus — are a risk factor for subsequent asthma development⁸⁵, although whether severe respiratory viral infections are initial drivers of atopy or act to exacerbate existing allergic sensitization is less clear. Similarly, airway infections with the bacterial pathogens *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae* have been significantly associated with wheeze symptoms during the first 3 years of life⁸⁶. In mouse models, neonatal exposure to mouse-specific pneumovirus species exacerbates airway inflammation in response to intranasal OVA or cockroach allergen exposure^{87,88}. Early airway-allergen sensitivity may also predispose to lung viral infection, as the induction of IL-33 in mice in response to cockroach allergen exposure inhibits antiviral IFN α production, thus resulting in an increased epithelial viral burden⁸⁷. Early life RSV infection in mice can impair breast milk-induced tolerance to OVA⁸⁹. In this model, RSV infection induced an IL-4 receptor subunit- α (IL-4R α)-dependent T_H2-like effector phenotype in lung FOXP3⁺CD4⁺ T_{reg} cells, thus increasing their GATA3 expression and T_H2-type cytokine production, and compromising their suppressive function⁸⁹.

Early life infections that promote tolerance. The timing of infection and the species of virus seem to be crucial in determining the effect of infection on allergic sensitization, as influenza A virus infection of 2-week-old suckling pups, but not adult mice, can protect adults from OVA-induced airway inflammation⁹⁰. Respiratory viral infections can alter the composition of the intestinal microbiota⁹¹, which raises the possibility that particular viral species affect the tendency for allergic sensitization by altering the function of the microbiota during a developmental time window in early life.

Several studies have found an association between childhood intestinal colonization with the bacterial pathobiont *Helicobacter pylori* and protection against atopic dermatitis, wheeze, allergic sensitization and asthma, although in many individuals the association is weak or not present⁹². Mouse models have demonstrated that *H. pylori* infection can protect against OVA-induced or HDM-induced allergic airway inflammation, with the strongest protection achieved when mice are

infected as neonates (that is, at 6 days old)⁹³. Mice that were orally infected with *H. pylori* as neonates showed a higher number of lung FOXP3⁺CD4⁺ T_{reg} cells than did uninfected mice. The depletion of T_{reg} cells during infection abrogated this protection against airway inflammation, and the transfer of FOXP3⁺CD4⁺ T_{reg} cells from the MLNs and Peyer's patches of neonatally infected mice — but not from uninfected mice — was sufficient to protect against airway inflammation in recipient mice⁹³. The oral administration of *H. pylori* extract could also reduce allergic inflammation that is induced by sensitization and challenge with OVA in adult mice, and similarly, this treatment was more effective at suppressing airway inflammation when given to 7-day-old mice than when given to adult mice⁹⁴. Protection against allergic airway sensitization resulting from the administration of *H. pylori* extract did not require the presence of FOXP3⁺CD4⁺ T_{reg} cells, but did require IL-10 — which was at least in part derived from CD11c⁺ cells — and IL-18 (REF. 94). Specifically, basic leucine zipper transcriptional factor ATF-like 3 (BATF3)-dependent CD103⁺CD11b⁺ DCs were recruited to the lungs following OVA challenge in mice that were neonatally exposed to *H. pylori*, and these DCs were required for *H. pylori*-mediated protection against allergic airway inflammation⁹⁴. In humans, colonization with *H. pylori* commonly occurs during early childhood, most often after the first year of life⁹⁵. It is likely that the age of exposure to *H. pylori* influences the relationship between *H. pylori* and allergy development, although this has not yet been established.

Colonization by helminths has been associated with reduced skin-prick test reactivity and reduced atopic wheeze symptoms during childhood⁹⁶. Animal models have been crucial in demonstrating a causal relationship between the presence of helminths and the inhibition of allergic inflammation⁹⁷. Paradoxically, helminth infections elicit many of the type 2 responses that are also responsible for the pathogenesis of allergy, including the production of T_H2-type cytokines and IgE, and the recruitment of mast cells and eosinophils. However, concurrent regulatory responses inhibit the efficacy of this type 2 response, which can both prevent the expulsion of helminths and inhibit allergic pathology in the host⁹⁸. Importantly, not all helminth species are associated with protection against allergies in human populations: the presence of *Ascaris lumbricoides*-specific IgE has been positively associated with allergic asthma, and this association may be due to the crossreactivity of IgE generated against *A. lumbricoides* tropomyosins with mite tropomyosins⁹⁹. Hence, prior exposure to *A. lumbricoides* may increase the likelihood of developing allergic symptoms, although ongoing *A. lumbricoides* infections in childhood have been associated with reduced atopic dermatitis and skin-prick test reactivity⁹⁶.

A few clinical trials have investigated the therapeutic effects of introducing live helminth infections into adult patients with allergies¹⁰⁰. Trials to date have shown no significant improvement in the symptoms of allergy or asthma during live helminth infection. However, it should be noted that trials have so far been conducted in small numbers of patients, used low doses of helminths

Pathobiont

An organism that can exist as an innocuous member of the microbiota but that in some circumstances can cause pathogenesis.

and involved adult patients who had ongoing allergic symptoms. Animal studies showing the protective effects of helminths during allergy have used high doses of helminths that would not be considered safe for use as a live therapeutic in humans. For this reason, a current focus is on isolating the immunomodulatory molecules that are produced by helminths¹⁰¹, as this would circumvent the need for high-dose live infections. The isolation of protective helminth-derived molecules may provide prophylactic therapies for use in children who are at a high risk of developing allergy or asthma.

A recent study demonstrated that intraperitoneal injection with a hookworm secreted protein (namely, anti-inflammatory protein 2 (AIP2)) was able to suppress antigen-specific IgE responses and lung eosinophilia in an OVA-driven mouse model of lung inflammation¹⁰². AIP2 was taken up by MLN CD103⁺ DCs and upregulated their RALDH2 activity, resulting in an increased frequency of FOXP3⁺CD4⁺ T_{reg} cells in the small intestinal lamina propria and trachea. Crucially, AIP2 seemed to exert long-term protection against airway inflammation that is mediated by MLN cells, as MLNs transferred from AIP2-exposed mice to MLN-excised mice were able to protect against subsequent airway inflammation that was induced by OVA 6 weeks after MLN transfer. Taken together, these findings suggest that exposure to helminth-secreted products may exert long-term tolerogenic properties on intestine-draining lymph

nodes¹⁰². Concurrently, helminths may contribute to the generation of a tolerogenic microbiota¹⁰³. The presence of helminths induces compositional changes in the microbiota, both in humans and in animals¹⁰⁴. In a mouse model, the transfer of a *Heligmosomoides polygyrus*-modified faecal microbiota (without the transfer of a live helminth infection) could inhibit HDM-induced airway eosinophilia¹⁰³. Mice that acquired a *H. polygyrus*-modified microbiota had elevated SCFA levels in their caeca, and a live *H. polygyrus* infection was unable to suppress HDM-induced airway inflammation in mice that lacked expression of the SCFA receptor G protein-coupled receptor 41 (GPR41; also known as G protein-coupled oestrogen receptor 1)¹⁰³. Together, these data indicate that one mechanism by which helminth infection can inhibit allergic airway inflammation is by promoting the outgrowth of SCFA-producing microbiota species.

Conclusion

It is becoming increasingly clear that environmental factors during the first year of life have long-term health consequences. Recent work that has coupled observations in large human cohorts with the use of animal models has been extremely valuable in the identification of causal factors that promote tolerance to foreign antigens or drive allergic sensitization or exacerbate existing allergic disease. Given that there are notable differences in the quality of immune responses to foreign antigens and stimuli even in the few weeks after birth in mice (FIG. 3), future work should focus on developing an understanding of how tolerance to foreign antigens is generated during a developmental time window in early life and how this time period translates into the setting of human neonates.

Allergens are not sterile exposures, and the microbial context in which they are first recognized by the immune system — alongside the timing and the route of first exposure — can influence the direction of the resulting immune response. Early mucosal exposure to potential allergens, particularly in the context of specific microbial exposures, seems to protect against allergic sensitization. The early life environment drives the structure of the microbiota, which can prime the immune system and promote tolerance. Furthermore, the composition of the microbiota can influence the colonization dynamics of infectious pathogens, and infectious pathogens themselves may promote tolerance or exacerbate existing allergic disease.

Several studies have reported the existence of distinct microbiome compositional states during the first year of life that each result in distinct metabolic profiles and that are associated with differential risks of allergy development later in childhood^{53,54,79}. Validating key species or metabolites that are indicative of particular microbiome states would allow for the development of therapeutic interventions to promote tolerogenic pathways before the symptoms of allergic disease become evident. Immunomodulatory microorganism-derived and helminth-derived molecules have great potential as therapeutics, as well as for prophylactic use in infants who are at a high risk of developing allergic disease.

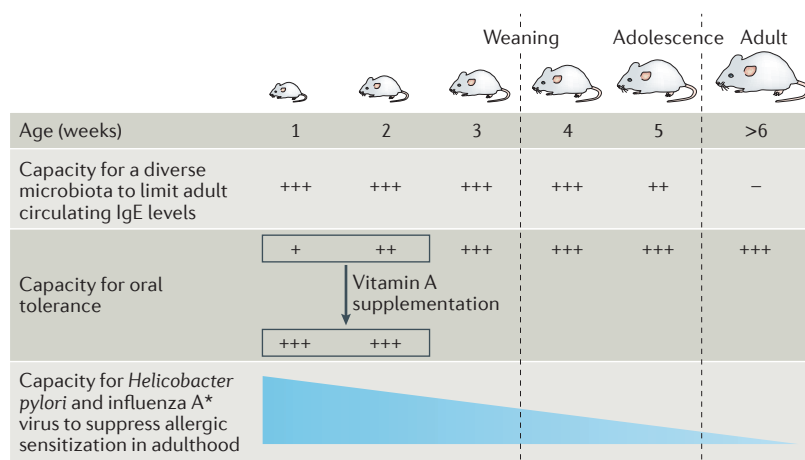


Figure 3 | Developmental time windows in early life affect adult allergic sensitization in mice. Neonatal mice differ from adult mice in their capacity for generating oral tolerance, and in the effect that colonization by the microbiota or infectious pathogens has on allergic sensitization. The colonization of germ-free mice with a diverse microbiota at 1 week post-weaning can limit adult circulating IgE levels, yet colonization during adulthood cannot⁵⁹. In the period immediately after birth, mice have an impaired ability to generate oral tolerance owing to low serum levels of retinol, but supplementation of vitamin A via the mother's breast milk can boost serum retinol levels and increase tolerogenic capacity to the levels found in adult mice²⁸. Early life infections with certain pathogens, including *Helicobacter pylori* and influenza A virus, are more effective at inhibiting allergic sensitization during the neonatal period than when the same infections are given to adults^{90,93,94}. How these developmental time windows translate into the setting of human neonates is not yet clear, although the microbiota composition in human infants in the first 3 months of life can be predictive of atopy in later childhood^{53,54}. *Of note, infection with viral species other than influenza virus, particularly respiratory syncytial virus and human rhinovirus, has been linked with exacerbated allergic symptoms in both infancy and adulthood^{85,87–89}.

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