Allergy, Immunity and Tolerance in Early Childhood

The First Steps of the Atopic March

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Introduction

One understands life only backwards; to live, you must do it forwards.
Søren Aabye Kirkegaard

The term "allergic march" refers to a characteristic sequence of phenotypic changes of various manifestations in infancy, childhood, and adolescence that seem to be co-expressed or genetically linked. Despite a wide range of variation in individual patterns of comorbidity, there appears to be a common developmental pattern including:

1. a sequence of individual immunoglobulin E responses starting with cow's milk and hen's egg proteins, followed by indoor and outdoor allergens
2. a sequence of clinical symptoms starting with eczema, which may be followed by rhinitis and asthma. In most cases, the first atopic manifestations can be observed in early infancy. The natural history of the disease has been carefully evaluated over the past two decades by various researchers. They have provided a remarkable gain in knowledge regarding the earliest changes during pregnancy and their predictive value, the role of genes, environmental exposure to allergens, microbial products and pollutants, and lifestyle and the role of the microbiome.

As it has turned out, atopic diseases have become a major health problem in childhood in many affluent countries, forcing general pediatricians and primary care physicians to come to terms with them. The burden of atopic diseases is frequently dramatic and the cost of health care to deal with this problem in many countries is highly relevant.

Currently, atopic manifestations represent a challenge for the pediatric allergist, who is not only responsible for early diagnosis and treatment, but has also a long-lasting interest in concepts for primary prevention. A number of highly interesting studies in animal models have led to promising concepts; unfortunately, only a few of them have been confirmed in humans. In this book, top experts in the field have been invited to contribute; presenting an update on accumulated knowledge of the natural history of the disease, as well as hopes and disappointments surrounding allergen-specific or nonspecific early interventions, including studies of microbial products. Not least in its achievement, this book outlines gaps in knowledge and presents novel hypotheses that might stimulate research and patient care in years to come.

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The Maturation of Immune Function in Pregnancy and Early Childhood

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Introduction

"The first 1000 days" has become a core focus and a prominent catch phrase in growing efforts to understand the developmental programming of disease predisposition in early life. Spanning all aspects of health, the strong concept of the developmental origins of health and disease is based on clear evidence that events and exposures in early development can have lasting and sometimes latent effects on later health.[1,2] Although the early focus of this research was on later-onset cardiometabolic diseases, it is clear that earlier-onset noncommunicable diseases (NCDs) such as allergic diseases share similar risk factors and should be regarded as a core part of this agenda.[3] Understanding the early environmental influences on early immune development is also a key element in disease prevention.

The immune system has a critical role in the development, homeostasis, and function of virtually all organ systems. Subtle early variations in the pattern of immune response can predispose to disease and influence both the propensity and the dynamics of inflammation in later life.[4] The modern epidemic of infant allergic disease has drawn considerable attention to the importance of early immune development and the potential impact of modern environmental changes on the maturing immune system. In addition to its obvious role in the rising predisposition to allergic and autoimmune disease, early immune dysregulation is now implicated in a range of NCDs, including predisposition to mental health disorders and cardiovascular disease.[3,5] With economic prosperity and lifestyle changes, the rising propensity for inflammation is implicated in the rising burden of chronic disease and all-cause mortality in modern societies. It is therefore critically important to understand the normal processes and pathways that underpin the normal immune development, how these are modified by adverse early environmental exposures to lead to disease, and how these may be favorably modified to reduce that risk. Optimizing immune health in early life will reduce the risk of allergy and immune diseases, but it is also likely to reduce the burden of many other chronic inflammatory diseases. Because immune function is so responsive to the early environment, and because immune

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disease manifests so early, this provides an important early indicator for the effects of environment on human health.

Immune development in health and disease

Healthy immune maturation depends on efficient communication between the two pillars of immune regulation, the *innate and adaptive immune systems*, which are composed of a large number of involved cells (details are given in Ref. 6).

In brief, innate immune cells such as monocytes, granulocytes, dendritic cells (DC), natural killer (NK) cells, mast cells, thrombocytes, and locally relevant cells (e.g., pulmonary alveolar macrophages) are present during healthy immune maturation and are mostly active in some allergy-affected organs such as, for example, the skin and the respiratory tract.[7]

In addition, innate lymphoid type 2 cells, NK2 subsets, and regulatory NK and NK22 cells were shown to be involved in functional T cell responses, the production of immunoglobulin E (IgE), and the function of the epithelial cell barrier, are thus possibly involved in allergy development.[8,9] Also, precursors of innate bone marrow-derived mucosal DC and the NLRP3 inflammasome may be involved in either inflammatory signaling cascades after virus infections or airway inflammation,[10] both of which are relevant for allergy development.

In close connection with innate immunity, the key players of adaptive immune regulation, namely different T cell subpopulations composed of Th2, Th1, and T_{reg} but also Th17, Th9, and Th22, and CD8+ cells, B cells, and regulatory B cells, are critical for healthy immune maturation and thus protection against allergic diseases.[7]

Early life immune regulation

The prenatal period is instrumental in shaping a child's immune system ("programming") influenced by a wide variety of factors elucidated below, including microbiome, nutrition, smoke exposure, and infection, among many others (Figure 1). This window of opportunity is thus critical for a wide range of risk and protective influences discussed in more detail below. Multifaceted effects on early immune programming can occur prenatally that are critical for effects on local tissues and relevant for risk for or protection from immune-mediated diseases, which may occur only several years later. Thus, an efficient interplay between innate and adaptive immune regulation can shape the maturing immune system, keeping it balanced over numerous years during childhood. Any default regulation affecting solely parts of the system or even cells can result in different immune-mediated diseases such as infections, more chronic diseases such as, for example, allergies, autoimmune diseases, or lack of tolerance.

Whereas bidirectional interactions between the fetus and mother seem critical for postnatal immune regulation, human studies on causal effects are complex because of multifaceted influences that are difficult to study at this time of maturation.

In addition to genetic factors such as "immutable footprints", epigenetics, the environment, and their interactions influence early immune programming, subsequently

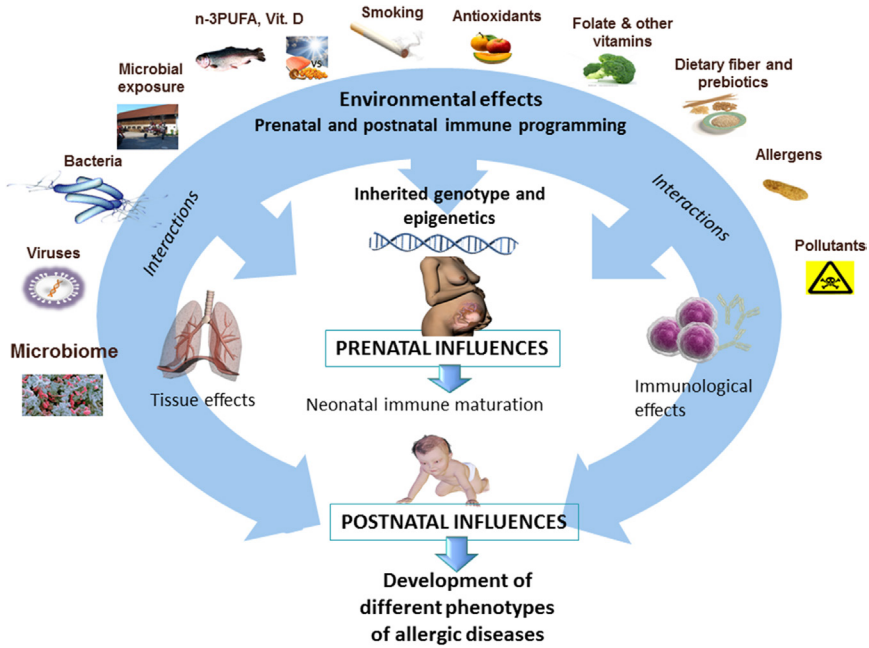


Figure 1 Influences on prenatal and postnatal immune programming for the development of allergic diseases. A wide range of environmental factors acting prenatally and/or postnatally are known to influence the maturation of immunological competence combined with genotype and epigenetics, and hence to modulate the risk for the development of allergic diseases. Reproduced with permission from Holt, Sly, Prescott, 2011. Early life origins of allergy and asthma (Figure 3). In: Stephen T., Holgate, Martin K., Church, David H., Broide, Fernando, D. (Eds.), Allergy: Principles and Practice, fourth ed. Elsevier Inc., Martinez.

affecting anatomical structures such as the mucosa and epithelium, and influencing barrier function.

The exact nature underpinning intrauterine modulatory mechanisms may be composed of the following: Although the amniotic fluid has been shown not to be sterile, direct and indirect modulation via fetoplacental transfer may occur. Decidual tissue maternal immune cells including macrophages, $CD8^+$ and γδ-T cells, and large granulated lymphocyte cells are able to induce rejection of paternal histocompatibility antigens. Maternal–fetal tolerance to paternal alloantigens is actively mediated, involving pT_{regs} (peripheral T_{regs}) distinctly responding to paternal antigens for tolerance induction.[11] Generally, maturation of the infant adaptive immune system occurs from the 15th to 20th week of gestation and can be Ag-specific.

Postnatal immune maturation influences are comparable to before birth, with the major difference in the absence of direct maternal environment. Whereas effects on immune programming most likely happen continuously with different thresholds on several types of immune cells, numerous factors induce changes directly in the organs subsequently affected by later disease. For allergic diseases, for example,

airway antigen-presenting cells are most likely involved in local damage during airway inflammation and are critical to programming of adaptive T cell responses after migration to the lymph nodes.

Regarding innate priming, children during the first year of life are not present in the airways without an inflammatory trigger.[12] During respiratory infection, mature DCs are revealed,[13] indicating that local pulmonary inflammation can influence DC maturation followed by T cell activation.

Although early respiratory tract infections (e.g., rhinovirus-induced) are linked to allergic inflammation, this early priming in the airways seems to be specific to the infectious agent.[14] Taken together, early childhood exposure to infectious agents affects immune programming, locally and systemically, inducing immune networks and subsequently influencing T_{regs} and Th1/Th2 cell fate that is important for healthy immune balance. It is still unknown which type of infections in the skin and upper and lower respiratory tract are important for immune programming. Different viruses or bacteria in close interaction with genetic, epigenetic, and environmental factors may influence early immune maturation in distinct ways, either predisposing for disease development or protecting against it.

Interplay of innate and adaptive immune regulation in organ-specific responses

The nasopharyngeal tract, extending via the glottis and trachea to bronchi and bronchioli of the lung, exerts an efficient defense system against the multitude of airborne pathogens to which it is regularly exposed.[6] **Innate immunity** acts via mechanical, physical, and chemical barrier functions, fighting against invading microorganisms. Its main goal is to decide between innocuous and hazardous microorganisms, avoiding invasion of particles larger than 5 μm into the upper airways.

Regarding the skin, innate mechanisms regulate via the inflammasome and influence thymic stromal lymphopoietin induction; antimicrobial peptides and human β-defensins are involved in the pathophysiology of allergic skin diseases.[15,16]

Whereas **adaptive immune** regulation is closely linked to innate mechanisms, its onset happens in a slower fashion, needing a few days for efficient defense. Induction through cellular activation after innate immune regulation occurs primarily for respiratory tract infections. Here, humoral and mucosal immune regulation is important in parallel to T cell–induced immunity.

In this context, childhood asthma as a complex multifaceted disease entity characterized by inflammation, airway remodeling, and airway hyper-responsiveness is currently viewed as a syndrome with distinct disease entities.[6] Different endotypes describing distinct functional mechanisms are likely to be mediated by different innate-adaptive interplay, critically influenced during early immune maturation.

Although T_{reg} are now described as critical players in the pathophysiology of allergic asthma, their dysregulation is associated with predisposition to respiratory infections early in life, with deficient IgE class-switch and reduced induction of tolerance.[17]

At birth, decreased T_{reg} numbers were associated with increased asthma risk, whereas increased T_{regs} were linked to protection from asthma.[18,19] This asthma-protective effect via T_{regs} was persistent at age 4 years in the European PASTURE birth cohort.[20] In parallel, T_{regs} protected against allergic sensitization early in life (first year) in a high-risk asthma cohort in the United States, associated with an increase in T_{regs} after allergic sensitization and eczema as a potential counterregulation.[21] In the German Cross-sectional Childhood Asthma Study, allergic asthma was associated with increased T_{regs} during manifestation of disease, pointing to a potential feedback mechanism to control inflammation.[22] Extending this, nonallergic asthma was induced by neutrophilic Th17-shifted immune regulation.[22]

Relevant for immune maturation, some childhood asthma phenotypes are actually similarly present in adults, such as eosinophilic and neutrophilic asthma, among others.[23] Adult asthma has been further characterized by Th2 high and low phenotypes, by measuring *POSTN* (periostin), *CLCA1*, and *SERPINB2* in airway epithelial brushings or interleukin (IL)-4, IL-5, and IL-13 in sputum cells.[24] This has not yet been confirmed in children, but it accounts for different maturational effects among children and adults.

Taken together, multifaceted regulation of innate and adaptive immune mechanisms is relevant for asthma development and may be distinct for different asthma phenotypes.

Key influences and determinants of early immune function and regulation

A broad range of factors can influence patterns of immune development during the first 1000 days, and possibly even before this. Each of these, discussed in turn below, may have differential effects at different stages of development, and some may even be important before conception through the effects of parental health. Relatively little is known about potential transgenerational effects of environmental exposures in humans, although animal models have elegantly demonstrated that grand-maternal environmental exposures can be potentially inherited through epigenetic modifications.[25] This includes genes that predispose to the allergy/asthma phenotype. Although the significance of this is not known, it highlights the growing complexity of investigating the impact of a changing environment on the risk of disease.

In addition to direct effects on immune maturation, many types of environmental exposure implicated in the risk of allergic disease, such as nutrition, the microbiome, and pollutants, also affect the development and function of many organ systems. This may contribute to the organ-specific manifestations of disease. It may also explain the highly variable expression of disease, because a large and diverse range of genetic polymorphisms may contribute to differential vulnerability of different tissues and immune pathways in different individuals.

Genetic factors

Although familial predisposition has been well documented for allergic diseases, the multifactorial nature of these conditions has made it difficult to identify specific genes with genetic polymorphisms that have either clear causative effects or predictive value. Similarly, although a large number of genes have been identified in Genome Wide Association Studies (GWASs) on asthma, there is wide variation among individuals and between populations, which explains between 35% and 95% of the heritability of asthma.[26] In part, this appears to be caused by complex interactions with environmental exposures and variable effects according to the environmental setting. This has been well demonstrated with genetic polymorphisms linked to early immune function. A well-described example is seen in genetic variants in the innate Toll-like receptor (TLR)-related microbial receptor for endotoxin, CD14. Specifically, the CC allele has been associated with an increased risk of allergic disease, but only in children living in environments with low microbial burden.[27,28] In a high microbial environment, carrying the CC allele has the opposite effect and appears to increase the risk of allergic disease.[29,30] This indicates that the effects of specific genetic polymorphisms can depend on the environment and similarly appear to vary with a wide range of potential environmental exposures (such as smoking, other pollutants, and specific dietary nutrients).

However, some current studies point to the importance of distinct polymorphisms for diagnosis and treatment strategies in childhood asthma, For example, a new susceptibility gene, *CDHR3,* and four previously reported asthma susceptibility loci (*GSDMB*, *IL33*, *RAD50*, and *IL1RL1*) were associated with severe, recurrent exacerbations in the Danish $COPSAC_{exacerbation}$ cohort, with substantially larger effect sizes than in previous asthma GWASs.[31]

Thus, whereas early measures of immune function in early childhood have been associated with both genetic polymorphisms and a familial history of allergic disease, many other factors in early life influence immune development. With advances in precision (personalized) medicine, it is hoped that genetic markers may better predict both disease risk and specific vulnerability to environmental risk factors; this is still out of reach with the current technology.

Direct effects of maternal allergy on immune maturation

Whereas familial allergy risk indicates a clear role for genetic risk, maternal immune phenotype may have direct effects on early immune programming. This is based on observations that maternal allergy is a stronger determinant of allergic risk than paternal allergy[32–34] and that maternal allergy may also contribute more strongly to reduced neonatal Th1 interferon (IFN)-gamma responses associated with allergic risk.[35] This suggests direct maternofetal interactions in utero or other maternally imprinted effects. There is some evidence that allergic women have modified immune interactions with the fetus in pregnancy, with lower Th1 IFN-gamma responses to human leukocyte antigen-DR mismatched fetal antigens compared with nonallergic women.[36] These factors may affect the cytokine milieu at the maternofetal interface and could be implicated in the attenuated neonatal Th1 responses observed commonly in infants of atopic mothers,[37] a recognized risk factor for infant allergic disease.[38–40] This suggests that

the endogenous effects of the maternal allergic phenotype may compound the increasingly proallergic exogenous environment. The rise in maternal allergy may become a compounding factor in the rising rates of infant allergy, amplifying the effect of environmental changes and potentially influencing the age of onset, phenotype, and severity of disease in her offspring.

Maternal environmental exposures and immune maturation

Most environmental risk factors implicated in the allergy epidemic have demonstrated effects on early immune development, beginning in utero. This includes microbial exposure, early nutritional patterns, smoking, and other environmental pollutants, with demonstrated effects on both animal models and human observational and/or intervention studies.[41,42]

Microbial exposure is arguably the strongest environmental stimulus for immune maturation, particular of regulatory and Th1 adaptive responses. Whereas most studies investigating the early immunomodulatory mechanisms have focused on *postnatal* microbial exposure, it is increasingly clear that the maternal microbial environment during pregnancy is also important in early immune programming.[43–46] In normal healthy pregnancies, microbes can be detected in amniotic fluid,[47,48] placental[48] and fetal membranes,[48,49] cord blood,[50] and meconium.[51–53] This reveals that the womb is not sterile after all, and that antenatal microbial exposure provides an important initial source of immune stimulation for the fetus. Experimental models confirm that exposure to bacterial endotoxin, probiotic bacteria, or other apathogenic bacteria has immunomodulatory effects and protects the offspring against an allergic phenotype.[54–56] These effects appeared to be mediated by activation of maternal innate (TLR) pathways,[56] with associated epigenetic effects on the regulation of Th1 gene expression in the newborn offspring.[57]

Bavarian farmhouses provide one of the best natural experiments to see the effects of microbial exposure on humans.[43,58,59] Children growing up in this setting have a reduced risk of allergic disease,[60] and *antenatal* exposure affords greater protection from allergy than postnatal exposure alone.[58,59] Biological data collected from cord blood samples also support this antenatal effect. At birth, newborns from farming families have increased numbers and function of regulatory cells (T_{reg}) and lower type 2 immune responses.[19] At age 4, T_{reg} function is still more efficient in farm milk–exposed children, which points to a strong persisting effect during immune maturation.[20] They also show higher Th1 responses, which are likely to be important for suppressing the development of allergy in the critical period after birth.[61] Most of these protective effects, most particularly against asthma, appear to result from contact with cows and straw and consumption of unpasteurized farm milk[62]—all major indicators of the high microbial diversity that characterizes the farm environment. Furthermore, prenatal farm exposure was associated with a distinct epigenetic methylation pattern of asthma- and allergy-related genes such as genes from the ORMDL family, RAD50, IL13, and IL4.[63] Thus, part of the farm effect may be mediated by epigenetic regulation.

Maternal nutrition has another significant influence on immune development and allergy risk.[64–66] Both specific nutrients and common dietary patterns have major

effects on all aspects of fetal development, including immune function. A particular dietary pattern that has shown protection from allergy and other NCDs is the Mediterranean diet. Protection against childhood wheezing and other allergic manifestations[67–69] may be related to higher levels of fiber, omega-3 polyunsaturated fatty acids (n-3-PUFA), and antioxidants in fresh fruits and vegetables (vitamins A, C, and E) and prebiotics,[66] all of which may have direct effects on immune function and anti-inflammatory effects on tissues. In particular, we have shown how increasing n-3-PUFA levels of diets in both pregnancy[70] and infancy[71] can have favorable effects on infant immune function (increasing Th1 responses and reducing Th2 responses).

Adverse exposures in pregnancy may also influence fetal immune development, the most obvious and well-studied of which is maternal smoking, with toxic effects on placental function and many aspects of fetal growth and development. This includes specific effects on lung growth and asthma risk[72] and on immune function.[73,74] Oxidative stress produced by cigarette smoking and air pollution can have significant epigenetic effects including remodeling of proinflammatory genes.[42] Mice exposed to diesel exhaust particles show an increased production of IgE with associated underlying epigenetic effects.[75] In humans, exposure to traffic particles in pregnancy has also been associated with epigenetic changes and has increased risk of developing asthma symptoms in children.[76] The many thousands of modern chemicals and pollutants that contaminate modern homes, food, clothing, and water sources, accumulating in human tissue with age, also may have adverse effects on human immune development,[77,78] although this is still ill-defined. Notably, many of these and other contaminants have been associated with epigenetic effects[79,80] including effects on global deoxyribonucleic acid methylation patterns at the low-dose exposure found in the ambient environment.[81]

A number of other exposures in pregnancy are also likely to influence immune function (reviewed elsewhere[65]), including maternal stress and hypothalamic–pituitary–adrenal activation, which are closely linked to placental and immune function. Medications commonly used in pregnancy, such as antacids, reflux medications, paracetamol, and antibiotics,[82–84] may carry an increased risk of some allergic diseases. Finally, there are emerging possible risk factors for allergy that are of uncertain significance, including higher risk of asthma in children conceived by in vitro fertilization.[85] Although this can be partly explained by the higher risk of neonatal complications, lower parental fertility appears to be a factor.[85] This could implicate a range of other modern environmental factors contributing to changes in reproductive health. All of these relationships serve to highlight the importance of the in utero environment in early immune programming as an essential prelude to environmental encounter after birth, shaping many other aspects of development and future health.

Early postnatal exposures and immune maturation

The concept of a window of tolerance came from observations that introducing complementary feeding before 3–4 months or after 6 months of age has been associated with increased allergy risk.[86] This is based on only observational data rather than controlled clinical trials, but it has led to concepts such as: (1) regular exposure to ubiquitous proteins such as foods and other innocuous environmental proteins may be

part of developmental processes that promote subsequent clinical tolerance; and (2) factors such as immature gut barrier, gut inflammation, or less tolerogenic conditions during initial allergen encounter may lead to disruption of normal tolerance mechanisms. For this reason, there is great interest in conditions in the early postnatal environment that are most likely to promote or disrupt a tolerogenic milieu, particularly in the infant gut. In this regard, breastfeeding and infant dietary patterns (discussed further in Chapters 16 and 17) are particularly important because of direct influences on postnatal immune maturation and secondary effects on colonization patterns.[66,87,88] Other risk factors such as atopic dermatitis (Chapter 3) may increase the risk of early transcutaneous sensitization through an immature skin barrier.[89]

After birth, the gut is the major interface between the host immune system and the microbial environment as a major driver of Th1 and T_{reg} maturation. Thus, perinatal factors that modify colonization during this early period have been implicated in the rise of allergic disease, including caesarean section, use of antibiotics, and the diversity of the maternal microbiome.[90] Reduced diversity in the first month of life is associated with an increased risk of a range of allergic outcomes,[91–94] which suggests early effects on immune maturation. There is also preliminary evidence that reduced diversity (in infants delivered by caesarean section) and delayed Bacteroidetes colonization are associated with reduced Th1 responses in the first 2 years of life.[95] In parallel, increased diversity of food within the first year of life might have a protective effect on asthma, food allergy, and food sensitization and is associated with increased expression of a marker for regulatory T cells.[96] In contrast, children living in a high microbial environment (e.g., exposed to farm milk during early life) show increased T_{reg} activity and lower risk of asthma as they approach school age.[20]

Colonization of the *upper* gastrointestinal is also of emerging importance. In traditional environments, colonization with *Helicobacter pylori* occurs during infancy, from around 4–6 months of age, and persists unless eradicated with antibiotic treatment. These bacteria are emerging as important initiators of immune regulation in the upper gastrointestinal tract,[97] favoring immune tolerance to evade our innate and adaptive immune responses. This allows them to remain in their habitat for decades while conferring host benefit—inhibiting autoimmune and allergic T cell clones and protecting against allergies, asthma, and inflammatory bowel diseases,[98] particularly with early colonization.[99–101] *Helicobacter pylori* provides another example of how disruption of mutually beneficial relationships may contribute to a range of immune and metabolic diseases. All of these factors provide potential avenues for immune modulation for disease prevention.

Modulating early immune development to promote immune regulation

As we learn more about the normal immune development and the environmental determinants, there will be more opportunities and more strategies to treat and prevent allergic disease. A detailed discussion of the merits of various allergy prevention strategies can

be found in Chapters 16–22, but here we briefly consider the likely avenues based on emerging evidence.

Although the evidence has been limited, the most promising strategies to improve early colonization may lie in the strategic use of prebiotics and probiotics. So far, most studies using these products for allergy prevention have focused on improving *postnatal* colonization. Probiotics supplementation has generally been studied only from *late* pregnancy and/or the early postnatal period. Prebiotics have been investigated only in the postnatal period. Commencing the use of these products much earlier in gestation to modulate maternal colonization may be more effective in influencing important earlier phases of immune maturation in fetal life. In particular, prebiotic soluble fiber (oligosaccharides) promotes microbial diversity by stimulating the growth of commensals and providing the substrate for anti-inflammatory short-chain fatty acid (SCFA) production by bacteria. This is likely to have more global effects on gut homeostasis, restoring intestinal dysbiosis as well as maintaining epithelial barrier integrity.[46,102]

Studies of prebiotic oligosaccharides in pregnancy are also limited. In animal models, prebiotics in pregnancy alter colonization and metabolic homeostasis[103] and reduce eczema-like inflammation in offspring.[104] To our knowledge, the only randomized controlled trial to use prebiotics in human pregnancy was too small (n = 48) to reliably assess immune effects on the fetus or clinical effects, but achieved favorable changes in maternal gut microbiota.[105] In a clear proof of concept, preliminary studies (personal communication, Charles Mackay) provide tantalizing evidence that a high-fiber diet in pregnancy and lactation significantly reshapes bacterial ecology and prevents the allergic phenotype of the offspring, with reduced eosinophils, reduced allergic cytokine responses, and airway hyperresponsiveness. Direct administration of SCFA (acetate) had a similar effect as an important mediator of systemic dietary effects.

This highlights maternal nutrition as an important general strategy to promote immune health in all individuals. Other nutritional agents that are likely to be an important part of this approach include diets rich in n-3-PUFA, vitamin D, grains, fresh fruit and vegetables, and less processed foods.[106] This will also have benefits for metabolic regulation in addition to the likely benefits on immune function.[106] Other examples of general strategies to promote immune health include promoting breastfeeding, avoiding adverse exposures (such as cigarette smoke), and reducing stress.

On the other hand, there are also likely to be more targeted strategies in the future aimed at immunomodulation, which may focus on specific pathways and/or specific individuals. An example might be primary (prophylactic) use of immunotherapy, in particular, for high-risk groups, such as aeroallergen immunotherapy in children with food allergy, or strategies to improve barrier function in children with eczema. Vaccination strategies are also being explored. In collaboration with Barry Marshall (who first discovered *H. pylori*), we are currently exploring the immunomodulatory effects of attenuated or killed *H. pylori* strains and their capacity to reduce and prevent the symptoms of allergic disease.

An attractive option would be to model the asthma-protective farm effect into prophylactic or intervention strategies. A potential cocktail containing asthma-protective farm exposure may present a tantalizing option for asthma prevention. Lyophilized

extracts of bacterial strains, in particular containing aeropathogenic bacteria, were suggested as protection against acute respiratory infections and possibly also for asthma.[46] Yet, how to optimize the microbiome to the most favorable, ideal colonization pattern still needs to be deciphered considering specific environmental exposure, among other factors.

Overall, for effective prevention, a combination of various factors from genetics to epigenetics, to different exposures such as nutrition, prebiotic, and smoking, and to orally administered bacterial extracts requires further investigation. Extending this, patient-individualized prevention taking into account specific control of environmental factors in patients with susceptible genotypes may prevent the manifestation of asthma.

Furthermore, intervention studies composed of a combination of factors may be more promising, because persistent asthma might result from complex interactions between respiratory viruses, allergens, and immune regulation.[6,107]

This raises a series of issues that will become increasingly relevant as technologies evolve, including how to target interventions, how to identify specific groups at risk, and how to refine strategies according to the level of risk. For generic and noninvasive immunomodulatory strategies to improve early immune health, it can be strongly argued that a ”whole-population” approach is both justified and logical because of the general health benefits that these are likely to have. Improving intestinal health is also beginning to fall into this category as better strategies and more evidence of the multisystem benefits continue to emerge. However, for other more costly, specific, or invasive strategies, a more targeted approach will be appropriate. For example, if allergen-specific immunotherapy is employed to prevent sensitization to common aeroallergens, this would be more appropriately targeted only to those at highest risk.

Conclusion

To keep healthy immune maturation in balance, environment combined with a given genotype participates in shaping early innate and adaptive immunity for healthy development. Although microbes are currently an attractive possibility influencing several facets of immune maturation, possibly during pregnancy, multifaceted regulation encompassing the mucosa, epithelium, gut, and immune system seems crucial. Together with currently known influences, a detailed insight into the key mechanisms mediated by microbial exposure can guide us toward novel and safe therapeutic and preventive strategies for healthy immune maturation.

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Immunological Aspects of the Atopic March

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Introduction

The atopic march is generally used to describe a clinical picture: the progression of atopic disorders, from eczema in young infants, to allergic rhinitis, and finally to asthma in older toddlers and children. This is a potentially powerful concept because it suggests possibilities for prognosis as well as intervention. However, the earliest sign of the atopic march, eczema, is not a specific marker of atopy. Therefore, an additional early criterion, the presence of allergen-specific immunoglobulin E (IgE), has usually been added even though the pathophysiological relevance of this early IgE was (and is) often not clear. The general impression is that the spectrum of IgE specificities develops in parallel with the clinical manifestations. This has evoked the suggestion of an additional, "immunological," atopic march starting with IgE to foods, followed by IgE to indoor allergens, and finally resulting in IgE to pollen and other outdoor allergens. This immunological atopic march may have an additional level of diversification: the complexity of the IgE response to a single allergen source such as grass pollen increases with time, as indicated by the number of epitopes recognized per component as well as the number of allergenic components per allergen source.

Although the causal relations among all these evolving processes (if any) remain largely unclear, each may contribute to establishing a more reliable prognosis as well as developing modalities for intervention.

In this chapter, we will first paint a general picture of observed antibody patterns. Next, we will describe how these developmental patterns might reflect immunological processes, with emphasis on the IgE-producing cells: B cells and plasma cells. Finally, we will describe some of the published birth cohort data and discuss what type of data still need to be collected to decide on the existence and relevance of the immunological allergic march.

Global inventory of the developmental pattern of allergen-specific antibody

The kinetics of IgE production is our main topic. However, it is illustrative to discuss not only the plasma levels of specific IgE (sIgE), but also to look at the IgE/IgG ratio to identify similarities and differences among IgE and IgG plasma levels. This kind of

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information is the starting point for trying to understand these plasma patterns from the perspective of the antibody-producing cells. The pattern described below is as observed in central and northwestern Europe. What is needed (but not yet available) is a more detailed description of the locations, specificities, and dynamics of the production of sIgE during infancy and childhood. We might then contrast this with those of IgG antibodies to allergens and nonallergens in atopic and nonatopic children.

The first sIgE antibodies that are associated with the atopic march are to cow's milk and chicken egg white. These antibodies are accompanied by high levels of IgG antibodies. These IgG antibody levels are similar among IgE-positive and IgE-negative children. Both IgE and IgG antibodies usually decline after age 2 years. Immunoglobulin E antibodies often become undetectable at age 4 years but IgG antibodies remain demonstrable for many years.

The pattern is different in the next phase of the allergic march (age 2–6 years), which at least in Europe typically involves IgE to house dust mites. In this situation, the IgG levels are significantly lower than in the case of milk and egg. The IgE/IgG ratio is often >0.1 for mite, whereas it is mostly <0.01 for milk and egg. Immunoglobulin G levels to the major mite allergens are much higher in IgE-positive patients than in IgE-negative subjects.[1] The IgE levels to mites tend to increase for several years. Details of this increase are being studied (as discussed below), but is seems likely that it is the result of not only an increase in antibodies of the initial specificities (more of the same) but also of antibodies with new specificities. These new specificities can be to other epitopes on the same mite molecules (epitope spreading), but also to epitopes on additional mite molecules (molecular spreading; Posa et al. Manuscript in preparation) and even to allergens from indoor allergen sources other than mites, such as mammalian pets. Exposure levels to pets tend to be higher than to mites, but the IgE responses are less frequent and the sIgE levels are lower. It is more common to observe substantial levels of IgG antibodies to pets in the absence of IgE antibodies to the same pet than levels found for mite allergens.

The third phase overlaps (age 3–7 years) with the second one and consists of IgE antibodies to outdoor allergens, mostly pollen from trees and grasses. This antibody response is much more like that found to mites than that to mammalian pets: IgE/IgG ratios often higher than 0.1 and little IgG in the absence of IgE.[2] The antibody response to pollen is characterized by a postseasonal increase.[3]

Tentative interpretation of phases observed in the serological picture

Antibody levels in plasma depend on many factors. Some factors that are important in the initial sensitization phase are:

- the number of virgin B cells activated by antigen contact with appropriate help from allergen-activated T cells and accessory cells
- the extent of developmental stages of the activated B cell: proliferation, class switching, secondary selection after somatic hypermutation, and differentiation to semidormant memory cells or to plasmablasts and plasma cells

- the lifespan of the main steady-state cells: the memory cells and the plasma cells
- the antibody production rates of these cells during the steady state
- the half-life of free antibodies in circulation, which is a function of extravasation, receptor binding, and catabolism.

Upon repeated exposure, the picture becomes even more complicated by the potential recruitment of memory cells, which may expand and differentiate to become antibody-secreting cells directly or after secondary class switching. This process can now be influenced by the B-cell receptor itself and also by circulating antibodies interacting with Fc receptors directly on the B cell or indirectly on accessory cells.

We will limit the discussion to six features related to the production of sIgE in the context of the immunological atopic march:

1. The low plasma level of IgE
2. The lack of true IgE memory B cells
3. The distinction between direct and indirect class switching to IgE
4. The contribution of mucosal tertiary lymphoidal activity
5. The differences in the lifespan of IgE-producing plasma cells
6. The phenomenon that the presence of specific IgE increases the risk of induction of IgE to new allergen epitopes

1. The low plasma level of IgE

The low plasma level of IgE (even in an atopic patient) is the result of at least three factors: (1) the brevity of the IgE half-life in plasma, which is more than 10 times shorter than that of IgG; (2) the low efficiency of the class switch to IgE, which is much more demanding than that to other isotypes[4]; and (3) the peculiar fate of the IgE-switched B cell, which is markedly different compared with that of the IgG-switched B cell.[5]

2. The lack of true IgE memory B cells

Unless the IgE-switched B cell rapidly becomes a plasma cell and finds a suitable survival niche, it will quickly die.[5] Thus, IgE memory B cells are extremely rare. Circulating IgE-positive B cells are mostly plasmablast type rather than B cells. Persistent IgE production reflects the activity of long-lived IgE-producing plasma cells and the activity of (mostly short-lived) plasma cells, derived from indirect class-switching (IgG memory cells to IgE rather than naïve IgM B cells to IgE). For a more extensive discussion of these issues, see [6] and [7].

3. The distinction between direct and indirect class switching to IgE

Immunoglobulin E produced by plasma cells that are directly derived from naïve IgM-positive B cells (i.e., by a direct μ to ε class switch) has less chance for proliferation and somatic hypermutation. Moreover, these IgE-switched B cells are less likely to be clonally related to IgG-switched B cells generated at the same time. Immunoglobulin E produced via this direct switching route might be an important source of the IgE responses to mite and pollen. In contrast, IgE produced by plasma cells derived from IgG memory cells is likely to have more somatic hypermutation and be clonally related to IgG antibodies produced by the IgG clone members. Because of this clonal relation, competition between IgE and the excess of IgG is likely to lower the pathogenicity (efficiency) of IgE antibodies to interact with the antigen/allergen. Immunoglobulin E produced via this indirect switching route might be an important source of IgE antibodies to milk and egg in early infancy and to animal pets later on.

4. The contribution of mucosal tertiary lymphoidal activity
   The potential lymphoidal activity of mucosal tissue has been well established for IgA. Mucosal antibody production is less obvious for IgE, probably because mucosal tissue (particularly in the gut) is not well suited for IgE class switching. However, upon TH2-driven mucosal inflammation, local IgE class switching and IgE production have been clearly established.[8] It is likely that this local IgE production is the major source of the postseasonal increase in pollen-specific IgE. It is probably involved in the IgE repertoire diversification phenomenon.[9] This effect of mucosal TH2-driven inflammation is a possible driving force in relation to the principle described as "IgE breeds IgE."[10] It will be interesting to see whether late additions to the allergenic spectrum have a more substantial IgG contribution and are less persistent than the initial specificities.

5. The importance of differences in the lifespan of IgE-producing plasma cells
   The important contribution of long-lived, radioresistant plasma cells in the bone marrow was established by Benner.[11] A major contribution to persistent IgE production was also established for long-lived IgE-producing plasma cells in the bone marrow in the early work of Holt et al.[12] The elegant work of Luger et al.[13] provided much more detailed information and showed that the dichotomy between short-lived (<3 months) and long-lived (>1 year, possibly lifelong) is too simple. The authors described mucosal IgA-producing plasma cells as surviving for many months outside the bone marrow.[14] It would be interesting to see to what extent the semipersistent (<4 years) IgE antibody response to milk and egg might be as a result of similar IgE-plasma cells surviving in the (more TH2-accommodating?) infantile gut.

6. The phenomenon in which the presence of specific IgE increases the risk of induction of IgE to new allergen epitopes
   As mentioned above, a local allergic reaction, particularly in mucosal sites, may have an important role in the evolution of the IgE repertoire. In addition, the physical conglomeration of distinct allergens on a single allergen-delivery particle might have a role. Examples are particles carrying allergens from a single source (examples are pollen grains and mite fecal particles) or from multiple sources (an example is dust particles). After triggering mast cell-driven local inflammation, IgE antibodies to one of these components may cause the influx and activation of various cellular players involved in generating IgE antibodies with specificities to other components of the allergen-delivery particle.

The trends of IgE levels to allergenic sources

Even when examined at the extract level, the natural history of IgE sensitization is a dynamic process characterized by a steady increase in the prevalence of sensitization combined with a relatively high remission rate of the weakest IgE responses, especially at younger ages.[15–18]

In the Multicenter Atopy Study (MAS) cohort, the incidence of sensitization to animal food allergens (milk and egg) peaked in the first 2 years of life and declined shortly thereafter.[19] Early sensitization to egg was found to be a good predictor of subsequent sensitization to airborne allergens as well as of asthma.[20] Immunoglobulin E sensitization to milk and egg persisted after age 5 years in only 20% of children. Instead, the incidence of sensitization to vegetable food allergens (soy and wheat)

increased progressively in the first decade of life. In the first years of life, primary sensitization to soy and wheat, characterized by high and persistent levels of IgE antibodies, was observed in only a few children. By contrast, frequent but weaker IgE sensitization to soy and wheat appeared later in childhood, always in association with sensitization to grass or birch pollen, as a result of cross-reactivity. In these cases, the signal of IgE antibodies recognizing grass or birch extracts was always stronger than that of IgE antibodies recognizing soy or wheat extracts.[21]

Sensitization to airborne allergens frequently starts at preschool age. In countries where exposure to mites is higher, such as New Zealand,[22] the prevalence of sensitization to indoor allergens in children is higher than that to pollens.[22,23] The dynamic evolution of specific IgE is a function of the genetic predisposition of the child, combined with environmental exposure to risk and protective factors.[24,25] In the MAS cohort, a half of the monosensitized subjects acquired a second sensitization within 3 years. This monosensitization window period is important from a clinical point of view, because specific immunotherapy may have a stronger efficacy if started in the monosensitization or even presymptomatic stage.[26,27] Earlier sensitization has a stronger impact than late sensitization in many respects. Early sensitization to mite and cat allergens was characterized by a faster and steeper increase in serum-specific IgE levels than sensitization to the same allergens starting later in childhood. These early-sensitized children had a stronger tendency to become polysensitized and to develop respiratory allergic disease persisting at least up to age 13 years.[18]

The old concept that sensitization against airborne allergens is inevitably persistent has been challenged by data from the birth cohort studies. Data from the Tucson birth cohort showed that sensitization against Bermuda grass, observed at age 6 years, is frequently lost at age 11 years.[15] Similarly, in the BAMSE cohort study about 10% of children with IgE sensitization at age 4 years were no more sensitized at age 8 years.[16,28] In the MAS cohort, these observations were confirmed and expanded by showing that weak sensitization (0.35–1 kU/L) detected at the age 2 years is lost at age 5 years in over 60% of cases (Table 1). The likelihood of sensitization remission declined with age in all three cohorts.[16,21,29] Moreover, no remission of sensitization to airborne allergens was observed in the MAS cohort when serum IgE antibody levels were higher than 3.5 kU/L. This threshold may have some clinical relevance but it cannot be extrapolated to a clinical setting to predict sensitization persistence, because these cutoff points are always context dependent and may change with populations and settings.[30,31] Therefore, studies in patients cohorts are needed to test whether persistence of sensitization against airborne allergens is predictable through levels of serum IgE antibodies.

Immunoglobulin E trends examined at a molecular level

Our understanding of IgE-mediated allergies has greatly improved since the advent of component-resolved sIgE testing (molecular allergology). This approach is based on the use of single allergens rather than extracts for the diagnosis (component-resolved diagnosis [CRD]) and potentially also for therapy (component-resolved therapy) of

Table 1 **Frequency of remission of IgE sensitization to inhalant allergens in the MAS cohort, by age**[a]

IgE antibody initial levels (kU/L)	Allergen	Frequency of remission[a]					
		Positive at 2 years but <0.35 kU/L at 5 years		Positive at 5 years but <0.35 kU/L at 7 years		Positive at 7 years but <0.35 kU/L at 10 years	
		n/N	*%*	*n/N*	*%*	*n/N*	*%*
>0.35; <1.0	All	18/28	64.3	11/41	26.8	23/73	31.5
	Mites	3/4	75.0	0/6	0.0	5/16	31.3
	Cat	2/3	66.7	2/7	28.6	4/9	44.4
	Dog	–	–	1/7	14.3	5/16	31.3
	Birch	3/5	60.0	3/7	42.9	4/10	40.0
	Grass	10/16	62.5	11/42	26.2	5/22	22.7
>1.0; <3.5	All	5/20	25.0	3/35	8.6	6/59	10.2
	Mites	0/2	0.0	0/3	0.0	1/9	11.1
	Cat	1/4	25.0	0/4	0.0	0/8	0.0
	Dog	–	–	1/4	25.0	0/10	0.0
	Birch	0/6	0.0	0/12	0.0	3/13	23.1
	Grass	4/8	50.0	3/35	8.6	2/19	10.5
>3.5	All	0/7	0.0	0/79	0.0	0/101	0.0

MAS, Multicenter Allergy Study.

[a]Number of sera with undetectable IgE antibody per total number of sera with the given concentration at the previous time point (2 years for 5 years: 5 years for 7 years: 7 years for 10 years) (e.g., 35 sera had a concentration of serum IgE against grass pollen at age 5 years between 1.0 and 3.5 kU/L; three of these [8.6%] had no detectable IgE against grass pollen at age 7 years).

allergic diseases. The application of CRD to birth cohort studies is contributing to the progress of our knowledge in this area.

1. *Cross-sectional studies*. On a molecular level, allergic IgE responses have been first investigated in cross-sectional studies. A huge cross-sectional study described the differences in molecular profiles of sensitization in over 23,000 allergic patients stratified by age at examination.[32] This study showed that the spectrum of sensitization to individual allergen components varies markedly with age, suggesting a different behavior in the lifetime sensitization process. Similarly, a cross-sectional study showed that children are sensitized to fewer components than are adolescents.[33]
2. *Birth cohort studies and other longitudinal studies*. A cross-sectional study cannot establish whether sensitization profiles are a stable hallmark of individual patients or whether they change dynamically over time. In adulthood, new sensitization is rare even at the molecular level, and remission has become more common.[34] By contrast, the IgE response is much more dynamic during childhood.[35] In particular, the IgE response against *Phleum pratense* evolves in many allergic children from a simple, often single-component sensitization stage to an oligomolecular sensitization stage, to terminate in a polymolecular sensitization stage ("molecular spreading" [see below]). Interestingly, it has been shown that in over 75% of German grass pollen allergic children participating in the MAS birth cohort study the first positive sample contained IgE antibodies recognizing Phl p 1 (a sort of initiator of this IgE response) and in 26% of them this was the only component that was recognized at that time. This may imply that Phl p 1 is a valuable target for early intervention.[35,36] The response may then involve Phl p 4 and Phl p 5; then also Phl p 2 and Phl p 6; and finally, Phl p 11, Phl p 12, and Phl p 7. Only a few children develop a full-blown response with involvement of all eight allergenic molecules of *Phleum pratense* that were investigated in this study. The development of the IgE spectrum to include house dust mite allergens was investigated in a cohort of high-risk children by Calkhoven et al. in 1991.[37] The hypothesis was that whether a primary allergen could be identified that initiated the IgE antimite response. At that time, only two major allergens had been identified, Der p 1 and Der p 2. Analysis of the first mite-positive serum sample did not provide clear support for a preferential primary allergenic specificity. Interestingly, the earliest serum with sIgE to mite extract was negative for both Der p 1 and Der p 2 in six of 18 children.[37]
3. *Molecular spreading of IgE responses*. The increase in pollen-specific IgE in serum is not caused solely by an increase in the amount of IgE with preexisting specificities, but also by an increase in the number of molecules recognized in pollen extract, and presumably also by an increase in the number of epitopes recognized per allergen (Figure 1). Interestingly, this allergen march occurs mostly in the preclinical stages of sensitization and starts with IgE to Phl p 1 even up to 5 years before the child starts to perceive disease symptoms (Figure 2). Preclinical IgE sensitization, characterized by weak and simple IgE responses, is progressively increasing in concentration and molecular complexity and develops into the clinically manifest stage of the IgE response, when inflammation is clinically perceived as a disease. In the first years after the onset of allergic rhinitis, a slower increasing trend in the number of molecules recognized by IgE is observed while the overall serum concentration of IgE antibodies against *P. pretense* grows at a consistent pace.[35] This phenomenon has been described as molecular spreading,[35,38] in analogy with the epitope spreading phenomenon in the T-cell response during the evolution of an autoimmune reaction. An interesting question is whether the preclinical molecular spreading phenomenon is limited to grass pollen or whether it is a general characteristic of all IgE

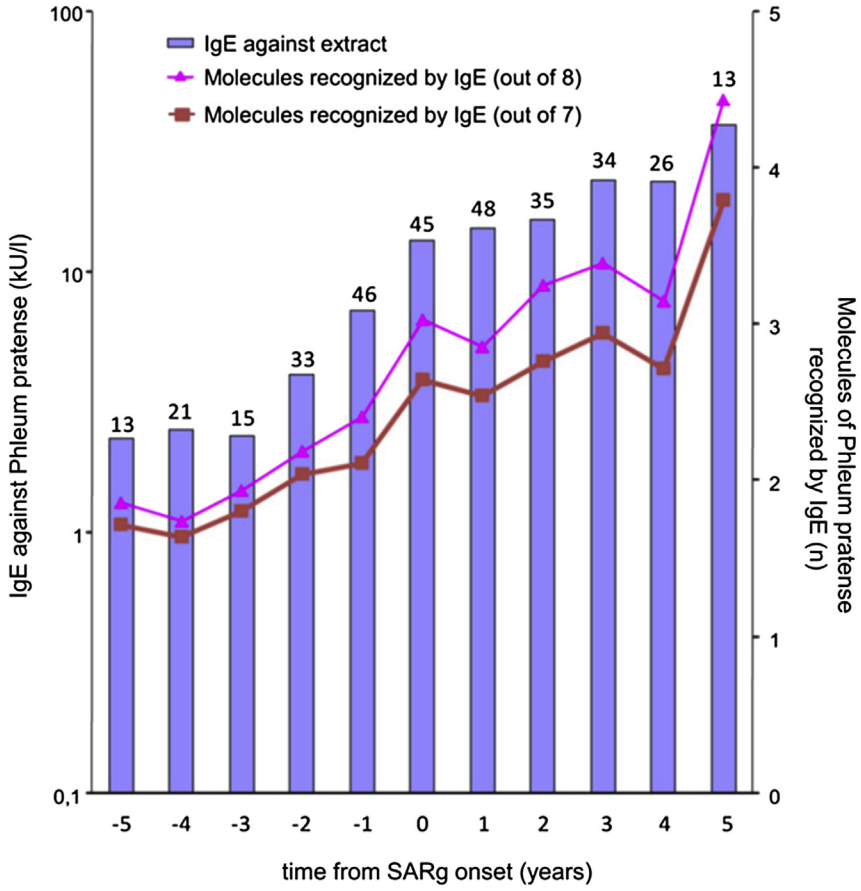


Figure 1 Immunoglobulin E to *P. pratense* by time from onset of grass-related seasonal allergic rhinitis. Bars show the geometric mean levels of IgE antibodies to *P. pratense* (extract) at each point in time in IgE antibody-positive sera. The number of tested children is indicated over each bar. Lines show the average number of all eight (triangles) or only seven (squares; Phl p 6 excluded) allergenic molecules of *P. pratense* recognized by IgE antibodies at each point in time in IgE antibody-positive sera.

responses to complex allergenic sources. Data from the cross-sectional studies mentioned earlier suggest that this phenomenon also occurs in IgE responses against other allergenic sources; longitudinal studies are in progress.

4. *Heterogeneity of IgE sensitization profiles*. When examined in a classic enzyme-linked immunosorbent commercial diagnostic assay based on allergenic extract of timothy grass, grass pollen allergic patients look the same from a qualitative point of view: that is, they are all positive (although with different degrees of positivity). By moving from extract to molecular analysis, the population's apparent homogeneity is substituted with a remarkable heterogeneity. A series of 39 different IgE sensitization profiles could be observed in a cross-sectional study of only 176 Italian children allergic to grass pollen (Figure 3).[39] The same population of

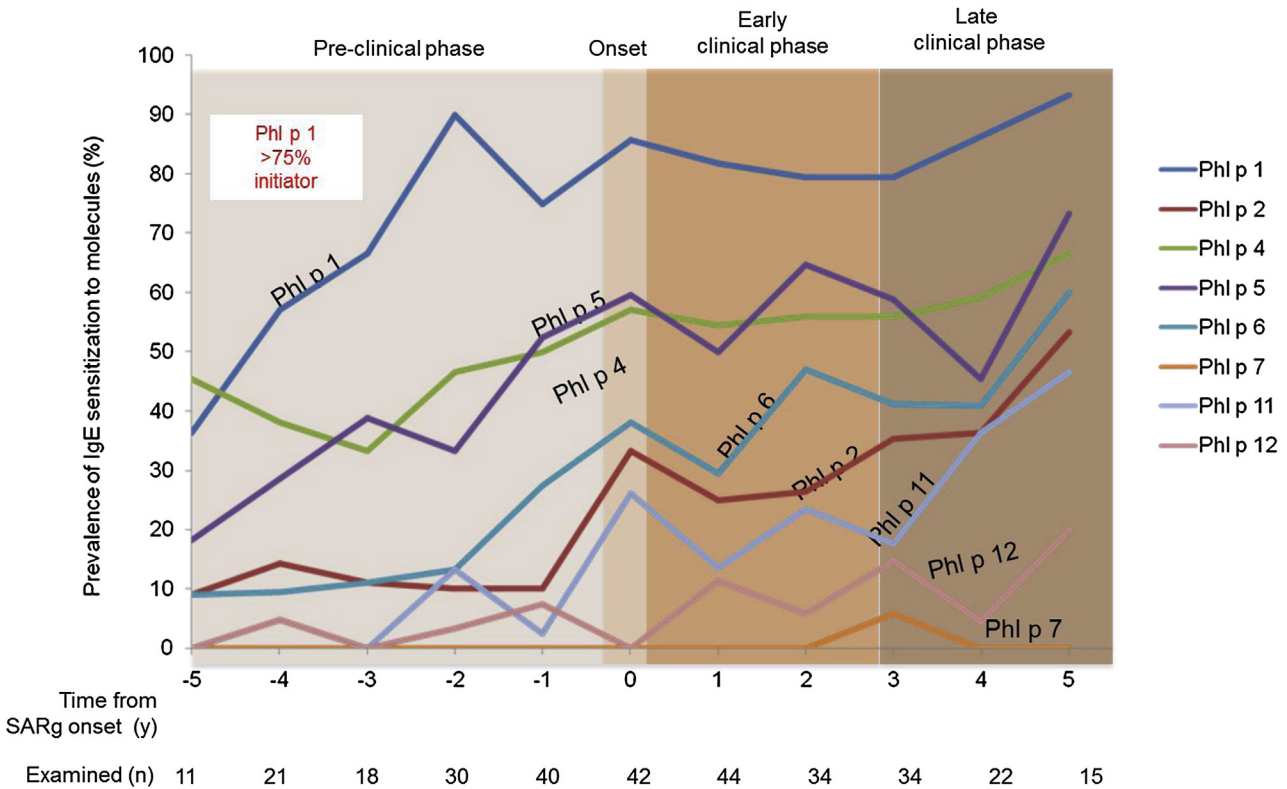


Figure 2 Immunoglobulin E to *P. pratense* allergenic molecules from the onset of grass-related seasonal allergic rhinitis. Lines show the prevalence of IgE sensitization (ISAC class ≥1) to the eight *P. pratense* allergenic molecules in children whose sera were available at each time point. The number of children examined at each time point is indicated under the *x*-axis. Clinical stages of seasonal allergic rhinitis are also indicated.

APCS code	pt 8 0.35	rPhl p 1	rPhl p 2	rPhl p 4	rPhl p 5	rPhl p 6	rPhl p 7	rPhl p 11	rPhl p 12	n pos. mol.	n	%	cum. %
128	10000000	●								1	36	20,8	21
248	11111000	●	●	●	●	●				5	21	12,1	33
160	10100000	●		●						2	10	5,8	39
184	10111000	●		●	●	●				4	8	4,6	43
186	10111010	●		●	●	●		●		5	8	4,6	48
251	11111011	●	●	●	●	●		●	●	7	8	4,6	53
192	11000000	●	●							2	7	4,0	57
216	11011000	●	●		●	●				4	7	4,0	61
249	11111001	●	●	●	●	●			●	6	7	4,0	65
250	11111010	●	●	●	●	●		●		6	7	4,0	69
32	100000			●						1	5	2,9	72
224	11100000	●	●	●						3	5	2,9	75
152	10011000	●			●	●				3	4	2,3	77
185	10111001	●		●	●	●			●	5	4	2,3	79
208	11010000	●	●		●					3	3	1,7	81
218	11011010	●	●		●	●		●		5	3	1,7	83
48	110000			●	●					2	2	1,2	84
64	1000000		●							1	2	1,2	85
144	10010000	●			●					2	2	1,2	86
162	10100010	●		●				●		3	2	1,2	87
187	10111011	●		●	●	●		●	●	6	2	1,2	88
193	11000001	●	●						●	3	2	1,2	90
217	11011001	●	●		●	●			●	5	2	1,2	91
225	11100001	●	●	●					●	4	2	1,2	92
16	10000				●					1	1	0,6	92
34	100010			●				●		2	1	0,6	93
58	111010			●	●	●		●		4	1	0,6	94
96	1100000		●	●						2	1	0,6	94
129	10000001	●							●	2	1	0,6	95
130	10000010	●						●		2	1	0,6	95
132	10000100	●					●			2	1	0,6	96
156	10011100	●			●	●	●			4	1	0,6	97
188	10111100	●		●	●	●	●			5	1	0,6	97
194	11000010	●	●					●		3	1	0,6	98
232	11101000	●	●	●		●				4	1	0,6	98
240	11110000	●	●	●	●					4	1	0,6	99
254	11111110	●	●	●	●	●	●	●		7	1	0,6	99
255	11111111	●	●	●	●	●	●	●	●	8	1	0,6	100
0	0									0	3	1,7	

Figure 3 Profiles of IgE sensitization to eight *P. pratense* molecules in 176 sensitized children. Profiles of IgE sensitization to eight *P. pratense* molecules in 176 children with an IgE reaction to *P. pratense* and complete dataset. The antigen-presenting cell code and absolute and cumulative frequency are shown. The profiles are ordered by declining frequency and the point at which the arbitrary threshold of 80% of the patient population has been reached is marked in red (light gray in print versions).

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grass pollen-allergic children included patients sensitized to only one molecule (in most cases, Phl p 1) and patients sensitized to all eight allergenic molecules examined.

Discussion

It is still uncertain whether the immunological atopic march is a sequence of causally related events. This is an important issue because if it is (and only if it is), it opens ways to intervention and possibly even prevention. Currently available data do not allow conclusions regarding the mechanisms involved. Although the progression of atopic diseases in different target organs may all be independent consequences of the same cause (i.e., genetic predisposition to atopy and/or to barrier defects), the expansion of IgE sensitization within the same allergenic source may be a phenomenon triggered by one or more initiator molecules, producing local conditions facilitating further IgE production (IgE breeds IgE concept[36]).

What kind of additional information is needed to better address these questions? First, we would need birth cohort studies with shorter intervals between blood samples (<1 year) to allow more accurate association analyses between symptoms and serology during the early steps of the sensitization process. Then, we would need to express our sensitization results in quantitative assays instead of the dichotomous classification mostly used. It is essential to compare IgE results with IgG antibody data in patients and controls, and to look for an explanation of the striking differences among allergens in the IgE/IgG ratios. Using quantitative sIgE assays, it will become possible and important to calculate the contribution of IgE to nonidentified allergens (i.e., the difference between total and specific IgE) and find out how much we do not know at different phases of the atopic march. As mentioned earlier, it is particularly important to investigate whether sensitization to egg and milk is causally linked to further sensitization to airborne allergens or whether events occurring in different mucosal tissues (airways and gut) are indeed unrelated or indirectly related through a common atopic genetic background or to a genetically determined barrier defect. This could be investigated, for example, by comparing the development of IgE responses in children with or without critical filaggrin mutations. All of these questions deserve an answer that might possibly be derived from biological samples in existing biobanks from birth cohort, but presumably they will require new prospective studies.

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Atopic Dermatitis in Infancy and Childhood

3

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Definition

Atopic dermatitis (AD) is the most common inflammatory skin disease in childhood, with an onset usually in early infancy.[1–3] It imposes a considerable burden on patients and their families and frequently represents the first chronic health problem for many families.[4,5]

The clinical manifestation of AD may vary according to age, with a more general manifestation in infancy (aside from the diaper region, the face, head, and neck are included) (Figure 1) and a more flexural manifestation (elbows and knees) later in life. It goes along with itch, which may contribute to sleep disturbance and impaired quality of life for both children and their families.

Manifestations are caused by inflammation of the skin, which involves the epidermis. Infiltration of T lymphocytes as well as immunoglobulin E (IgE) binding antigen-presenting dendritic cells and eosinophils drives this inflammatory process. The influence of mediators, particularly TH2 cytokines such as interleukin 4 and 13 during the acute and subacute period, has been named responsible for a transient downregulation of barrier proteins of the skin in affected patients (Figure 2).[2,6]

Family history and genetics

Parental atopy is associated with the risk and severity of early atopic dermatitis in the offspring, with the strongest effect being for parental atopic dermatitis.[7–10]

Recent studies have elegantly demonstrated that in some geographic locations more than 20% of patients afflicted with AD have a genetic predisposition for this disorder. The most common gene associated with AD is Filaggrin (FLG), which produces profilaggrin, which is processed in the stratum granulosum into the FLG protein.[11] Filaggrin contributes to the barrier function of the stratum corneum of the skin. It has been shown that the stratum corneum of affected but also clinically unaffected skin of patients with AD is abnormal, with reduced expression of FLG and FLG-like proteins.[12] This skin barrier is presumed to protect the skin from penetration

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Figure 1 Infantile manifestation of atopic dermatitis.

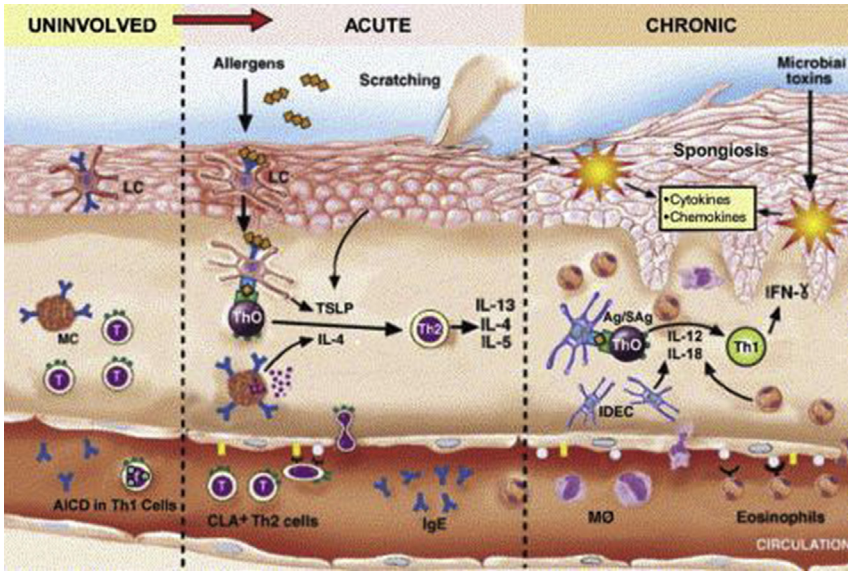


Figure 2 Immunological pathway involved in the progression of AD.[2] LC = Langerhans cell, MC = mast cell, TSLP = human stromal lymphopoietin, AG = antigen, SAG = superantigen, AICD = activation-induced cell death, CLA = cutaneous lymphocyte antigen, MO = monocyte.

by environmental irritants and allergens.[13] Although FLG loss-of-function mutations have been extensively described in individuals of European and Asian descent, these mutations obviously vary by race. In an African American cohort with AD, two additional FLG mutations were associated with more persistent AD, which suggests that mutations arose in different populations during different time periods and were propagated selectively because of a multitude of factors that might include natural selection, genetic drift, or a population bottleneck effect.[14] Regardless of FLG mutations, FLG deficiency has been observed in patients with atopic dermatitis, which suggests that the absence of FLG is a key factor in the genesis of this skin condition.[15] Applying

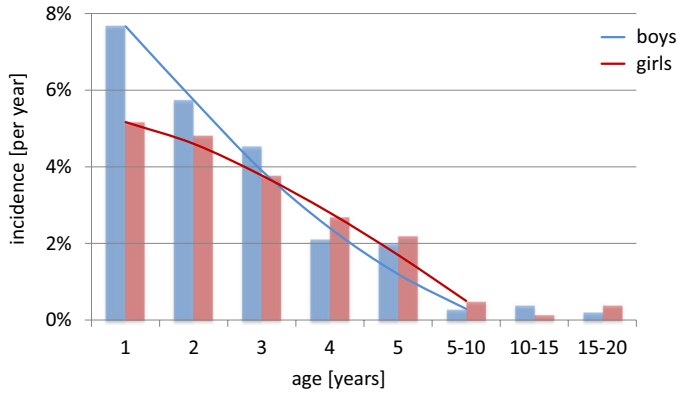


Figure 3 Annual incidence of atopic dermatitis in childhood and adolescence. Data from MAS.[8]

single-molecule direct ribonucleic acid sequencing to analyze the whole transcriptome, researchers demonstrated that the extracellular space and lipid metabolism in atopic skin pathology is important independent of the FLG genotype, and that an aberrant defense response is seen in subjects with FLG mutations.[16]

Natural history of disease

The natural history of atopic dermatitis has been studied extensively during the past 2 decades in a variety of birth cohort studies that have been followed longitudinally up to adolescence. In the German Multicenter*Allergy*Study (MAS) cohort, 13.4% of all children developed the classical manifestation during the first year of life. By the age of 2 years, the lifetime prevalence amounted to 21.5%.[7,8,10,17]

The highest incidence of atopic dermatitis is observed in the first 12 months, with a clear dominance of male sex (boys 7%; girls 5%). The annual incidence decreases by school age to less than 0.5% in both boys and girls (Figure 3).[8,18]

More than 40% of children with infantile manifestation develop complete remission after age 2 years. Almost one-fifth of children with early atopic dermatitis have persisting symptoms every year up to age of 7. In addition, intermittent manifestation has been observed in 30.8% of children with infantile manifestation (Figure 4).

Scratching of the inflamed skin has been considered a marker of severity. About one-third of families report scratching and another third report infrequent scratching.[10] To score the severity of disease, a number of instruments have been proposed and validated (SCORAD and EASI) that have turned out to be useful in describing the longitudinal development of the disorder.[19,20]

Infants with atopic dermatitis who had been observed over 18 months in a placebo-controlled trial with levocetirizin[21] turned out to lose severity (during 18 months, approximately 18 points on the SCORAD scale). This loss of severity of

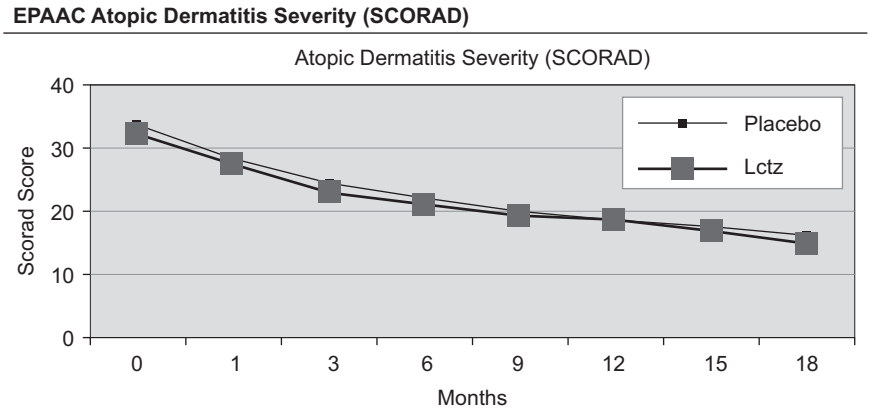


Figure 4 Loss of severity over time in a cohort of children with AD treated with either placebo or levocetirizine.
Data are unpublished from the EPAAC trial.[21]

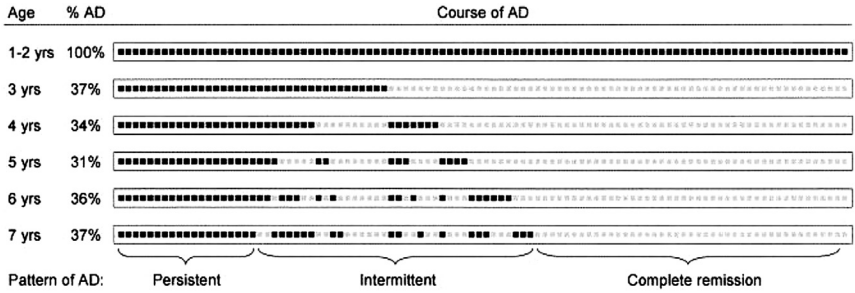


Figure 5 Natural history of AD from infancy to childhood, obtained from the prospective German MAS.[10]

infantile atopic dermatitis seems to be characteristic for young infants despite the persistence of this chronic relapsing disease. In the long-term levocetirizine study, the observation was independent of any pharmacological intervention (Figure 5).

Another characteristic feature of atopic dermatitis in early childhood is the association with various comorbid or mostly subsequent manifestations such as food allergy, asthma, rhinitis, and urticaria.[22–26]

Immunoglobulin E–mediated sensitizations may induce allergic reactions, which in the case of atopic dermatitis may relate to independent comorbidities (urticaria or rhinitis) as well as factors that contribute to the severity of atopic dermatitis itself.

Allergic sensitization and allergy as comorbidity

Immunoglobulin E responses develop in a characteristic pattern, with reactions to food proteins such as hen's egg, cow's milk, or peanuts during the first year of life; pollen-associated food proteins in subsequent years; and IgE antibodies to indoor (house dust mites or pets) and outdoor (pollen) allergens some years later.[27]

Immunoglobulin E-mediated sensitizations are related to the severity of the disease as well the total serum IgE concentration: Multiple IgE responses are associated with higher prevalence and severity of the disease in the first 3 years of life.[8]

Only about 30% of young children with sensitization to food proteins show clinically relevant reactions in double-blind, placebo-controlled food challenges, which justifies dietary avoidance. A considerable proportion of children react to food proteins with urticaria or gastrointestinal or airway symptoms rather than worsening of atopic dermatitis.[28]

Rhinitis and asthma as comorbidity

Pooled data of 12 ongoing European cohort studies participating in the Mechanisms of the Development of Allergy project showed that eczema, rhinitis, and asthma coexist in the same child at age 4 and 8 years more often than would be expected if these diseases were independent. Common underlying mechanisms could exist among these different manifestations besides IgE sensitization and parental history of allergies.[29]

Based on the observation that there is a characteristic sequence of manifestations (eczema in infancy and airway disease later in childhood or adolescence), a number of interventional trials have been initiated to block the so-called "allergic march." Unfortunately, the results of these trials were not encouraging (see the chapter by Spergel and Warner). Cetirizine, levocetirizine, desloratadine, and pimecrolimus failed to reduce the incidence of allergic airway disease in children with infantile atopic dermatitis.

Urticaria as comorbidity

Acute and chronic urticaria are disorders that may be observed in children with atopic dermatitis more often than in unaffected children.[30] In prospective cohort studies, the incidence of urticaria was approximately 1% per year. The cumulative prevalence in children up to age 10 years was 14.5% for boys and 16.2% for girls.[31] The cumulated prevalence of acute urticaria over the first decade of life was associated with allergic sensitization to peanuts, soy, weeds, and flowers but not to inhalant allergens. Clinical eczema increases the risk for urticaria.[32] In a longitudinal study (ETAC) in which 817 children with atopic dermatitis between ages 12 and 24 months were randomized to receive either cetirizine or placebo for 18 months,[30] acute urticaria occurred in 16.2% of children.

In addition to acute urticaria, chronic spontaneous and chronic autoimmune urticaria are linked to atopic dermatitis. The natural history of this disorder tends to be benign, with a remission rate of about 50% after 3 years.[32]

Determinants of the disease

Socioeconomic status

The National Health Survey on children in Germany (KIGGS) confirmed the frequently reported observation that unlike many other health problems in childhood,

atopic dermatitis is not associated with poverty, but rather with high socioeconomic status.

In the German birth cohort MAS, this observation could be confirmed. The link between higher educational degree of the mother and infantile atopic dermatitis was statistically significant.[8]

Infant feeding

In 1936, two pediatricians in Chicago reported that of the 2061 infants they had seen in 5 years, infantile eczema occurred seven times as often in infants who received cow's milk formula and twice as often in partially breast-fed compared with fully breast-fed infants (see the chapter by Bergmann et al.) All infants had received small amounts of orange juice from week 4, cod liver oil from week 6, and solid food after 4 months. Breast-feeding might reduce infectious morbidity and even mortality in developing countries, but discussion on the protective role on atopic diseases is ongoing. In the German MAS study, the prevalence of atopic eczema in the first 7 years increased with each additional month of exclusive breast-feeding and the risk was even higher if parents had a history of atopic eczema, which suggests that mothers probably extended breast-feeding once the infant had developed eczema early in infancy because they believed that breast-feeding is protective. This phenomenon of reversed causation confuses the ongoing discussion about preventative infantile diets because randomized controlled trials on breast-feeding are considered unethical.[25] Guidelines on infant feeding recommend the use of hydrolyzed infant formula from either whey or casein as part of primary prevention of atopic dermatitis in case breast milk is not available. The recommendations are based on the results of a large randomized controlled trial in Germany (GINI), which demonstrated some effect on the incidence of atopic dermatitis, but not on the process of sensitization or the development of airway diseases.[33–35] Similar results have been reported from interventional studies with some probiotics (lactobacillus GG) or prebiotics (enrichment of the formula with indigestible oligosaccharides) in infant formulas. The mechanism of this effect is not completely understood; the intervention effect seems to be relatively weak. Although some decades ago the age at which solid food was introduced was considered to have a significant impact on the manifestation of AD, most recent studies do not confirm such an effect.[36]

Domestic environment

The level of domestic mite and pet allergen exposure, parental smoking, and maternal smoking during pregnancy, along with the number of older siblings, have not been found to have an effect on the manifestation or severity of early AD.[10]

Key elements of treatment

The aim of therapeutic interventions for atopic dermatitis is not to cure the disease but rather to control the symptoms.

International guidelines that are based on the results of evidence from placebo-controlled trials in the young age group recommend:

1. **Providing rehydration for dry, inflamed skin**
   Two placebo-controlled randomized trials in high-risk neonates demonstrated that parental instruction to apply full-body therapy at least once per day starting in the neonatal period has a protective effect on atopic dermatitis. Upon confirmation of these observations in larger trials, emollient therapy from birth may become a simple and low-cost intervention that could reduce the global burden of AD.[37,38]
2. **Anti-inflammatory topical treatment with topical corticosteroids or calcineurin inhibitors**
   Low-potency corticosteroids are recommended for maintenance therapy, whereas intermediate- and high-potency corticosteroids should be used cautiously in early childhood and only to treat severe exacerbations over short periods of time. In young children, it should always be taken into consideration that the degree of corticosteroid absorption through the skin and hands increases the potential for systemic adverse effects directly according to the surface area of the skin involved as well as the potency of the corticosteroid preparation.[2]

   Topical calcineurin inhibitors may be considered in children older than age 2 years. Tacrolimus ointment and pimecrolimus cream decrease the number of flares, reduce the need for corticosteroids, and control pruritus without causing skin atrophy. A published long-term safety study on pimecrolimus in 2439 infants[39] demonstrated safe application of intermittent long-term treatment with a calcineurin inhibitor in children before age 2 years.[4]

   Long-term treatment with antihistamines has failed to prove efficacy. H1 blockers are no longer recommended in recent guidelines; however, in young children with concomitant acute urticaria, long-term treatment with cetirizine significantly reduced the number of episodes with acute urticaria.[21]
3. **Diets**
   Dietary interventions are indicated only in children with proven food allergy. Considering the risk of potential nutritional defenses, clinicians should not recommend general elimination diets based only on skin or specific IgE test results. Even with positive skin test results, most patients will react to no or few food items on oral challenge.[28]
4. **Parent education**
   To achieve effective control of atopic dermatitis in infancy and early childhood, it is essential to educate parents and caregivers about the chronic nature of the disease and exacerbating factors, as well as the safety and side effects of medications. Training in skin care techniques, written treatment plans, and comprehensive information about the nature of the disease with individual trigger factors are essential in managing AD. Structured educational programs for children and their caregivers have been systematically proven to be of great value.[4]

Prevention

A number of preventative approaches have been studied. Recommendations for breast-feeding for 6 months or longer are not based on robust evidence. Because of other beneficial aspects of breast-feeding, pediatricians all over the world encourage mothers to breast-feed. Some guidelines, particularly in Europe, recommend feeding with hydrolysates prepared from whey or casein, if mother's milk is not available in sufficient amounts.[40] The randomized controlled GINI study in Germany convincingly demonstrated the reduction of atopic dermatitis in the first year of life during and after feeding with hydrolysates.[35] An effect on sensitization to food allergens or

the development of allergic airway disease was not observed. Results have been disappointing and mothers at risk should be discouraged from implementing these potentially risky diets.

A bacterial lysate from *Escherichia coli* and *Enterococcus faecalis*, applied orally for 6 months in young infants at risk, was studied in a large randomized, placebo-controlled trial. Although it was not effective in the whole study cohort, the incidence of eczema was reduced in children with only one atopic parent.[36]

Long-term outcome in adulthood

Most young children with atopic dermatitis grow out of the disease within the first 2 decades of life:

About 30% of all children with incidence of eczema in the first year of life will have remaining eczema by age 20 years despite a loss of severity (Figure 6). Later onset between the second and fourth years of life will further reduce chronification and improve the long-term outcome. It appears that boys have a slightly better prognosis than girls. In addition, predictors of persistence of atopic dermatitis and of disease severity were sensitization to cow's milk and hen's eggs and wheeze at school age.[8]

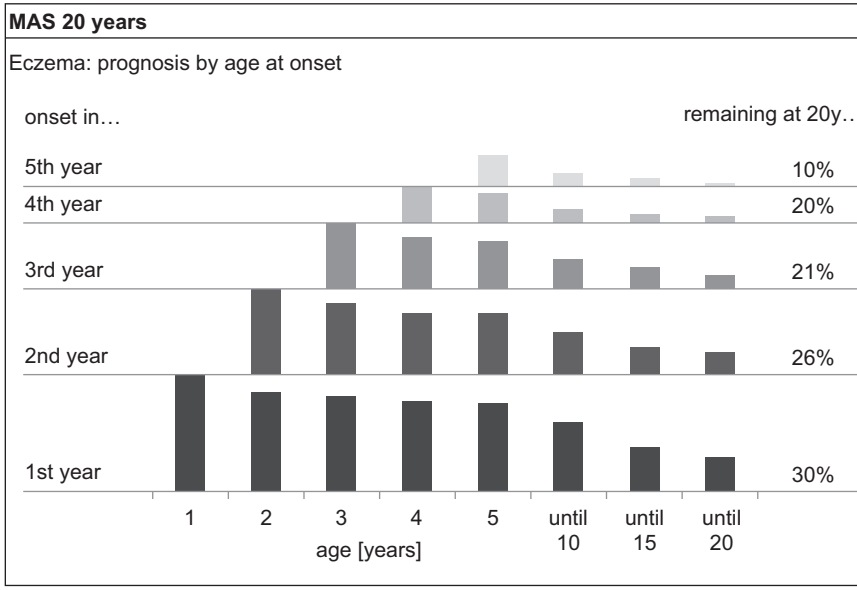


Figure 6 Atopic dermatitis: long-term outcome at age 20 years, by age of onset. Data are from MAS.[8]

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Adverse Reactions to Food

4

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Clinical presentation of food allergy in infancy

Infants and children who have food allergies may present with a heterogeneous pattern of clinical symptoms. Most food-induced allergic reactions are immunoglobulin E (IgE)-mediated (immediate type, type I hypersensitivity) as opposed to less frequent types: the non-IgE-mediated food allergy (cellular delayed type hypersensitivity) and a mixed type (IgE/non-IgE, often involvement of eosinophils or other cellular components).[1,2] The classification is mainly based on their different clinical features, the IgE level, or other features such as endoscopic findings.[1] In the following discussion, we will focus on IgE-mediated food allergies.

Food-induced IgE-mediated allergic reactions may affect every organ system. The skin is often involved and symptoms may include urticaria, angioedema, redness (flaring), and worsening of atopic dermatitis. Symptoms of the gastrointestinal tract include vomiting, diarrhea, or stomach pain; those of the respiratory tract are dyspnea, wheezing, stridor, cough, or rhinoconjunctivitis; and those of the cardiovascular system are hypotension, tachycardia/arrhythmia, and cardiovascular arrest.[3,4] In addition to objective symptoms, that is, subjective symptoms that may be hard to measure, are dizziness, tingling/burning in mouth and throat, itchiness of skin, eyes, or nose, general discomfort, dysphagia, and nausea. In addition, many symptoms are not specific and therefore it is sometimes difficult to truly connect symptom and diagnosis. For instance, vomiting and diarrhea might result from a gastrointestinal infection or be of psychological origin.[4] Symptoms may be mild, solitary, or combined with involvement of different organ systems and range in severity up to an anaphylactic, life-threatening reaction.[1] A specialty should be considered, especially in small children. A study noted different age-related patterns in the clinical presentation of children with severe allergic/anaphylaxis, indicating that infants (aged <2 years) with anaphylaxis more often experience hives and vomiting, whereas wheezing and stridor were more commonly observed in (anaphylactic) preschool-aged children (aged 2–5 years).[5] Another study reported that in younger age groups (aged 0–9 years in contrast to aged 10–17 years), the occurrence of respiratory symptoms seems to dominate, but circulatory symptoms seem to increase with increasing age in adults.[6] Nevertheless, it has been suggested that the rare reports of cardiovascular symptoms in younger children might "represent an age-related clinical pattern" or result from the less frequent measurement of blood pressure in younger children.[5] These data underline difficulties in recognizing food-induced allergic reactions and/or anaphylaxis in younger children.[5,7] Another

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explanation might be that specific allergens tend to involve specific organ systems more frequently than others.[3] Particular attention should be directed toward children with (coexisting) bronchial asthma because they may be at higher risk for more severe (anaphylactic) reactions.[8] Moreover, it has often been reported that severe food-induced allergic (anaphylactic) reactions are "dominated by respiratory compromise."[6,9]

Most IgE-mediated reactions occur immediately, that is, within a few minutes up to several hours after exposure to the ingested allergen.[8,10] A study using a modified oral food challenge procedure in children with peanut allergy showed that the median time from ingestion to clinical reaction was 55 min, ranging from 5 to 210 min. In addition, biphasic reactions with varying degrees of symptom severity can occur[11]. In biphasic reactions, with a prevalence of 2%–6% in the pediatric population,[8,12] "symptoms recur after resolution of the initial event in 1–72 h, with most developing within 8 h; in protracted reactions, symptoms do not respond to treatment and may last up to 72 h or more."[8,11] Because of the possibility of these biphasic and prolonged reactions, children should be observed in the hospital after severe food allergic or anaphylactic reactions. Guidelines from Great Britain recommend 6–12 h of observation; other countries differ slightly.[13]

Chronic reactions to food allergens are also described, resulting, for example, in worsening of atopic dermatitis. Other chronic gastrointestinal symptoms, such as poor weight gain, bloody stool, diarrhea, or constipation, might be caused by IgE-mediated food allergy but are more typical of non-IgE-mediated food allergy.

Even more complex, the severity of a single reaction in a single child cannot be predicted, because many factors such as the type and amount of an allergen in its matrix, how it is processed (roasted, boiled, or baked), and the means of uptake (oral, direct or indirect [breast milk]; or inhalation) may contribute. Moreover, risk factors have an important role.[14] Next to concomitant diseases such as bronchial asthma, which increase mortality, augmenting factors such as physical exercise or acute infectious diseases can lower the reaction threshold or make allergic symptoms more severe.[14] In addition, cofactors, defined as a subgroup of risk factors not acting on an immunological basis themselves seem to have a role; typical examples include certain allergens (e.g., peanuts or tree nuts), certain stages of development (e.g., adolescence), psychological factors (e.g., emotional stress), and drugs used on a daily basis (e.g., angiotensin-converting-enzyme inhibitors).[14] With respect to the influence of certain allergens on the severity of a food allergic reaction, a distinct distribution pattern has been observed in clinical settings after oral challenge tests.[3] The development of gastrointestinal symptoms occurred more often after challenges with hen's eggs compared with challenges with cow's milk, soy, and wheat, whereas the development of respiratory symptoms occurred more often after peanut challenges compared with challenges with cow's milk, hen's egg, soy, and wheat.[3] Interestingly, skin symptoms were present in nearly all positive food challenges.[3]

Key learning points

Clinical reactions to foods are heterogeneous: from mild to severe and life-threatening, affecting single or different organ systems, typically immediate but also late and chronic.

Epidemiology and natural course

Theoretically, an allergic reaction is possible to any food. Nevertheless, most reactions are caused by hen's eggs, cow's milk, peanuts, tree nuts, wheat, soy, sesame, and kiwi in young children, but fish, shellfish, peanuts, and tree nuts in older children and adults.[15] Although the prevalence of food allergies seems to be increasing worldwide, reliable data are scarce.[2,16,17] This is partly because the definition of food allergy varies and only few epidemiologic studies use the reference standard, the double-blind, placebo-controlled food challenge (DBPCFC) for food allergy diagnosis.[16] A systematic review and meta-analysis of the epidemiology of food allergy in Europe calculated the pooled lifetime and point prevalence of self-reported food allergy in children and adults at 17.3% (95% confidence interval [CI], 17.0–17.6) and 5.9% (95% CI, 5.7–6.1), respectively, but challenge-verified food allergy at 0.9% (95% CI, 0.8–1.1), with food allergy being more prevalent in the northern than southern Europe.[16] Challenged-verified data from a population-based cohort from Australia revealed an estimated prevalence rate of 3.0% for peanut allergy, 8.9% for raw eggs, and 0.8% for sesame in 1-year-old infants[18]; and estimates from the United Kingdom suggest that 2% of 8-year olds have peanut allergy.[19] Generally, estimations for self-reported food allergies from Canada report that 8% of the overall population have food allergy, 7.1% of which are food-allergic children (most common allergens are cow's milk [2.2%], peanuts [1.8%], and tree nuts [1.7%]).[20] Worldwide, certain geographic associations with food allergy have been described.[21] For example, the two most common food allergens are milk and eggs; the third varies among countries[22]: peanuts in the United States (US) and Switzerland, wheat in Germany and Japan, tree nuts in Spain, and sesame in Israel.[23,24] This is not only because of the prevalence of certain allergens and characteristics of a country's traditional eating habits, but also the "pattern of immunologic reactivity to individual allergenic components within the food."[21]

Today, an affected child seldom has just one food allergy. "A progression from milk and/or egg allergy in early infancy to additional food allergies, for example, peanut, is common. This phenomenon is analogous to the 'Atopic March' and is best described as the 'Food Allergic March.'"[15]

Many but not all children tend to outgrow cow's milk or hen's egg allergy and develop tolerance. Focusing at first on **cow's milk** allergy, one of the most common food allergies in children, recent data documented rates of tolerance of 19% by age 4 years, 42% by age 8 years, 64% by age 12 years, and 79% by age 16 years.[25] Others show even a higher rate of tolerance development.[26] Interestingly, most (75%) of children with cow's milk allergy seem to tolerate extensively heated (baked) milk products.[27] These children seem to outgrow cow's milk allergy faster; therefore, it has been suggested that at least two different phenotypes of IgE-mediated cow's milk allergy exist in children: a type with transient food allergy tolerating baked foods and a persistent type also reactive to baked milk.[27] Interestingly, when children who tolerate heated milk during an initial oral food challenge add baked milk frequently to their subsequent diet, the development of unheated milk tolerance seemed to occur more rapidly.[28]

Similar observations were made regarding **hen's egg** allergy: In a huge prospective, population-based cohort, challenge-confirmed hen's egg allergy at age 1 year resolved

in 66 infants (47%) (95% CI, 37%–56%) by age 2 years.[29] Eighty percent (95% CI, 73%–86%) of infants who reacted to raw egg white on baseline challenge were able to tolerate baked egg. Similar to cow's milk allergy, tolerance to baked egg at 12 months seems to predict the resolution of egg allergy; moreover, frequent ingestion of baked egg seems to accelerate the development of tolerance.[29]

With respect to other typical food allergens in young children, for example, **soy and wheat**, it is known that "most manifestations of food allergy are generated through *primary* food-specific sensitization," but *secondary* can occur when generated as a consequence of primary sensitization to cross-reacting pollen antigens via inhalation.[30] Approximately 0.4% of children have primary **soy allergy**.[31] Similar to cow's milk or hen's eggs, it is "generally thought that the majority of children with (primary) soy allergy develop tolerance in early childhood." In a study about the natural history of soy allergy, the Kaplan–Meier analysis predicted resolution of soy allergy in a highly atopic population in 25% by age 4 years, 45% by age 6 years, and 69% by age 10 years.[31]

The typical **wheat allergy** generally begins in early childhood. Rates of resolution of 29% by age 4 years, 56% by age 8 years, and 65% by age 12 years are described.[32] In a small Finnish study, the resolution was even better, with 84% in children aged 10 years and 96% of children aged 16 years.[33] In addition to this classic food allergy, other manifestations of IgE-mediated allergy are described in regard to wheat, such as exercise-induced anaphylaxis, occupational asthma (so-called baker's asthma) and rhinitis, and contact urticaria whereas these mainly occur in adulthood.[34]

In contrast to these food allergies, the probability of overcoming an allergy against fish, sesame, or peanuts and tree nuts at any age is dramatically lower. Studies document that around 20% of **peanut**-allergic children outgrow the peanut allergy,[35–38] but some reports suggest that up to 50% of peanut allergy is transient.[39–42] Moreover, peanut allergy may recur, with an estimated recurrence rate of 8%.[43] Roasted peanut is considered to be the most potent allergenic food and subsequent reactions are associated with fatal episodes of anaphylaxis.

Sesame allergy appears to have increased in many countries, with a common manifestation in the first year of life. Unfortunately, a minimum amount of allergen may cause an allergic reaction and only a few patients develop tolerance (80% rate of persistence).[44] Similarly, **fish** allergy is usually persistent; around 80% of patients are allergic even a decade after the initial diagnosis.[45] "Fish and crustaceans are among the four food groups most commonly provoking anaphylactic reactions."[45] However, data on fish allergy are limited.

Key learning points

Food allergies appear to have increased worldwide. Most allergic reactions in early infancy are caused by hen's eggs, cow's milk, peanuts, tree nuts, wheat, soy, fish, and sesame. Of these food allergies, cow's milk, hen's eggs, wheat, and soy are considered transient with regard to the development of tolerance, whereas peanuts, tree nuts, sesame, and fish are considered allergens with higher allergenicity and a more persistent course.

Pathogenesis

The immunopathogenesis of food allergy is subject to a complex of interactions between genetic and environmental factors. Identification of the major player is of great interest to find preventive strategies. Food allergy is often one of the first manifestations of the atopic march. The atopic march describes the subsequent age-related observation of atopic disorders and accompanying sensitization to food and aeroallergens associated with atopic dermatitis and/or wheeze within the first years of life, and often chronic allergic asthma, allergic rhinitis, and other clinical manifestations of atopic disease in later life.[46] The strong connection between the atopic entities is also noted when looking at coexisting atopic diseases in children with food allergy.[24] As in the study on cow's milk allergy, 49% of 807 cow's milk-allergic children (median age, 13 months) enrolled had coexisting asthma, 40% had allergic rhinitis, and 71% had atopic dermatitis.[25]

The pathogenesis of the atopic march became clearer when the strong relation between filaggrin (FLG) gene mutations and atopic eczema was documented[46,47] (Figure 1, Ref. 48).

Filaggrin is believed to have a key role in maintaining an effective skin barrier against the environment. Consequently, impairment of FLG may enable allergens to penetrate the skin of patients with atopic dermatitis and induce an allergic sensitization.[46] Filaggrin mutations adjusted for atopic dermatitis were associated with food sensitization (odds ratio, 3.0; 95% CI, 1.0–8.7; $P = .043$), which fits nicely with the hypothesis that a barrier defect promotes sensitization. Finally, the longitudinal relationship among three common FLG gene loss of function (FLG-LOF) mutations and food allergy was explored[49] and demonstrated an association with FLG-LOF mutations and eczema and food allergen sensitization, but also a role in the development and persistence of food allergy. Moreover, FLG mutations and food sensitization represent two distinct mechanisms interacting in the pathogenesis of asthma.[50]

Several studies have demonstrated that atopic dermatitis is a strong risk factor for IgE-mediated food allergy.[51] Other barrier defects such as tight junctions defects (e.g., claudin-1) have been discussed as other or further predisposing genetically based factors.[52] Overall, it is difficult to imagine skin barrier dysfunction without

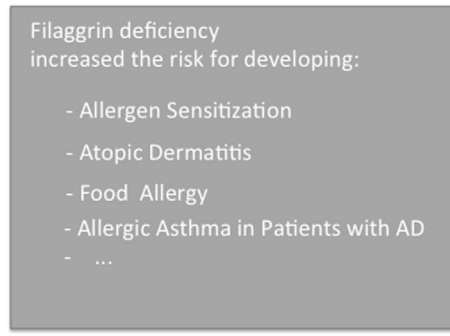


Figure 1 Association between filaggrin and skin and allergic diseases.

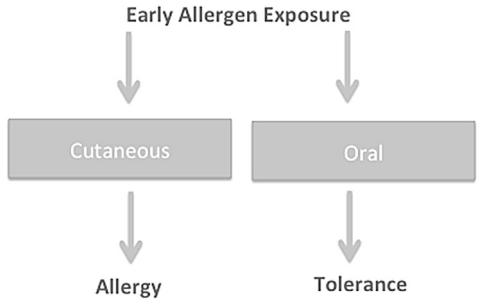


Figure 2 Dual-allergen exposure hypothesis for the pathogenesis of food allergy. Simplified from Ref. 21.

atopic dermatitis, and therefore barrier dysfunction should be added to the list of risk factors for food allergies, in addition to heredity, route, and timing of exposure to food allergens (e.g., introduction of solid foods [see **Chapter 16**, dietary intervention in infancy]), vitamin D sufficiency, dietary fat, antioxidants, obesity, exposure to infection, and other factors.[21] G. Lack proposed the "dual-allergen exposure hypothesis" for the pathogenesis of food allergy, implicating allergic sensitization after cutaneous exposure of inflamed skin, whereas tolerance occurs after oral exposure to food[21] (Figure 2, Ref. 21).

This hypothesis is supported by the observation that peanut allergen can be detected in house and bed dust.[53] Because infants spend a large proportion of time in bed, cutaneous exposure to peanut protein via the skin with barrier dysfunction may promote specific sensitizations. In addition, T-cell–reactive peanut-derived epitopes were cloned from the skin of a 6-month-old infant with atopic dermatitis, even though the mother had not eaten peanuts during the pregnancy or lactation period, nor had the child ingested peanuts during the first few months of life.[54] The second part of the dual-allergen exposure hypothesis, which proposes that early and **frequent** oral consumption of (allergenic) foods might induce tolerance, is based mostly on observational studies[24]; however, intervention studies are under way (see **Chapter 21**).

Altogether, the hypothesis of the atopic march is backed by genetic as well as epidemiologic observations. It has been shown that hen's egg-specific IgE at age 12 months is a valuable marker for subsequent allergic sensitization to other allergens that cause asthma, allergic rhinitis, and atopic dermatitis.[55] Moreover, a Swedish study showed that having a food allergy or being food sensitized is next to atopic dermatitis, parental allergic diseases, and male gender as risk factors for allergic rhinitis at 4.5 years.[56,57]

Key learning points

Pathogenesis is multifactorial; genetic and environmental factors are involved. An impaired barrier function of the skin seems to have an important role in the development of food sensitization and subsequent atopic diseases.

Diagnostic

Accurate diagnosis of a suspected IgE-mediated food allergy is important not only to prevent children from experiencing severe, life-threatening allergic reactions, but also to avoid unnecessary diets.

Key elements in the diagnosis of food allergy are:

- Clinical history and examination
- Determination of specific sensitizations
  - Food-specific IgE
  - Skin prick test (SPT)
- Oral food challenge

Careful evaluation of clinical history including the child's dietary history (breast milk or formula, introduction to solid foods, and observed reactions to foods), family history, physical examination (examination of nutritional status and growth, atopic signs such as eczema or dermatitis, allergic rhinitis, and asthma) combined with the determination of food allergen-specific sensitizations are the first steps in the diagnostic workup.[58] **Food-specific IgE** (sIgE) antibodies might be measured in serum but sensitization may also be detected in the skin using **SPT**. The atopy patch test is not recommended in routine diagnostics.[58,59] It is important to emphasize that detection of food-specific IgE or a positive SPT by itself confirms only a specific sensitization, that is, the presence of food-specific IgE. For the diagnosis of a clinically relevant food allergy, either a clear recent history (e.g., generalized urticaria and wheezing within 30 min after consuming a peanut snack for the first time) or an oral food challenge test is often necessary. The reference standard for the diagnosis of food allergy is the DBPCFC. Because this procedure is time-consuming and subjects the patient to the risk of an anaphylactic reaction, numerous attempts have been made to estimate the probability of a positive challenge outcome based on immunologic parameters such as SPT or sIgE.

- The **SPT** is easy to perform, rapid, and inexpensive. Native allergens seem to be superior to commercially available extracts.[60–62] The SPT has a high sensitivity but low specificity.[62] Predictive decision points for a positive outcome of food challenges have been calculated for some foods such as hen's eggs and cow's milk, based on wheal size and skin index rather than simply recording the result as positive or negative.[60–62]
- With regard to **food-specific IgE**, numerous studies performed worldwide in several centers calculated diagnostic decision points for some (but not all) foods (eggs, milk, peanuts, and tree nuts), which "provide a useful tool for predicting outcome, especially if the level is high (>20 kUA/L)."[63] Nevertheless studies have not always been consistent and it was not possible to attribute one specific cutoff value that provides optimum specificity and sensitivity for any single food across all populations (especially including different age groups) (Table 1). However, all studies showed a direct correlation between increasing concentrations of food-specific serum IgE and the probability of a positive reaction upon food challenge.[61] The benefit of measuring total IgE in addition to specific-IgE remains controversial, but it is not recommended in the *National Institute of Allergy and Infectious Diseases* Guidelines.

Table 1 Specific IgE concentrations with 95% positive predictive values, age-dependent

Age	Allergen		
	Eggs	**Milk**	**Peanuts**
Age <2 years	2 kUA/L	5 kUA/L	Not clear
Mean age 5 years	7 kUA/L	15 kUA/L	15 kUA/L

kUA/L = Allergen-specific KU/L.
Modified from Ref. 64.

Both methods have advantages and both should be used and individually adapted to each patient.[63] Importantly, although results of SPT and sIgE are often used interchangeably in clinical practice and allergy research, discordant results exist between these methods.[62,65] In children from a Danish at-risk birth cohort, food allergen sensitization at age 6 months and 1.5, 4, and 6 years has been detected by both methods and it was found that "the prevalence of food sensitization increased during childhood when diagnosed from sIgE (7.8–12.1% to 15.0–18.9%, $P<0.0001$), but decreased when diagnosed from SPT (5.3–5.1% to 3.7–3.0%, $P=0.05$)."[66] Therefore, it has been recommended that the tests should not be used interchangeably; especially in children with a suggestive history who have a negative test result with one test, the alternative test should also be used.[65]

Although SPT and sIgE are established tools in the detection of IgE-mediated food allergy, they have disadvantages, including the fact that positive test results to tolerated foods are common; results can be affected by factors such as age, reagents used, and cross-reactivity between proteins found in pollens and foods. Also, test results do not accurately predict the severity of an allergic reaction.[67] Therefore, a lot of interest has been focused on **allergen component-resolved diagnostics (CRD)**, an analysis of component proteins in allergenic foods and pollens. In CRD, specific components of pure allergenic proteins, produced by purification from natural allergen sources or recombinant allergenic proteins produced by expression of allergen-encoding complementary deoxyribonucleic acid, are used for analysis compared with crude allergen extracts consisting of a mixture of allergenic and nonallergenic components.[67] The method is theoretically attractive, and because of its potential higher accuracy, IgE-based allergy diagnostics may be improved.

So far, the most experience has been with CRD and **peanut** allergy, in which food challenge outcomes are compared with specific-IgE levels for the peanut seed storage proteins Ara h 1-3, -4, and -6, the lipid transfer protein Ara h 9, and the PR-10 protein Ara h 8, which has high homology to the birch pollen protein, Bet v 1. Studies assume that[2]:

- Sensitization to the birch protein (Bet v 1) homolog Ara h 8 is believed to indicate only a good prognosis.[68]
- Ara h 2 (Ara h 1–3, seed storage proteins) seems to be a good predictor of allergic reactivity, probably better than standard peanut testing.[69,70]

- Results regarding the predictive value vary among studies.[70,71] Importantly, "binding to Ara h 1, Ara h 2, or Ara h 3 does not necessarily diagnose a severe reaction and lack of binding to these proteins has been observed in some patients with severe reactions to peanut."[2] "Of note, the predictive value of SPTs remains competitive with these serum tests."[2,72]

A study by Vereda et al.[73] documented geographic differences in children with peanut allergy: Spanish patients were primarily sensitized to lipid transfer protein Ara h 9, Swedish patients—with milder symptoms/oral allergy symptoms—to the birch pollen-related protein Ara h 8, and US patients—with severe symptoms—to seed storage proteins Ara h 1–3.

- Regarding **cow's milk** allergy, there are conflicting data regarding whether measurement of a specific IgE to singular allergen components such as casein (Bos d8), β-lactalbumin (Bos d 4), β-lactoglobulin (Bos d5), bovine serum albumin (Bos d 6), immunoglobulin (Bos d7), or lactoferrin are superior to the measurement of specific IgE to crude cow's milk allergen extract as used in current immunoassays. One study suggests the Bos d 8 may better predict oral food challenge outcomes,[74] whereas another found no benefit through analysis of commercially available allergen microarray assay using Bos d 4, 5, 6, and 8.[75]
- In **hen's egg** allergy, some authors have suggested that IgE binding to ovomucoid is superior to standard tests,[76,77] whereas others found no benefit of CRD.[75,78]
- Regarding **wheat** allergy, wheat-dependent exercise-induced anaphylaxis has been associated with sensitization to omega-5-gliadin (Tri a 19),[79] but this entity is more common in late childhood or adulthood. In contrast, in children with primary wheat allergy, measurement of omega-5–specific IgE did not correlate with the outcomes of oral food challenges in wheat-sensitized patients with suspected wheat allergy.[80]

In general, "CRD suffers from some of the same limitations seen in the standard tests. For example, the degree of binding is relevant, specific test platforms might differ, and there could be differences depending on geography, age, and target organ reactivity."[2] In a systematic review and meta-analysis, the authors compared SPT, specific-IgE measurement, component-resolved diagnosis, and atopy patch test with the reference standard of DBPCFC of different foods and came to a similar conclusion.[81] The authors concluded that "SPT and specific-IgE (and probably also component-specific-IgE) have good sensitivity, but poor specificity with wide variation in estimates for each of the food allergies investigated."[81] To date, many studies have shown the value of CRD in the diagnosis of food allergy, especially in peanut allergy, but more studies are needed to establish its position and versatility as a routine diagnostic. Any method should be selected and individually adapted to the single patient. Nevertheless, for verification of the diagnosis *food allergy*, in most cases the oral food challenge is necessary.

Importantly, measurements of food-specific IgG and IgG4 levels have not been helpful in predicting clinically relevant food allergy.[63] Other tests such as basophil activation assays or diagnosis with T-cell proliferative responses likewise have not been shown to have a place in routine diagnosis of food allergy.

The reference standard in diagnosis of food allergy remains the **DBPCFC**. Open or single-blind challenges may be acceptable under special conditions.[63] The blinding enables correct identification of allergic symptoms and rules out asymptomatic sensitization and functional symptoms; double-blinding means that neither the patient nor the supervising medical team (doctor or nurse) knows the order of the given foods

that contain the masked allergen or just a placebo preparation that mimics the allergen dosages. Single doses are given in increasing concentrations every 20–30 min. The challenge is the reference standard to diagnose and monitor food allergy, or to prove oral tolerance. In December 2012, the PRACTALL[63] consensus report about standardizing DBPCFCs was published. In the following section, we rely on the PRACTALL report, which was composed by experts from the US (AAACI) and Europe (EAACI) and describes in detail the safe conduct of DBPCFC. In this chapter, we will provide an overview of the central elements of the test procedure.

Briefly, prechallenge assessment includes[63]:

- Avoiding the food to be challenged for at least 2 weeks; a stable baseline, that is, no active symptoms, with regard to atopic disease is recommended.
- Medications that may interfere with interpretation of challenge results (such as steroids and antihistamines) or affect safety (such as β-agonists) should be discontinued in advance if possible.
- Challenge is preferably performed on an empty stomach.

Challenge procedure:

1. Challenges should be "conducted in a location that is well-equipped to deal with anaphylactic reactions by physicians and staff who are well-trained in dealing with such reactions." Emergency medications with appropriate doses should be prepared and made readily available.[63]
2. Administration of active and placebo challenges should be on separate days, or challenges should be separated by at least 3 h.
3. Preparation of challenged food should mask the natural taste, odor, texture, and color to optimize blinding.
4. Suspected food or placebo should be given to the patient, starting with a low dose that is then gradually increased until the cumulative dose is achieved. A general challenge schedule consisting of 3, 10, 30, 100, 300, 1000, and 3000 mg food protein at intervals of at least 20 min is recommended.[63]
5. A 2-h observation after ingestion of the last dose is recommended. Especially in peanut allergy, IgE-mediated reactions might occur after 2 h.[82]

Assessment and interpretation of results include:

6. Intense monitoring of the patient through the test, including clinical examinations, assessment of respiratory, and cardiovascular parameters.
7. Food allergy challenges are usually stopped if objective clinical reactions are observed or the last dose is consumed without clinical symptoms.[58]
8. Scoring (e.g., with the scoring systems provided in the PRACTALL[63] report).
9. Observe for late reactions to food, especially in patients with atopic dermatitis (AD), and biphasic reactions.

Because patients may develop tolerance spontaneously, reevaluation by oral food challenge should be performed at regular intervals "to avoid inappropriate or unnecessarily lengthy dietary elimination diets that may impair the quality of life, affect normal growth, and lead to unnecessary healthcare costs."[58]

In this context, measurement of food allergen-specific IgE levels over time are sometimes helpful in determining the development of tolerance; however, an oral food challenge is the only method to prove it. "In cow's milk or hen's egg allergy, intervals for reevaluation might be every 6–12 months, while for peanut and tree nut allergy,

[oral food challenges] every 2 years in the absence of an accidental reaction would be more appropriate."[58]

Key learning points

The correct diagnosis of food allergy is important but difficult. Patient history, measurement of specific serum IgE to crude allergen extracts and allergen components, and SPT are helpful tools and might reduce the need for an oral challenge test. The reference standard remains the DBPCFC.

Therapy

Strict **avoidance of causal food(s)** is one of two backbones in the therapy of food allergy (long-term management).[83] Allergen avoidance is often difficult because many food allergens are ubiquitous in the food supply and contamination of other nonallergenic food might occur. Therefore, patients, parents, and caregivers need to be educated on how to read ingredient labels, how to prepare food alternatives, what is to be considered when eating away from home, and risk of cross-reactivity and cross-contact. Moreover, in case of multiple food allergies, a balanced diet under the supervision of a dietician may be necessary to avoid malnutrition or nutritional deficiencies. With regard to current feeding recommendations for infants with cow's milk allergy, please refer to Chapter 16 (Dietary interventions in infancy: food allergen avoidance, hydrolysates, preprobiotics), in which the use of and evidence for cow's milk formula alternatives is discussed (Figure 3).

The other backbone is patient/parent education to promptly **treat allergic reactions** (short-term management). Assessment of the risk of severe reactions is crucial in successfully managing patients with food allergy. Risks vary in different patient subgroups and depend on various risk factors; for example, patients with previous anaphylactic reactions, bronchial asthma, or mastocytosis seem to have a higher risk of

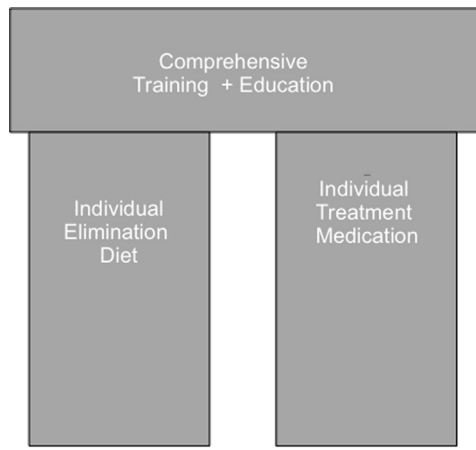


Figure 3 Backbone strategies in managing food allergy.

severe reactions compared with other patients. Other augmenting factors may worsen symptoms and include nonsteroidal anti-inflammatory drugs, exercise, and infections.[14,84] Other cofactors may also have a role; for example, it has been observed that certain allergens such as peanuts cause more severe reactions.[3,14]

Data from a prospective observational study of allergic reactions to foods in preschool children allergic to egg or milk showed that 53% of patients reported more than one reaction in an observation period of 36 months.[85] Most reactions (71%) were accidental and were attributed to unintentional ingestion, label-reading errors, and cross-contact. Eleven percent of reactions were severe but just 30% were treated with epinephrine. Therefore, education and training of indexed patients including their caregivers are a fundamental part of managing food allergies, including a **personalized emergency management plan,** which takes into account the "age of the patient, literacy of patient and family, type and range of food allergy, concomitant disease, geographic location, and access to medical support."[58] Training should cover patient-specific avoidance strategies at home and in the wider environment, interpretation of warning signs, and when and how to treat reactions including use of self-injectable adrenaline if appropriate.[58,84] It has been shown that a structured patient education program on knowledge, emergency management skills, and psychological parameters may be beneficial in managing anaphylaxis by increasing patients' empowerment to prevent and treat the disease.[86]

Medical treatment includes[84,87]:

- **First-line treatment:** ***inject*** epinephrine: "Intramuscular adrenaline (1 mg/mL) should be given at a dose of 0.01 mL/kg of body weight (maximum dose: 0.5 mg/dose). For the patient adrenalin-auto-injectors are available with fixed dosages (0.15, 0.3, and 0.5 mg [in Europe]). When using adrenaline auto-injectors it has been suggested to use the 0.15 mg dose in patients weighing between 7.5 and 25 kg and move to the 0.3 mg dose at 25–30 kg" to optimize dosing[88]
- **Second-line treatment:** inhaled β-2 agonists for wheezing; inhaled adrenaline for stridor; remove the trigger, call for help, position
- **Third-line interventions:** H1- and H2-antihistamines, glucocorticoids (age-dependent dosages).[87]

Immunotherapy for food allergy

Because some foods have a poor prognosis, including potential life-threatening consequences, considerable research effort is focused on immunotherapy in food allergy.

Interestingly, as discussed above, roughly 70% of cow's milk- or egg-allergic children are able to tolerate the allergen in baked foods such as cake and biscuits.[89,90] Children who tolerate baked eggs or cow's milk seem to resolve the allergy to the native allergen more quickly.[28,91] It has been shown that infants with proven tolerance to baked hen's eggs at age 1 year have a 50% likelihood of developing tolerance to raw hen's eggs by age 2 years compared with a 13% resolution rate in baked egg-allergic infants.[29] Moreover, this effect seems to be associated with the frequency of oral consumption of baked eggs.[29] Because comparable immunological changes have been observed in children who eat regular baked hen's eggs or cow's milk compared with those undergoing oral immunotherapy,[89] baked foods may be regarded as a form of immunotherapy.[90]

Generally **allergen-specific and allergen-nonspecific therapies** need to be distinguished.[92] The ultimate goal of all therapies is to induce tolerance. With respect to allergen-specific therapies, the specific allergen is usually given to the patient by the subcutaneous, oral, or sublingual route. So far, most clinical trials have been conducted with peanuts, hazelnuts, hen's eggs, or cow's milk. In general, oral immunotherapy (OIT) with food allergens leads to desensitization and reduced allergic symptoms for children and adults.[93] Nevertheless, an increased risk of side effects (local and systemic) needs to be considered.[93] Sublingual immunotherapy (SLIT) also seems to be effective but overall, protection appears to be less than that seen with OIT.[93] For example, in cow's milk allergy, although it is associated with more systemic side effects, OIT appeared more efficacious than SLIT.[83,94,95] Subcutaneous immunotherapy in food allergy has been performed mainly in adults and has been associated with severe adverse reactions; therefore, data in children and infants are rare.[83]

Other issues are the long-term efficacy and side effects of specific immunotherapy. One uncontrolled study demonstrated sustained unresponsiveness after peanut OIT in about one-half of subjects treated for up to 5 years.[96] However, aside from immediate adverse reactions, it has been shown that the development of eosinophilic esophagitis develops in about 2.5% of children undergoing OIT.[97]

More studies are necessary to assess these issues. At this time, OIT is not recommended as routine treatment.[98,99] "In light of its potential benefit, it should be performed only in highly specialized centers, with expert staff and adequate equipment, and in accordance with clinical protocols approved by local ethics committees."[58]

Alternative strategies to specific immunotherapy attempt to induce desensitization via T cells without activating an IgE-mediated allergic response by using tolerogenic peptides. These strategies are currently being investigated in phase 1 studies (recombinant modified peanut proteins Ara h 1, Ara h 2, and Ara h 3[100] or in murine models).[101,102] Unfortunately, rectal administration of modified peanut proteins resulted in frequent adverse reactions, including severe allergic reactions in 20% of patients.[100]

Other nonspecific immunotherapies include treatment with anti-IgE antibodies (omalizumab; Xolair; TNX-901) and humanized monoclonal anti-IgE antibodies.[83] In these trials, it was possible to increase the thresholds of reactivity to food allergens in a subgroup of patients.[103,104] With the combination of anti-IgE and OIT, a rush oral desensitization over 7–11 weeks could be performed but site effects were still present.[105] Although promising, "the cost and availability of anti-IgE therapy within public healthcare systems are likely to be significant limiting factors."[90]

Another nonspecific strategy is the use of a Chinese herbal formula, FAHF-2, which has been used in a phase 1 study. Nevertheless, this therapy is currently being investigated and is far from routine clinical use.[106] In addition, there are insufficient data at this time to recommend the use of prebiotics or other supplements for the long-term management of food allergy.[83]

Key learning points

Management strategies in food allergy include a diet eliminating the responsible food allergen(s) and medical treatment in case of accidental allergen ingestion. Education and training of patients, family members, and caregivers is essential to ensure its

implementation of appropriate elimination diets and emergency measures. Immunotherapy for food allergy is currently only a research intervention and treatment is often associated with significant adverse effects, but on the whole, results are promising.

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Viral-Induced Wheeze and Asthma Development

5

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Introduction

Wheezing illnesses occur in up to 50% of children by age 6 years[1] and are almost always initiated by viral infections. Respiratory syncytial virus (RSV) and rhinovirus (RV) infections are the two major causes of preschool wheezing illnesses, and there are a number of other viruses that cause smaller numbers of cases. Viral lower respiratory illnesses (LRI), with or without wheezing, are the most frequent cause of hospitalization in young children, and unfortunately specific treatments are lacking. Thankfully, for most children virus-induced wheezing episodes are transient and cease to occur after the preschool years. However, some children who wheeze in early life go on to develop persistent childhood asthma. It is important to define risk factors for the transition from recurrent virus-induced wheeze to persistent childhood asthma, to develop new strategies for asthma prevention. This review will focus on the epidemiology and etiology of virus-induced wheezing in early life and the relationship of viral LRI in early life to the subsequent development of asthma.

Longitudinal studies of childhood wheezing

One of the first large birth cohort studies in the United States to evaluate the natural history of childhood wheezing and risks associated with wheezing and asthma development was the Tucson Children's Respiratory Study.[2,3] An important finding of the study was the description of distinct wheezing phenotypes that occur during childhood: transient, nonatopic, and atopic.[4] Investigators later modified and identified four distinct wheezing patterns: never wheeze, transient early wheeze, late-onset wheeze, and persistent wheeze. Never wheeze was described as never having had a lower respiratory wheezing illness from birth to age 6 years. Transient early wheeze was described as having a wheezing lower respiratory illness before age 3 years only. Late-onset wheeze was described as wheezing at age 6 years only, and persistent wheeze was described as having a wheezing lower respiratory illness before age 3 years and wheezing after age 6 years.[5] Long-term follow-up of children in Tucson and other cohorts indicate that children with a persistent wheeze phenotype are at greater risk for subsequently developing asthma. Interestingly, the risk of asthma is not linked to viral infections per se; rather, the clinical response to infection seems to be most important. For example, the German Multicentre Allergy Study (MAS)

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Table 1 **Modified asthma predictive index**[10]

Modified asthma predictive index		
≥ 4 wheezing episodes in a year, plus:		
At least 1 major	**OR**	**At least 2 minor**
Parental asthma		Wheezing unrelated to colds
Atopic dermatitis		Eosinophils ≥ 4% in circulation
Allergic sensitization to at least one aeroallergen		Allergic sensitization to milk, eggs, or peanuts

reported that frequent episodes of upper respiratory illness marked by runny nose were inversely related to the development of asthma at age 7 years, whereas viral wheezing illnesses were positively related to the same outcome.[6]

The Tucson study also developed an Asthma Predictive Index based on risk factors for the transition between wheezing in infancy and asthma (Table 1).[7] This index can be used in the clinic to predict which children with recurrent wheeze have a high versus low probability of developing asthma in the next few years. It has been validated in other populations,[8] and a version modified to include more objective criteria has been used in clinical research studies to identify high-risk preschoolers.[9,10] Additional indices have been developed from other study populations, with similar performance characteristics.[11,12] Many of the risk factors for asthma onset after recurrent wheeze in childhood are related to personal or parental atopy (e.g., eczema, blood eosinophilia, or parental asthma).[13,14] Other factors include male sex, preterm delivery, and a history of wheezing apart from colds.[7,11,12]

Two of the longest longitudinal studies of wheezing illnesses and asthma were conducted in Australia and New Zealand. The Melbourne Asthma Study followed a group of children from age 7 years through adulthood. At age 42 years, study participants who had asthma as children were most likely to have significant wheezing into adulthood, with remarkable tracking of lung function and asthma severity.[15] Participants of the Dunedin, New Zealand, birth cohort were followed from birth through age 26 years. Over 25% of children had wheezing that persisted from childhood to adulthood or that relapsed after remission. Other factors that predicted persistent wheezing or wheezing relapse in this group included atopic sensitization, airway hyperresponsiveness, female gender, smoking, and early age of wheezing onset.[16] These longitudinal studies with extended periods of follow-up suggest that outcomes of asthma in adulthood are largely predetermined early in childhood (Table 1).

Etiology of wheezing illnesses in infancy

Most wheezing illnesses in the first few years of life are caused by respiratory viruses (Table 2).[17–21] Many viruses can cause wheezing illnesses in infancy, but the two most common are RSV and RVs.[21] Other frequent causes of wheezing illnesses include

Table 2 Pathogens associated with acute wheezing illnesses in infancy

Viruses	Bacteria
RSV	*Streptococcus pneumonia*
RV	*Moraxella catarrhalis*
Metapneumovirus	*Haemophilus influenzae*
Parainfluenza viruses	*Bordetella pertussis*
Coronaviruses	
Enteroviruses	
Bocavirus	
Influenza viruses	
Polyomaviruses	

metapneumovirus, coronaviruses, parainfluenza viruses, and bocaviruses. Coinfections with more than one virus are most common in young children and can result in greater severity of illness.[19,22] Prolonged illnesses are often caused by sequential infections with more than one virus.[23]

Respiratory syncytial virus

Respiratory syncytial virus is a medium-sized, negative-stranded ribonucleic acid virus of the family *Paramyxoviridae*, which causes a range of respiratory illnesses in infants and children. It can present as a benign upper respiratory illness or as severe bronchiolitis, especially in high-risk infants with prematurity of chronic lung disease. Respiratory syncytial virus infections are the most common cause of bronchiolitis in the first year of life, and nearly all children have had an RSV infection by age 3 years.[24]

Rhinoviruses

Rhinoviruses are members of the genus *Enterovirus* of the family *Picornaviridae* and are classified into three species (A, B, and C). More than 160 RV types have been identified to date.[25] Rhinoviruses are responsible for most upper respiratory tract infections (common colds) and, after RSV, are the viruses most often detected in wheezing infants. For years it was assumed that RV infection was confined to the upper airway and did not affect the chest except under unusual circumstances. Initial studies demonstrated optimal RV replication at 33–35 °C, and it was assumed that higher temperatures in the lower airways would limit RV replication. Contrary to these initial assumptions, direct measurements in the lower airways have shown that large and medium size airways are at the ideal temperature for RV replication.[26] Furthermore, some RV types, including C-species viruses, replicated equally well at 33 °C and 37 °C.[27,28] In addition, cultured lower airway epithelial cells support RV replication in vitro at least as well as, and perhaps even better than, cells derived from the upper airways.[29,30] Rhinovirus has been detected in sputum and bronchial biopsy specimens after experimental inoculation of

the upper airway[31–33] and is frequently detected in lower airway biopsies from infants with recurrent wheezing.[34] These findings provide strong evidence that RV can infect the lower airways, especially in young children. The species of RV affects its virulence, because RV-A and RV-C cause more severe illnesses in infants[35,36] and are more likely to cause exacerbations of childhood asthma.[37,38]

Bacteria and wheezing illnesses

It has been demonstrated that bacterial infections may also be associated with wheezing illnesses. The bacteria most commonly implicated include *Streptococcus pneumoniae, Moraxella catarrhalis,* and *Haemophilus influenzae*.[39] Furthermore, in a Danish birth cohort study, colonization with these common bacterial pathogens soon after birth was associated with increased incidence of recurrent wheeze and asthma in early childhood.[40] Viruses may precede overgrowth of respiratory pathogens, and both the virus and secondary changes in airway bacteria may contribute to the severity of acute illnesses.[41–44] However, serious bacterial infections accompanying bronchiolitis are rare,[45] and a meta-analysis found no evidence that antibiotics such as azithromycin or ampicillin are efficacious for treatment of bronchiolitis.[46] Because of the close relationships between viral and bacterial infection in the pathogenesis of other diseases such as otitis media and sinusitis,[47,48] understanding the role of airway bacteria in acute wheezing illnesses is a priority.

Risk factors for virus-induced wheezing

There are a number of risk factors for acute wheezing illnesses in early childhood (Figure 1).[1,49–51] These include factors related to genetics and lung structure and function (e.g., small lung size, prematurity). Environmental factors that can influence wheezing illnesses include siblings, day care attendance, stress, and exposure to

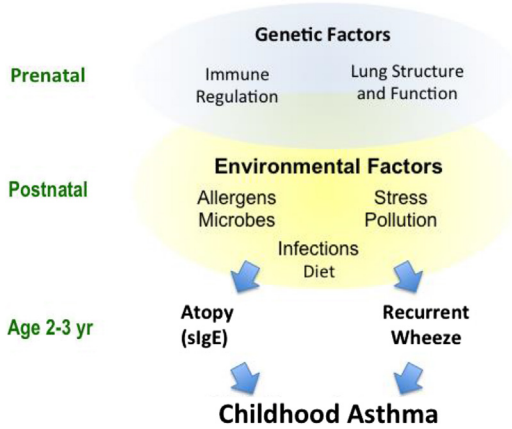


Figure 1 Risk factors for virus-induced wheezing and childhood asthma.

tobacco smoke, especially during the prenatal period. Other risk factors for viral LRI with wheeze include male sex, race, and low socioeconomic status. For RSV bronchiolitis, the relationship of birth date to peak RSV season is important; a birth date that occurs approximately 120 days before the peak of RSV season confers the highest risk.[51] For RV-induced illnesses, moderate-to-severe colds are more likely to occur in the wintertime, even after controlling for seasonal variation in the prevalence of different RV species.[35] Immunologic risk factors for viral LRI include low interferon responses from cord blood mononuclear cells and reduced numbers of plasmacytoid dendritic cells (pDCs), which are important sources of type I interferons.[52–55]

Genetics

Immune pathways that modify the risk of bronchiolitis have also been identified using genetic studies (Table 3). Several studies using a candidate gene approach have implicated genes encoding cytokines, chemokines, and antiviral effectors.[56–62] In addition, several studies have linked the severity of RSV illness to variation in surfactant and vitamin D pathways.[63] Fewer genetic associations of RV-induced wheezing have been identified. A study of infants hospitalized for their first episode of wheezing identified viral etiology and observed these infants for 2 years to assess recurrent wheezing. Investigators found that recurrent wheezing after RV bronchiolitis was associated with IL4RA, IL4RG, and MAP3K1 polymorphisms.[56] Finally, polymorphisms in the 17q12 region have been strongly linked to RV wheezing illnesses and to childhood asthma. In the Childhood Origins of Asthma (COAST) birth cohort study, there was an interaction between these outcomes; 17q12 genotype was strongly related to asthma, but only in children who had experienced RV wheezing illnesses in the first 3 years of life.[64]

Role of atopy

Personal or family history of atopy is an important risk factor for recurrent wheezing. Infants whose parents, especially mothers, had atopy or asthma are at increased risk for viral wheezing.[65] Sensitization to food proteins or aeroallergens in infancy is a risk factor for recurrent wheezing episodes.[66] Viral respiratory illnesses and wheezing

Table 3 Genes associated with viral-induced wheezing in infancy[56–58,61–64,110–113]

Cytokines and chemokines	Receptors	Other
Genes associated with RSV wheezing		
IL4, IL6, IL10, IL13, IL19, IL20, IFNG, IFNA5, CCL5, CXCL8, TGFB1, IL4RA, TNF	TLR4, CCR5, CD14, VDR	SPA, SPD, MBL, VDBP, JUN, NOS2, ADAM33
Genes associated with RV wheezing		
IL10	IL4RA	MAP3K1, 17q21

are more common in infants with atopic dermatitis, especially when the dermatitis is persistent.[67] Likewise, mutations in the *FLG* gene (encoding filaggrin) are associated with more significant atopic dermatitis and also with early wheezing illnesses.[68]

Microbial exposures

In addition to bacterial pathogens, there is interest in defining relationships between microbial exposures and colonization and the risk of wheezing illnesses. In western Europe, rural infants from farm families are less apt to develop transient wheeze, likely of viral origin.[69] Findings from other studies suggest that farm-related microbial exposures are likely to mediate protective effects against allergies, wheezing, and asthma.[70,71] Children growing up in poor urban neighborhoods have different exposures, with high rates of cockroach allergens, stress and violence, and pollutants. Even so, high-level exposure to microbes in house dust in the first year of life is associated with a reduced risk of atopy and atopic wheeze.[66] Children with the lowest risk of atopic wheeze are those who have increased exposure to both microbes and allergens.[66] These findings suggest that environmental microbial exposures in early life could be important in modifying the immune response to respiratory infections, either directly or indirectly through effects on atopy.

Progression from virus-induced wheezing to asthma

Role of atopy

Longitudinal cohort studies have established that there are strong and dose-related effects of early life sensitization on the development of asthma. For example, prospective studies of high-risk children in Perth, Australia demonstrated that serum immunoglobulin E (IgE) levels to house dust mite at age 2 years had a dose-related association with recurrent wheeze at age 5 years.[72] In this study, the number of LRIs and mite-specific IgE both contributed to risk for subsequent wheezing episodes.[72] In the MAS cohort, children with atopic wheeze lost lung function relative to children with nonatopic wheeze after age 5 years and lung function was lowest in children who were both sensitized and had exposure to the same allergen.[73] A latent class analysis of children in a population-based cohort identified multiple early sensitization to allergens as an important risk factor for recurrent wheezing and asthma at age 8 years.[74] Children who were polysensitized to multiple allergens before age 2 years have especially high risk for developing virus-induced wheezing episodes and subsequently, asthma. Polysensitization was also related to lower lung function and airway hyperresponsiveness.[74]

Respiratory syncytial virus and asthma

Respiratory syncytial virus bronchiolitis is associated with an increased risk for recurrent wheezing in the months after the acute infection, and up to half of children who are hospitalized with RSV bronchiolitis are subsequently diagnosed with asthma.[75]

These associations have been consistently observed in birth cohort studies and those with long-term follow-up of hospitalized infants. For example, Sigurs and colleagues conducted a case–cohort study of 93 Swedish children hospitalized for RSV bronchiolitis in infancy and 47 matched control children. Those with early RSV bronchiolitis were found to have recurrent wheezing or asthma, allergic sensitization, airway obstruction, and airway hyperreactivity in early adolescence.[76] This same group of children were followed into early adulthood and found to have an increased prevalence of allergic asthma.[77] Relationships with atopy and asthma have also been evaluated in birth cohort studies, with slightly different findings. For example, the Tucson Children's Respiratory Study observed children from birth and found that RSV lower respiratory illnesses in early childhood were a risk factor for recurrent wheezing up to age 11 years, but not age 13 years.[78] Unlike the Sigurs study, there was no association between RSV bronchiolitis and the prevalence of atopy later in childhood. Similarly, data from a large British birth cohort study (Avon Longitudinal Study of Parents and Children) found that hospitalization with RSV bronchiolitis in infancy was associated with asthma (odds ratio [OR], 2.5 [1.4, 4.3]) but not with atopy (OR, 0.7 [0.2, 1.7]) at age 7 years.[79]

There have also been efforts to identify risk factors for development of asthma after hospitalization with RSV bronchiolitis. In a prospective cohort study of 206 infants hospitalized with RSV, risk factors for subsequent asthma development included maternal asthma, exposure to dog allergen, and allergic sensitization by age 3 years.[75]

Intervention studies for RSV

Palivizumab is a monoclonal antibody to the RSV F protein that inhibits binding of the virus to cellular receptors. When administered preseasonally to high-risk infants, palivizumab reduces the rate and severity of RSV infections and the incidence of RSV bronchiolitis. Because of the high cost of this medication, its use is limited to infants who are at increased risk for adverse outcomes such as hospitalization for bronchiolitis.

Several studies have now tested whether prophylaxis with palivizumab reduces subsequent rates of recurrent wheezing and childhood asthma. A prospective nonrandomized study involving centers in Canada and Europe explored the influence of RSV prophylaxis on subsequent wheezing and found an 80% decrease in the risk of recurrent wheezing from ages 2 to 5 years in nonatopic children, but no effect in atopic children.[80] More recently, healthy preterm infants born at 33–35 weeks' gestation were enrolled in a double-blind, placebo-controlled trial of palivizumab prophylaxis and were monitored for 1 year to determine effects on recurrent wheeze. All of the children were age 6 months or less at the start of RSV season. In this study, palivizumab treatment led to a 61% relative reduction in the total number of wheezing days in the first year of life.[81] A case–control study in Japan enrolled a similar population of 444 infants with mild prematurity, 349 of whom were treated with palivizumab. The children were monitored for 3 years, and recurrent wheezing was significantly less common in the treated versus untreated groups (6.4% versus 18.9%; $P < .001$).[82]

Rhinovirus and asthma

Several studies have identified a relationship between the viral etiology of wheezing illnesses in infancy and the risk of developing asthma. In a study of children aged less than 2 years who were hospitalized for acute wheezing illnesses, RV was the predominant virus seen in these children after age 5 months. When followed up 5 years after hospitalization, 60% of children who wheezed with RV in the first 2 years of life had asthma.[83] Researchers from the same group found that hospitalization for RV-induced wheezing before age 2 years predicted the risk for asthma during the teen years.[84] The COAST birth cohort is a high-risk cohort of children observed prospectively through adolescence. In this cohort, outpatient RV wheezing illnesses during the first year of life are a significant predictor of recurrent wheezing through age 3 years.[19] Furthermore, RV wheezing illnesses in infancy significantly predicted development of asthma at age 6 years, especially among children with evidence of aeroallergen sensitization by age 3 years.[85] Similar findings were reported in an Australian birth cohort study involving high-risk children; early life infections with RV or RSV were associated with an increased risk of asthma, but only in children who had developed aeroallergen sensitization by age 2 years.[13] Several studies have linked the susceptibility of RV-wheezing illnesses to allergic predisposition in infants of atopic families.[86] These findings suggest that RV wheezing illnesses and early onset of atopy are both strongly associated with childhood asthma.

Do severe viral respiratory infections in infancy cause asthma?

Observational studies have defined a strong and consistent association between viral LRI with wheezing in infancy and subsequent childhood asthma, but this type of study cannot definitively establish causality. There are at least three possible hypotheses to explain the nature of this association: (1) there is a common predisposing factor that increases the risk of both viral wheezing and asthma; (2) children who are predisposed to asthma are more likely to wheeze during viral infections; or (3) viral respiratory illnesses contribute to the causation of asthma. There are data to support each of these possibilities, as discussed in the following sections.

Hypothesis 1: Infants who are already predisposed to asthma are more likely to wheeze when they develop viral illnesses. This hypothesis implies that asthma, or at least a predisposition to asthma, can be established early in life or perhaps during the prenatal period. Viral respiratory infections, which are a significant source of lower airway inflammation, merely reveal the underlying asthma and serve as a signaling event rather than having a causal role. In other words, children who are predisposed to virus-induced wheezing are also predisposed to asthma.

In support of this theory, asthma risk can be passed from parents to offspring through genetics (and perhaps epigenetic modifications) and also from mother to child via environmental exposures (e.g., tobacco smoke) in utero. To investigate maternal effects on RV- and RSV-induced wheezing illnesses, the Tennessee Children's Respiratory

Initiative conducted a longitudinal analysis of mother–infant dyads in which mothers were classified in four groups according to the presence or absence of atopy and asthma.[65] Results indicate that clinically significant RV illnesses during infancy were associated with a maternal history of atopic asthma. Maternal atopic asthma was also associated with increased severity of RV infection in infancy, which suggests that infants with RV wheezing in early life are more likely to have a familial predisposition to atopic asthma.[65] This association could help to explain why infants who experience RV wheezing illnesses are most likely to develop asthma subsequently.

Several twin studies have investigated a causal link between RSV infection in infancy and childhood asthma, but the results supported that susceptibility to asthma predetermines RSV infection.[87–89] In addition, studies of infant lung function suggest that reduced neonatal lung function is a risk factor for virus-induced wheeze[90,91] and asthma later in childhood.[92,93] The results of these studies provide evidence that predisposing factors such as lung function or familial atopy can influence the risk of virus-induced wheezing and asthma.

Hypothesis 2: Viral respiratory illnesses contribute to the causation of asthma. This hypothesis implies that viral infections can damage the structure or alter the function of the lower airways in such a way as to promote asthma. In fact, viruses that infect the lower airways lyse airway epithelial cells and induce inflammatory responses that cause further damage to the airways. The potential to cause persistent effects on the lower airway is greatest when viral LRIs occur in early life during rapid lung growth.[94] Animal models provide evidence that viral LRI in early life can have long-lasting effects on airway epithelial cell and macrophage inflammatory responses,[95] and can induce abnormalities in the morphology of small airways.[96]

Respiratory syncytial virus infections are seasonal, and analysis of the seasonal patterns of birth date, RSV wheezing illnesses, and incident asthma support a causative effect of viral illnesses on subsequent asthma. In a study involving over 95,000 infants in a Tennessee Medicaid database, the risk of developing asthma corresponded with the timing of infant birth in relationship to the winter virus peak.[51] Infants born approximately 4 months before the winter virus peak had the highest risk of developing RSV bronchiolitis and also the highest asthma risk (adjusted OR, 1.29; 95% confidence interval, 1.19–1.40) at age 5.5 years.

Intervention studies provide valuable insights into disease causation, and as outlined earlier in this review, studies of prophylactic use of palivizumab provide convincing evidence that prevention of RSV LRI in the first year of life reduces the risk of recurrent wheeze for 1–5 years.[80,81] To date, palivizumab has not been shown to prevent allergic asthma, and so far studies of effects on asthma have been restricted to infants who were born prematurely. Nonetheless, these findings demonstrate that prevention of acute virus-induced lung injury leads to beneficial effects on airway physiology for several years.

Clinical evidence supporting an association between RV wheezing illnesses and subsequent childhood asthma is strong, but in contrast to RSV, currently no interventions can prevent RV illnesses to assess effects on asthma. Nevertheless, other experimental evidence strongly suggests that RV infections can contribute to causation of asthma. One unique feature of RV infection is that with over 160 types,[97,98] a child who

is susceptible to RV LRIs with wheezing can develop multiple infections per year. RV infections probably cause less lower airway damage per infection compared with more virulent viruses such as RSV and influenza, but cumulative damage to the airways from multiple RV infections could be substantial. Notably, RV infections can induce factors (e.g., *vascular endothelial growth factor*, transforming growth factor-β)[99,100] that have been linked to airway remodeling, and in murine models, RV infections of young animals can induce mucoid metaplasia and airway hyperresponsiveness.[101]

Hypothesis 3: There is a common predisposing factor that increases the risk of both viral wheezing and asthma. This is a joint effect theory, in which a predisposing factor would cause viral wheezing *and* asthma, but viral wheezing is not causal for asthma. A number of factors could increase the risk of both virus-induced wheeze in early life and childhood asthma. Examples include atopy, immunologic factors (low interferon responses or bias toward type 2 T-cell responses), and lung factors (low lung function at birth or poor epithelial cell barrier function). It would be difficult to prove this theory because one would need to prove that the predisposing factor caused both viral wheezing and asthma, and that an intervention targeting viral wheezing would not affect asthma incidence.

Atopy, virus-induced wheeze, and the complex causality of childhood asthma

The three theories listed above are not mutually exclusive, and each could contribute to the association between viral wheezing and childhood asthma. As stated above, there is evidence that predisposing factors increase viral wheezing, and that viral wheezing can damage airway structures and promote remodeling. With the growing understanding that asthma has multiple endotypes and that the genetic and environmental influences (including viral respiratory infections) on childhood asthma onset are complex, there is no doubt that asthma causation is also multifaceted.

As an example, RSV and RV are the two viruses most closely associated with wheezing, but differences in the viruses and natural histories of illness likely translate into different relationships with asthma causation. Respiratory syncytial virus is the more virulent of the two viruses, and babies who develop severe RSV illnesses are at risk for recurrent wheezing that may persist for several months up to 10 years. Mechanisms for post-RSV recurrent wheezing are still speculative, but likely involve airway injury, and remodeling of the airway structures and perhaps neural elements.[102,103] After the initial infection, activation of the adaptive immune response reduces the risk of a second RSV LRI, and serious illnesses with subsequent RSV infections are uncommon.

The effects of RV and interaction with host effects could be different. RV wheezing illnesses are less common that RSV illnesses until year 2. There appear to be some common risk factors for wheezing with RV versus RSV (e.g., small lung size[90,91]), but there are some differences as well. Atopy appears to be an important risk factor for RV wheezing, but it is less of a factor for wheezing with other viruses.[104] Similarly, maternal asthma increases the likelihood of wheezing with RV infections but does not affect the risk of wheezing with other viruses.[65] Interestingly, for children who progress from RV recurrent wheeze to persistent asthma, the number of RV wheezing illnesses

in the first few years increases, and this is in distinct contrast to the stepwise reduction that is observed in children who do not develop asthma.[85] This temporal increase in susceptibility to RV wheezing occurs concurrently with increasing sensitization to aeroallergens. In fact, a temporal analysis in the COAST birth cohort demonstrated that atopy generally precedes RV wheezing episodes, rather than the converse (wheezing episodes preceding the onset of atopy).[105]

These findings raise the possibility that atopy might impair the immune response to viral respiratory infections, and several potential mechanisms have been described. For example, pDCs are potent sources for type I and III interferons, which have strong inhibitory effects on RV infections. pDCs are responsive to allergic inflammation because they express the high-affinity IgE receptor (FcεRI) on the cell surface. Notably, cell surface expression of FcεRI is inversely related to the virus-induced interferon secretion.[106] Furthermore, cross-linking FcεRI markedly impairs interferon responses to influenza virus or RV,[106,107] suggesting that the combination of allergy and allergic inflammation could inhibit antiviral responses at the mucosal surface. In epithelial cells, allergic inflammation and RV infection can induce mucus metaplasia through mechanisms that involve induction of the transcription factors forkhead box protein A3 (FOXA3) and SAM pointed domain containing ETS transcription factor (SPDEF).[68,108] FOXA3 expression in epithelial cells can inhibit antiviral responses (e.g., type I interferons) and enhances expression of pro-allergic factors such as TSLP.[109] During acute viral infections, the function of FOXA3 may be to limit inflammatory responses as the infection resolves, but chronic expression in the context of allergic inflammation could instead inhibit antiviral responses, potentially leading to more severe illness.[109]

Considered together, effects of more severe infections with RSV, RV, and other pathogens (including bacterial) could represent repeated insults to the developing lung and immune system that promote the development of asthma. Respiratory syncytial virus bronchiolitis can cause significant damage to the airways during a particularly vulnerable period with respect to lung growth and differentiation. In infants who develop allergic sensitization, repeated RV LRI could provide repeated insults to the developing small airways. Repair of virus-induced damage may remodel the airways, thus increasing the risk for asthma, especially in children who are at increased risk because of genetic factors or exposed to adverse environmental stimuli.

Conclusions

There is a close relationship between virus-induced wheezing in infancy and the onset of childhood asthma. The associations are well documented, but important questions remain about causality. These questions are best answered with interventional studies; results of observational studies indicate that strategies to target respiratory viruses and those directed at prevention of atopy are warranted. Defining relationships between viral infection, atopy, and lung development in early life could lead to new strategies for the prevention or treatment of viral LRI and, ultimately, the prevention of childhood asthma.

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Infantile Lung Function and Airway Hyperresponsiveness

6

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Introduction

Obstructive airway diseases, including asthma and chronic obstructive pulmonary disease (COPD), are among the most common noncommunicable diseases. Asthma often presents early in life; for many, it persists into adulthood and may precede COPD.[1–3] In common with asthma, COPD is associated with reduced premorbid lung function,[2,4] and genes linked to asthma as well as COPD have been identified,[3,5] all of which point to early life pulmonary disease programming.[6]

Associations between asthma and other allergic (or atopic) diseases such as allergic rhinitis, rhinoconjunctivitis, atopic eczema, and food allergy are well-recognized.[7–12] These allergic diseases often start in infancy with atopic dermatitis (AD) and food allergy, followed by asthma and subsequently allergic rhinitis (AR) (the "atopic march," a term originating in Sweden by Kjellman and co-workers and later used extensively).[13–15] Common but not obligatory to the allergic diseases is the involvement of immunoglobulin E (IgE) and specific IgE antibodies to allergens. The initiation of allergic sensitization, which reflects an unwanted break in tolerance to harmless environmental proteins, is still poorly understood. Although allergic sensitization may develop in the first year of life, it is uncommon for infants to be sensitized to allergens in the first few months of life. On the other hand, many subjects with asthma or AD will never develop allergic sensitization (sIgE antibodies to allergens), but they may have manifestations of these diseases nevertheless. Furthermore, airway hyperresponsiveness (AHR) is a common but not obligate feature of asthma in infancy[16] and childhood.[17–20]

So why is it important to understand allergic disease in relationship to infant lung function?

First, reduced lung function in infancy is associated with later recurrent wheeze[21,22] and asthma.[23–25] Second, allergic sensitization is a strong risk factor for asthma[26–28] and has been associated with reduced lung function[29,30] in childhood. Third, the discovery that lung function is reduced at birth in adolescents with asthma, AD, and AR[9] supports the notion that aberrant lung development starts early, probably even in utero.[31] However, it is largely not known what these early factors are, what their individual or synergistic impact may be, and when they come into play.[31,32] Fourth, reduced skin barrier, found also with protein defects associated with filaggrin mutations, are associated with AD and asthma[33,34] but they explain only some cases of disease. Airway epithelial barrier defects, including both upper and lower airways, possibly associated

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with filaggrin mutations[35] suggest a common inflammatory asthma pathway.[36] A dysfunctional barrier in the skin and airways,[33,36] in concert with genetic and epigenetic variation,[37] is probably directly involved in early atopic development, and AD appears to precede allergic sensitization[38] as well as asthma.[39] However, the role of impaired lung function in this development is less well established. Fifth, because we still have not been able to identify meaningful asthma phenotypes that can improve our mechanistic and clinical understanding of different types of asthma, we need to explore the interaction between clinical disease manifestations, lung function development, and risk factors by and in infancy. This chapter will therefore describe lung function in infancy with relevance for allergic diseases.

Lung development

Lung budding starts during the fourth week of gestation, and at 6 weeks the first appearance of two separate lungs can be seen.[6] The airways continue to develop into approximately 17 bronchial generations by around 24 weeks' gestational age. At this time the terminal sacs are present, making gas exchange possible, and the airways will develop a further seven orders during postnatal life. From around 29–30 weeks' gestation, the primordial alveoli are present, whereas the characteristic mature alveoli develop mainly after birth[40] for some time during early childhood.[41] At full term (40 weeks' gestation), between 100 and 150 million alveoli are developed.[6] Although increases in lung volume mainly result from an increasing number of alveoli up to about 3 years of life, alveoli may still develop until age 8 years.[40] In contrast, the number of conducting airways is complete at birth and thereafter airways increase only in size.[42] After completion of the alveoli, lung growth is probably isotropic, with symmetrical development of lung size and airway size.[42] Thus, potential insults may affect lung development differently, depending on the time of exposure.

Lung function measurements

With some exceptions, there is a general lack of cohort studies evaluating lung function from early infancy into childhood and later adulthood,[43–45] and even fewer have measured lung function from birth.[9,46] This is mainly owing to feasibility and because most methods require sedation also for ethical reasons.[47] However, in recent years, several studies have included lung function measurements shortly after birth,[46,48,49] enabling follow-up in relation to respiratory outcomes. Specific details on available methods and factors that may affect results are outlined in updated and comprehensive review articles.[50–53] In this chapter, only a few methods will be discussed in detail, referring to the clinical outcomes of asthma or allergic diseases.

In the late 1980s and early 1990s, several studies measured flow volume loops using the rapid thoracic compression method (RTC) and demonstrated that measures of maximum flow at functional residual capacity (FRC) ($\dot{V}'\max_{FRC}$)[16,54] in infancy

were associated with later wheeze. Because such measures were limited to the tidal breathing range, they were termed "partially forced flow volume loops."[55] This method has been the most commonly studied in relation to later asthma outcomes.[43,49,56–62] Further development of the method included raising the lung volumes to achieve flow limitation, giving a more comparable measure to maximal forced flow volume loops obtained from schoolchildren onward.[63] This raised volume rapid thoracic compression method (R*V*′RTC) involves increasing the airflow at the airway opening until a preset pressure is achieved[63] to obtain flows that exceed those obtained by tidal volumes at FRC. Forced expiratory maneuvers are repeated with increasing pressure until distinct flow limitation is obtained.[63] Commonly reported parameters calculated from the R*V*′RTC are forced vital capacity (FVC), forced expired volume in 0.5 s (FEV0.5), forced expiratory flow at expired 50% of FVC (FEF50) forced expiratory flow at expired 75% of FVC (FEF75), and FEF when 25–75% of FVC has been expired (FEF25–75), in line with spirometry in older children. The method has been used also in prospective cohort studies with asthma or wheeze outcomes.[49,59,64–66] Studies suggest that flow values measured by the raised volume technique appear to correlate better with respiratory outcomes than does $V'\mathrm{max}_{\mathrm{FRC}}$.[59]

Because of the need for sedation with RTC measurements, the use of noninvasive techniques such as tidal breathing measurements by tidal flow volume (TFV) loops[16,67,68] or curves[50] was implemented in respiratory or allergy-focused birth cohort studies to examine larger study populations with lung function measurements in early infancy. Measures of tidal breathing patterns may be performed by placing the sensor at or over the airway opening by a pneumotachograph or an ultrasonic sensor, or by inferring respiratory movement by indirect measures obtained by sensors characterizing movement in the chest wall or abdomen. Such noninterventional tidal breathing measurements may be obtained in any subject regardless of respiratory state or arousal state, provided the subject is quietly breathing. During tidal breathing, respiratory control is an important contributor to neonatal lung function, affecting the respiratory cycle and thereby a breathing pattern that is different from that in adults.[69] This will have a bearing on measuring lung function in nonsedated neonates and infants by TFV loops. Tidal flow curves including TFV parameters represent an integrated response of the infant's entire respiratory system, including the neural respiratory control centers, vagally mediated stretch and chemoreceptor feedback control, higher cortical inputs, and mechanical properties of the thoracopulmonary system.[69] The TFV parameter most commonly associated with later respiratory morbidity is the ratio of time to peak tidal expiratory flow (t_{PTEF}) by total expiratory time (t_{E}), the t_{PTEF}:t_{E} ratio is thus a parameter reflecting airway size, chest wall, and respiratory control mechanisms. Findings similar to those observed using R*V*′RTC measures in relation to short-term and long-term outcomes of wheeze or asthma were found using these noninvasive techniques.[16,23,48,70]

Lung volumes are most commonly measured by (whole-body) plethysmography or multiple breath washouts (MBW) in infants, including measures of FRC, and are sometimes included in prospective studies related to asthma or wheeze outcomes.[48,50,71,72]

Passive respiratory mechanics may give rise to measures of respiratory resistance, compliance of the respiratory system (Crs), and specific airways resistance (sRaw), measured in several prospective cohorts with asthma or wheeze asthma outcomes.[23,67,73–75]

These parameters are a composite measure of flow changes at the airway opening and FRC, and may be performed through a face mask during tidal breathing or by plethysmography, both of which remove the need for specific respiratory maneuvers.[16,50]

From a relatively old technique,[76] new modifications producing lung clearance index (LCI) have been used in clinical trials of bronchial obstruction to assess small airway diseases[77,78] in infants,[79] but they are more often used to detect abnormal lung function in children with cystic fibrosis,[80–82] with particular usefulness in multicenter trials of young children.[82] The LCI is a measure of ventilation inhomogeneity derived from the MBW inert gas washout technique during tidal breathing. In children older than age 12 months, nitrogen washout is performed after inhaling pure oxygen, whereas in younger infants sulphur hexafluoride (SF_6) is used.[83] However, the MBW protocols strongly influence ventilation inhomogeneity,[84] and the ability to identify early small airway changes in infants related to later asthma is not yet clear.

Measures of AHR in infants depend on using available methods for measuring lung function, and on some sort of provocation, such as cold (dry) air[85] or a pharmacological agent such as histamin[16,86] or methacholine.[24,87–89] Although AHR measurements have been included in prospective studies,[16,24,57,90] there is a general lack of large prospective cohort studies in healthy infants with measures of AHR related to later asthma or allergic respiratory outcomes. Airway hyperresponsiveness outcomes have most often been assessed by provocation dose (PD) or concentration (PC) of the agent causing a certain percentage of drop in lung function variable, such as a $V'max_{FRC}$, reported as PC_{30}, denoting a 30% fall in lung function.

Lung function development

Lung function measured throughout childhood demonstrated substantial tracking that may be almost as great as tracking in height (tracking index, $\tau > 0.90$) when evaluating FEV_1 and FVC.[91] This means that early lung function predicts later lung function. Developmental paths (trajectories) have been demonstrated from infancy using tidal breathing, passive respiratory mechanics, and partially FEF volume curves through preschool years[92,93] and school age,[23] as well as by maximum expiratory flow volume curves (spirometry) into school age.[94,95] This tracking is largely confirmed in many prospective birth cohort studies evaluating lung function at different time points,[29,43,44,94] which appear to start at birth[9] and early infancy.[24,43,56]

In a study from Perth, Australia, lung function tracking from infancy was confined to children with persistent wheeze at age 11 years, although this was not found in non-wheezing children age 11 years when results were compared using the rapid thoracic compression technique to obtain partially FEF volume curves with regular FEF volume loops.[96] In contrast, comparing similar measurements, a study from Tucson, United States, demonstrated that persistently reduced lung function was associated with persistent but not transient wheeze from infancy to age 6 years.[97] Also, in the 22-year follow-up study, lung function measures in the lowest quartile at birth remained in the same lowest quartile compared with the upper three quartiles among the 83 patients who completed the 22-year follow-up study,[43] although a modest correlation between

maximum flow at FRC ($V'max_{FRC}$) and FEV_1 was reported (−0.19 and −0.19 at 11 and 22 years, respectively).[43] Similar correlations ($r^2 = 0.10$) between TFV ratios shortly after birth and midexpiratory flow values at age 10 years were reported from Oslo.[23] Some of the difference is clearly related to methodological differences, reflecting varying underlying features including lung, chest, and airway mechanisms; size; and control of breathing.[52]

However, some impact on postnatal lung development seems likely,[24,56,98,99] although the full extent of postnatal impact on lung function trajectories is not known. Findings suggest that tracking through childhood appears to be independent of asthma-like phenotypes,[9,23,97] and point to the importance of in utero lung growth and development and allergic disease programming.[48,100,101] So, what factors may influence development of airway function?

Postnatal impact on lung function development

Despite increasing evidence that reduced lung function at birth or in early infancy recruited from a healthy population may indicate later poor health, prediction of later disease or health on an individual level is limited.[102] In the Environment and Childhood Asthma (ECA) study, the positive predictive value for asthma at 10 years was low (14–31%) for all cutoff values for tidal flow volume ratios as well as for Crs[23] and not much better for other childhood respiratory outcomes in a Swiss study.[46,102] Yet, the associations between impaired lung function at the start of postnatal life and wheeze or asthma outcomes are well-established by various methods, as mentioned. The discovery that reduced lung function at birth was associated with asthma combined with allergic rhinitis and atopic eczema in 16-year-old Norwegian subjects[9] (Figure 1) points to the importance of understanding factors that may influence early lung development in utero.

Despite a strong element of tracking, some modification of lung function development through early childhood is reported.[24,56,99,103] The potential impact of postnatal environmental or other factors is not clear. Birth cohort studies including infant lung function are important for understanding the impact early in life on lung development in relation to allergic disease.[31,104] Tobacco smoking is the most well-established factor negatively associated with reduced lung function[3,24,105,106] from birth,[48,107,108] in early infancy[62,93,109] and later in childhood or adulthood.[110–112]

The adverse effect of air pollution on childhood lung function has been well documented,[113–116] and also in terms of reduced lung or airway growth.[116,117] Only a few studies have assessed the impact of air pollution on infant lung function.[118] A study from Switzerland demonstrated in 5-week-old babies that higher exposure to particulate matter in pregnancy was associated with increased minute ventilation and inflammation, suggesting a possible mechanism by which high levels of air pollution are linked to increased infant mortality.[72]

Respiratory infections in early childhood have been shown to reduce lung function in childhood in 7-year-old children in the German Multicenter Allergy Study (MAS) study[119]; respiratory syncytial virus affects lung function 22 years after bronchiolitis[120]

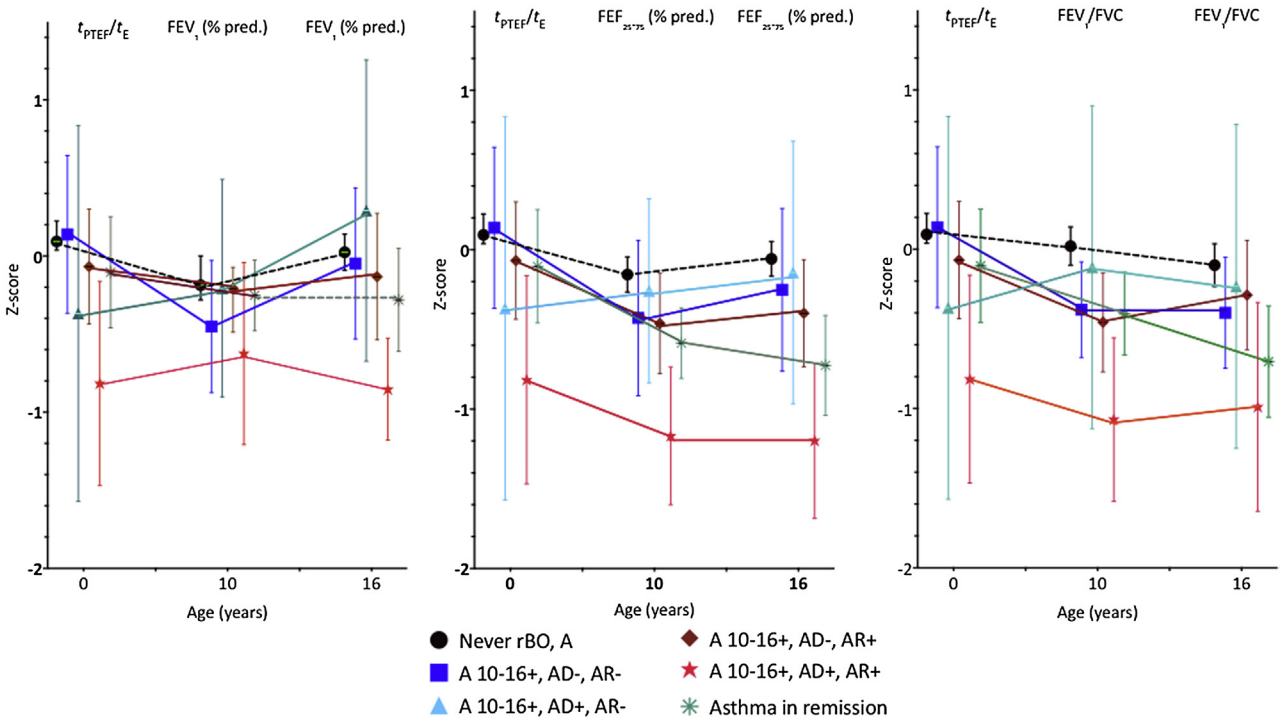


Figure 1 Lung function trajectories given by Z-scores from birth (t_{PTEF}/t_E) through age 10 and 16 years for FEV_1, FEF_{25-75}, and FEV_1/FVC for children who did not have recurrent bronchial obstruction or later asthma (reference group), versus children with asthma and various comorbidities (atopic dermatitis [AD] or allergic rhinitis [AR]) or asthma only before age 10 years (asthma in remission). Trajectories in children with asthma were significantly different from those in the reference group, and the trajectory of asthma with AD and AR was significantly different from all other groups.

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and severe childhood respiratory infections had an impact on adult lung function in the European Community Respiratory Health Survey.[121] However, in terms of the impact of respiratory tract infections on later lung function, the associations may have been casual rather than causal, owing to the lack of premorbid lung function information. In the ECA study in Oslo, the reduced lung function of children at age 10 years who had undergone recurrent or severe airway infections in early childhood was confirmed. However, the impairment of lung function was no longer significant after adjusting for neonatal lung function,[122] which is in line with the findings from Perth, where lung function impairment observed in children years after bronchiolitis was present before the respiratory infection.[75]

The role of allergy in lung function development is still not clear. Sensitization to indoor allergens developed in the first 3 years of life was associated with subsequent loss of lung function at school age in the MAS study, and concomitant high exposure to sensitizing allergens aggravated this process.[29] In this study, the key features of asthma (AHR and impairment of lung function) at school age were determined by early-life allergen sensitization and high allergen exposure during the first 3 years of life.[29] Also, in the Manchester Asthma and Allergy Study,[123] reduced lung function (sRaw) and atopic sensitization at age 3 years predicted the subsequent persistence of wheezing,[123] which suggests that both a primary lung determinant (reflected by impaired lung function in early life) and a systemic immune response (observable as IgE-mediated sensitization) contribute to the development of persistent wheezing during childhood. Few infants in these birth cohorts were sensitized to allergens during infancy, which raises the question of how much impact allergic sensitization may have on lung function or allergic diseases through childhood. The latter was assessed by a collation of birth cohorts in Europe and demonstrated that the independent effect of allergic sensitization on allergic comorbidities at age 6 and 8 years accounted for only 38% of the excess co-morbidity.[7] In fact, in the ECA study, the impact of allergic sensitization on lung function trajectories was minor, with no significant associations between sensitization by age 16 years and lung function trajectories (Figure 2). This is perhaps unsurprising because relatively few infants are sensitized to allergens during infancy. In the German MAS study (established in 1990), including high-risk infants, 16.4% of all infants were sensitized to at least one allergen, but the proportion increased to 26.7% by age 3 years.[124] With the strong element of tracking from birth, the question remains regarding how much impact later manifestations of allergic sensitization have on lung function growth through childhood.

The effect of breast-feeding on lung function was summarized in a review report including 10 studies assessing lung function from age 4 years onward.[98] The review suggested that all but one study found a positive association between breast-feeding and later lung function, particularly in relation to FVC, but uncertain interactions between breast-feeding and maternal asthma were shown. Weight gain is related to asthma in different ways. Rapid early weight gain increases the risk of early childhood wheezing illnesses and lower lung function,[125] whereas slow linear growth in utero, possibly a result of impaired placentation, increases the risk of later asthma and may be associated with impaired development of the airways.[100] None of the studies included lung function during infancy, and only two had more than one measurement time, which limited the possibility of assessing possible impact on lung growth.

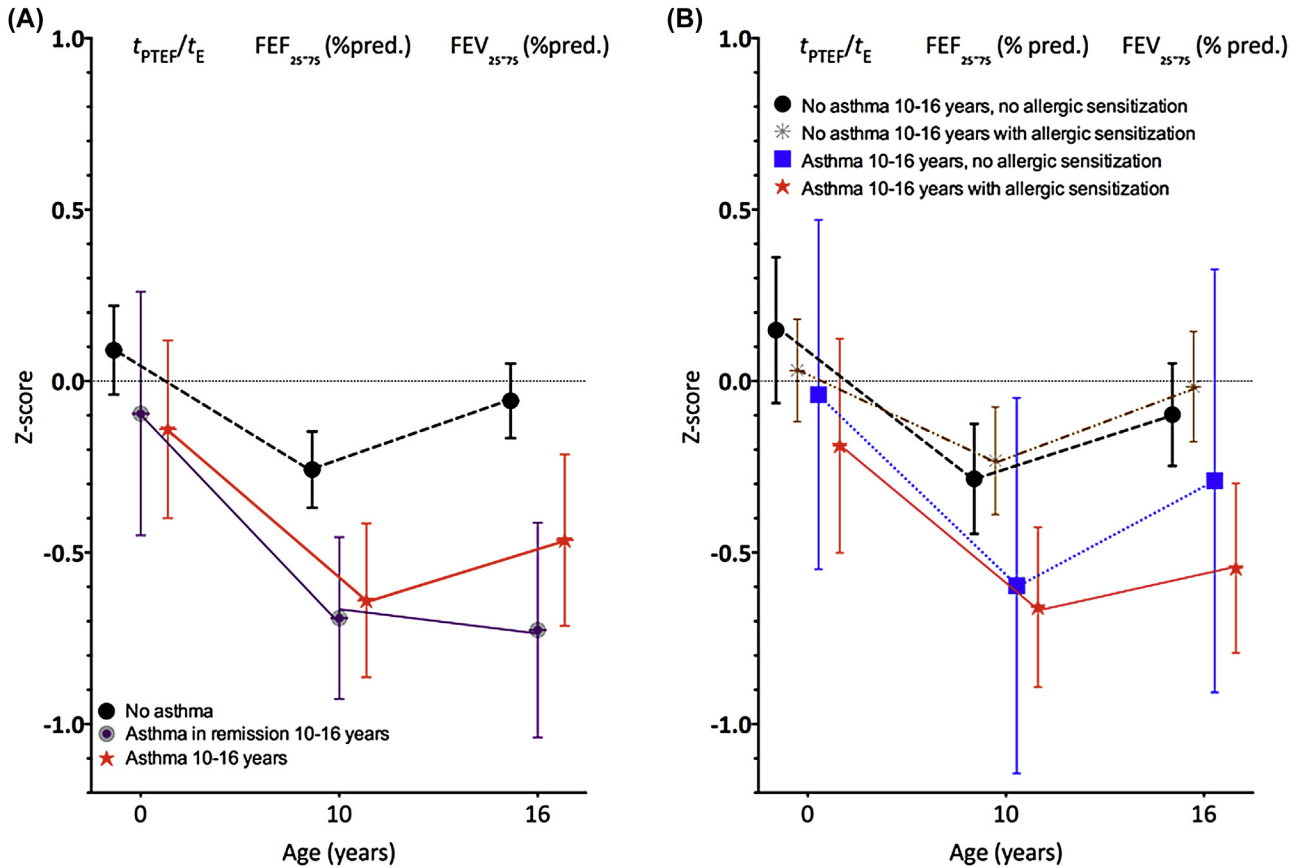


Figure 2 Lung function trajectories given by Z-scores from birth (t_{PTEF}/t_E) through age 10 and 16 years (FEF_{25-75}) in children who did not have recurrent bronchial obstruction or later asthma (reference group; black dot in [A] and [B]), as well as in (A) children with asthma in remission after age 10 years and those with asthma after age 10 years. (B) The same children are classified on the basis of the reference group as well as children with asthma after age 10 years stratified by allergic sensitization. Trajectories in both groups of children with asthma were significantly reduced compared with no asthma, but with no significant differences related to asthma phenotypes (A) or to allergic sensitization (B).
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However, the findings are supported by a study demonstrating that lower rates of fetal growth and higher rates of early infancy weight gain were associated with impaired lung development in 131 term infants with lung function measured by RTC as well as Crs at age 5–14 weeks.[126]

Only when premorbid lung function is included is it possible to estimate the true potential impact on lung function development exerted by environmental or other factors. This was shown in the Danish Copenhagen Prospective Studies on Asthma in Childhood study,[24] which suggested that by age 7 years, approximately 40% of the airflow deficit associated with asthma was present at birth, whereas 60% developed after birth. There is a paucity of studies including young adults with lung function measured during infancy as well as in early adulthood, with which the potential impact of postnatal environmental or other factors may be assessed. However, in Perth, Australia, it was shown that infant atopy and maternal asthma were associated with impaired lung function from infancy, that a certain improvement in lung function was observed through childhood, particularly in infant boys who were flow limited initially, and that in subjects with asthma the initial reduction in lung function appeared to be constant.[56] This is in line with findings that children from the ECA study in Oslo who had asthma with AD and AR had persistently impaired lung function from birth and through childhood. In studies assessing potential longitudinal deterioration or improvement in individual lung function, it has been suggested that the inappropriate use of size-corrected lung function measures may introduce erroneous conclusions regarding lung deterioration or improvement.[127]

Factors that may influence in utero lung function development

There is limited knowledge about when and how aberrant development starts during fetal life. Measurements related to lung development during fetal life are crucial to understand early deviation from normal development that is detected from early infancy. Exposure to tobacco in utero is well documented to be associated with reduced lung function at birth[107–109] in term[128] as well as preterm infants[109] and in infancy.[24,129,130] Also, involvement of at least three COPD genes has been implicated in lung development and lung growth, which points to reduced airway caliber in early childhood.[3] Yet, there is limited knowledge about when, how, and at what levels tobacco exposure affects lung development lung function at birth and possible involvement in the infant's lung response to smoke exposure in utero and in early life.[3] However, some studies may shed a light on the timing and possible mechanisms by which tobacco exposure appears to cause impaired lung development. Reduced t_{PTEF}:t_E was seen with exposure to tobacco in utero in preterm babies at 33 weeks gestation,[109] which suggests that the effects are not limited to late pregnancy. This is supported by a study of almost 2000 pregnant women, in which maternal smoking during the second and third trimesters but not the first trimester was associated with reduced lung function at 5 years.[131] A randomized clinical trial[48] revealed the role of potentially modifiable adverse in-utero exposures, investigating whether lung function at birth could be modified by supplementation of

500 mg vitamin C daily during pregnancy to mothers who did not want to stop tobacco smoking or were unable to do so. Among the 179 infants who underwent lung function measures at birth, use of 500 mg vitamin C supplement daily versus placebo was associated significant improvements of 11% and 10% in t_{PTEF}:t_E and Crs at birth, respectively.[48] Although there was a tendency toward higher Crs after 1 year, lung function measurements were no longer significantly different related to the vitamin supplement among 106 infants with measurements at age 1 year. Data from that study,[48] from a study of the association between prenatal exposure to air particular matter and lung function at birth,[72] and other data[32,114,118] point to a possible role of oxidative stress that may influence lung growth in utero.

Valuable insights into the type of lung function deficits resulting from early insult to the developing lung are provided in studies of extremely prematurely born children, in which reduced lung function was shown with varying deficits of spirometric values or respiratory diseases in later childhood[132–134] or early adulthood.[133,135] Neonatal anthropometric measures have been associated with lung function in infancy and childhood in infants who were growth-restricted in utero,[136] as well as in infants with birth weight within the normal range.[126] Fetal growth trajectories and childhood lung function and signs and symptoms of atopy and wheezing were observed by conventional ultrasonographic biometric measures.[131,137] Ultrasonographic evaluation of fetal lung size and blood flow is used to predict neonatal lung function in pregnancies with clinical risk of lung hypoplasia, for example, in pregnancies with a diagnosis of congenital diaphragmatic hernia or with preterm premature rupture of membranes.[138] Fetal thoracic circumference (TC) or area (with or without the heart area) increases almost linearly with gestational age. The ratio of TC to abdominal circumference is almost unaltered throughout pregnancy and is used as a predictor of lung hypoplasia.[139] However, there is a paucity of studies evaluating fetal lung function (lung size and Doppler blood flow velocity) in normal pregnancies with a potential impact on later respiratory disease and lung function development.

Infantile AHR

Few cohort studies with allergic disease outcomes have performed measures of AHR during infancy. In an Australian birth cohort study of 253 infants with histamine challenges, which assessed PC_{40} in early, middle, and late infancy, 122 were reassessed at age 6 years.[140] At age 6 years, infants with increased AHR had significantly more often lower FEV_1, physician-diagnosed asthma, and lower respiratory tract symptoms.[58] A further follow-up at age 11 years demonstrated that wheeze in later childhood was associated with increased AHR from age 6 to 12 months, but not in early infancy. Also, the persistence of wheeze from age 4 to 6 years demonstrated at age 11 years was most prevalent among children who had reduced $V'max_{FRC}$ at 1 month.[141] The researchers also found that 14 of 103 subjects with AHR measures in middle and late infancy as well as late childhood had persistent AHR on all occasions, whereas 15 had no AHR on any occasion. Thus, AHR changed in 72% and resolved in half the children. The observed associations between late infancy AHR and later asthma was independent of initial lung function ($V'max_{FRC}$), which suggested that AHR may result from a succession of respiratory insults through

infancy[141] and that AHR in early life defines a functional state associated with abnormal airway function, lower respiratory symptoms, and asthma in later childhood.[58]

In a smaller British study[57] of 73 infants with methacholine challenges followed to age 11 years, neonatal AHR (PC_{30}) was significantly reduced in children who wheezed during preschool age, only, but was not significantly associated with neonatal $\dot{V}max_{FRC}$ or PC30 in children who wheezed after age 4 years.[57] As in the British[57] and Australian[141] studies, the association between infant AHR and asthma later in childhood was confirmed in a Danish study.[24] In this study 317 infants born to mothers with asthma had, measures at 1 month of lung function by the R$\dot{V}$RTC technique and AHR by methacholine challenge assessed by $PD_{20}T_{CO_2}$ (the dose causing a 15% decrease in transcutaneous CO_2 measure) and spirometry assessed at age 7 years. However, in contrast to the study from Perth,[58] the Danish[24] cohort of high asthma-risk children showed an association with AHR at age 1 month, with asthma at school age. All three studies used different methods to measure AHR in infancy, and methodological variations may be related to later clinical outcomes.

From childhood and infantile studies, overall, AHR appears to be greater early in childhood and to decrease thereafter.[19,142] However, the mechanisms underlying AHR are poorly understood in terms of when and how AHR starts as well as the longitudinal development of AHR through childhood. Although there is a well-established observed association between AHR and allergic sensitization later in childhood,[143,144] the potential impact of allergy on AHR development is not clear.[19] A mouse study found that the development of AHR in a particular strain of mice was associated with activated CD4-T cells recruited to the airway wall, rather than with sIgE antibodies.[142] Clearly, more research is need to unravel when and how AHR develops in early life, as well as what factors may drive such development.

Summary and conclusion

Reduced lung function at birth or in infancy is associated with later wheeze and asthma, but it is a limited predictor of later asthma in individual children. Lung function appears to largely track from birth, although it is likely that a proportion of lung growth may be influenced postnatally by environmental factors. Postnatal influence on lung function through childhood should ideally be estimated only when premorbid infant lung function is available, to reduce the risk of casual rather than causal associations. The tracking of lung function observed in children with asthma, particularly combined with allergic comorbidities, appears to be established at birth and points to in utero programming of complex allergic disease expression. Several factors appearing to influence lung development in utero are measurable as infant lung function, including factors associated with somatic growth, including tobacco smoke and air pollution.

The current research focus[24,32,145–151] points to early programming of somatic and lung growth combined with immune plasticity and possibly involvement of oxidative stress and inflammatory changes in response to a suboptimal in utero environment.

Inadvertent effects on infantile lung development with likely lifelong consequences highlight the importance of (possible) early intervention[43] to reduce later disease

development. Lung function measurements in infancy may thus provide a unique opportunity to assess possible modifying environmental effects on lung development through childhood into adulthood.

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The Development of Allergic Rhinitis in Childhood

7

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Allergic rhinitis (AR) is an allergen-induced, immunoglobulin E (IgE)-mediated chronic inflammatory disease of the nasal mucosa. It represents one of the most prevalent manifestations of allergic disorders, usually starting in the first 2 decades of life. It is frequently accompanied by concomitant conjunctivitis.[1,2] Allergic rhinitis is characterized by nasal itch, anterior or posterior watery rhinorrhea, or nasal obstruction, which may occur in episodes or continuously. Pain, anosmia, or recurrent nasal bleeding is uncommon.[3–7]

Allergic rhinitis has been commonly classified as seasonal or perennial, according to symptoms related to seasonal exposure to pollen or other outdoor allergens, versus perennial rhinitis, which in most cases is related to indoor allergens such as house dust mites, pets, or cockroaches. This distinction has been challenged because it may not be applicable to all patients (concomitant sensitization to indoor and outdoor allergens) in all areas of the world (perennial occurrence of pollen). The general classification of allergic rhinitis has been revised by the Allergic Rhinitis and Its Impact on Asthma consensus group (ARIA), an initiative of the World Allergy Organization. Based on the duration of symptoms, ARIA classifies AR as intermittent or persistent, and depending on the impact of the disease on quality of life or daily activities, as mild or moderate/severe.[8,9]

Until recently there had been a lack of longitudinal investigations, particularly birth cohort studies, focusing on rhinitis. In the past decade we have obtained some information about the incidence, prevalence, and natural history of allergic manifestations of the upper and lower airways during the first 2 decades of life.[10–12]

Incidence

Birth cohort studies in which AR was based on parent-reported nasal symptoms have shown that the percentage of new cases with seasonal AR increases at a constant rate of approximately 2%/year between ages of 3 and 12 years. In the German Multicenter Allergy Study (MAS) cohort, the average yearly incidence rate was 2.3%, with a median age at onset of 7 years.[13]

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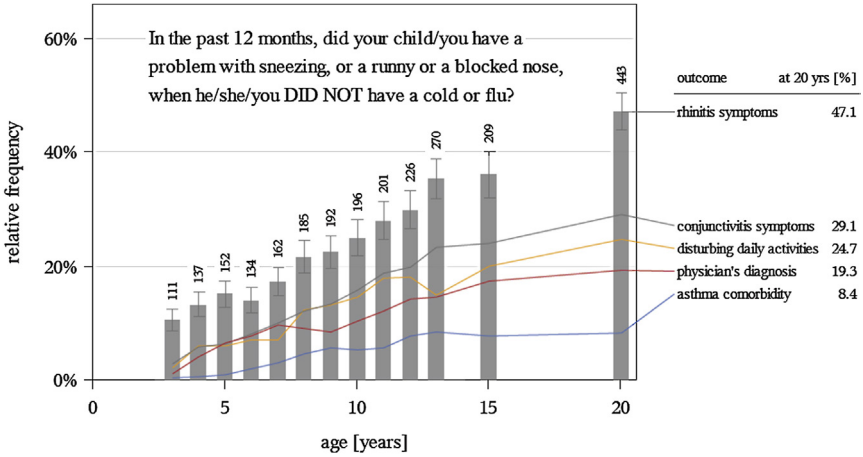


Figure 1 Prevalence of current (past 12 months) rhinitis symptoms, point-wise by age/assessment. Absolute number of cases above bars, 95% confidence intervals. Colored lines indicate fraction with additional features, based on questionnaire items. Asthma comorbidity is defined by current asthma symptoms, current asthma drugs, and/or previous/current physician's diagnosis. In the table, frequencies are at age 20 years.[17]

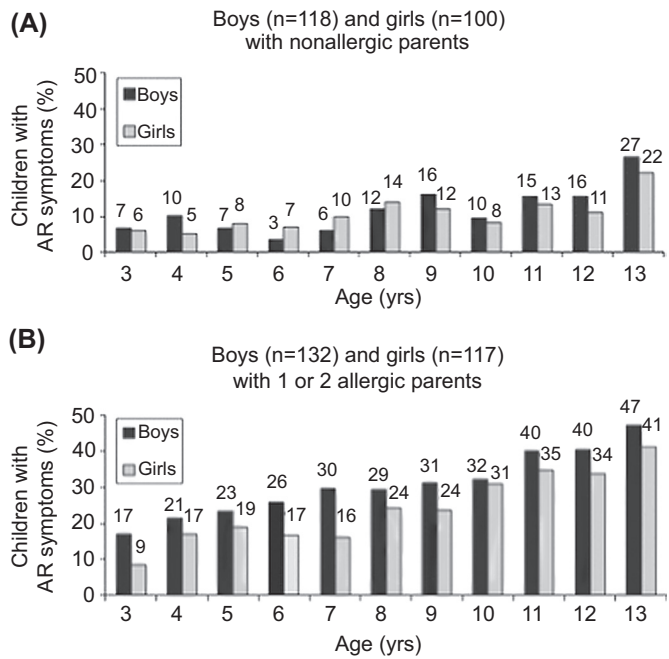


Figure 2 Sex-specific 12-month prevalence of AR symptoms up to age 13 years for children (A) with and (B) without allergic parents.[12]

Period prevalence

In the cross-sectional international study of asthma and allergies in childhood, which was initiated in many countries over all continents, the prevalence of current AR was much higher in older than in younger schoolchildren.[14–16]

Few anecdotal observations reported rare cases of seasonal manifestations in the second summer season of life. Between ages of 3 and 20 years, rhinitis symptoms in the MAS cohort rose from 6% to 47% (Figure 1). More than 60% of these also reported accompanying eye symptoms at age 20 years. Between one- and two-thirds of the children with persistent AR had severe persistent symptoms, according to ARIA, that also affected their daily life (Figure 2).

In children with at least one parent with a history of allergic manifestations, the 12-month prevalence of AR increased from 13% at age 3 years to 44% at age 13 years. A total of 50–70% of children with persistent AR had severe symptoms.[9]

Sex differences

In children with nonallergic parents, there seems to be no obvious difference between males and females in the prevalence of AR during childhood. Among children with allergic parents, there was a consistent male predominance in the prevalence in the first decade of life, as in other early manifestations of atopy: Boys seem to develop AR earlier, but girls catch up and show higher incidences during and after puberty, to reach comparable frequencies by age 20 years[12,20].

Comorbidity

In atopic children comorbidity is a characteristic feature already in the first years of life: Many children with allergic rhinitis had developed eczema in infancy before (Figure 3). Among children with any form of AR, the proportion of those who also

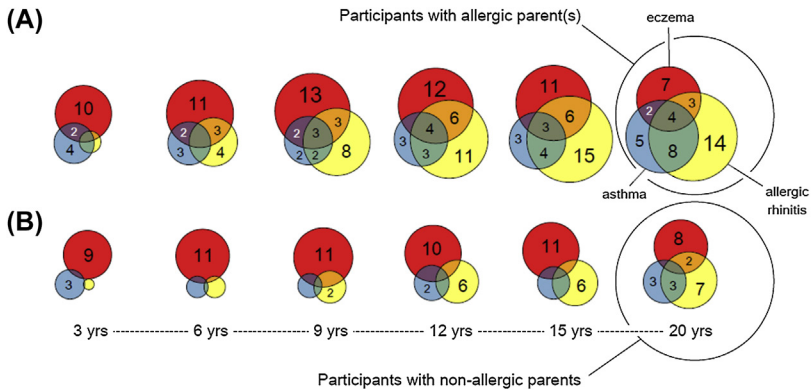


Figure 3 Developing atopic comorbidities of the airways and skin in children (A) with and (B) without allergic parents.[13]

wheezed was 15–35%. At most follow-up time points, more children with severe persistent AR also currently wheezed (up to 52%) compared with those with mild persistent or intermittent AR symptoms.[12,20]

Rhinitis in childhood is a strong predictor for adolescence- and adult-onset asthma (Figure 4).[13,21,22] In the German MAS findings, rhinitis in preschool children was demonstrated to be a risk factor for subsequent wheeze, but only when associated with allergic sensitization.[11,21,22]

These findings are in line with the concept of united airways. It suggests that in young children, as in adults, a progression from rhinitis to wheezing can frequently be found.[8,18,23] This progression could be mediated by common factors or by an interaction between the upper and lower airways. Several potential mechanisms might explain the observed association.

It has been proposed that an impaired mucosal barrier might lead to an increased allergen uptake and a mounting of IgE responses, which eventually result in allergic airway inflammation, airway hyperresponsiveness, and asthma. Conversely, lower airways might be more susceptible to viral infections that could interact with the process of allergic sensitization and thereby increase the risk of asthma. This process is supported by studies showing that mutations in the genes that code for filaggrin have an

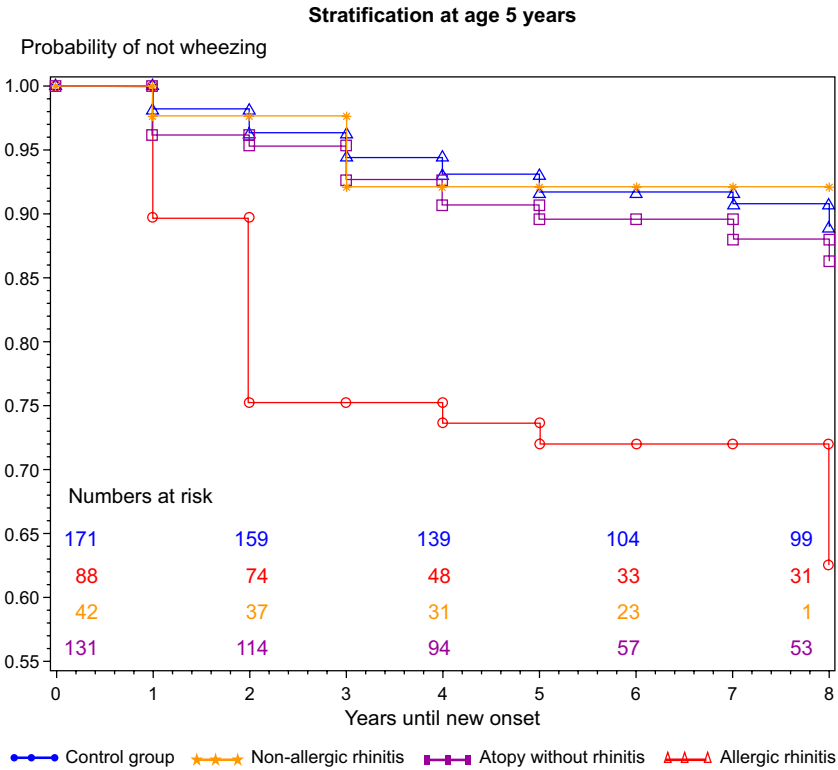


Figure 4 Probability of remaining free of wheezing, stratified by different phenotypes at age 5 years.[19]

important role in the integrity of the epithelial barrier, and were also associated with the prevalence of AR.[24]

Another hypothesis linking the upper and lower airways implies a shared airway inflammation throughout the respiratory system. Different parts of the respiratory system that have structurally and physiologically uniform disorders of the entire respiratory system present with similar inflammatory responses. Preschoolers with rhinitis might benefit from early assessment of allergic sensitization to identify those at high risk of developing wheezing.

The role of sensitization

The 12-month prevalence of sensitization to indoor and outdoor allergens rises from year to year in the first decade of life, reaching almost 60% of all boys and a third of all girls at age 13 years in children with a positive family history. At age 13 years in the German MAS report, 91% of 35 children with severe persistent AR were sensitized to at least one aeroallergen, whereas this proportion was only 70% among children with mild persistent rhinitis.

A systematic evaluation of the process of sensitization before and after the onset of classical seasonal symptoms was performed in grass and birch pollen allergies. Sera from sequential blood samples were tested for IgE antibodies against grass and birch pollen and against individual allergens. The process seemed to be initiated years before the onset of AR symptoms.[25,26]

The average number of grass pollen (*Phleum pratense*) allergens is also increasing with time, and in many cases IgE responses to grass pollen as well as specific grass pollen allergens can start years before perception of the first symptoms. In general, serum concentration increases before and during the disease process, and after the initiation of monosensitization to one of the major allergens, the process of molecular allergen-spreading becomes more and more complex with time. It has been assumed that the disease onset of AR in childhood occurs in the middle of a complex immunologic process that starts years before disease onset and may fully mature years afterward.

Once sensitization to grass pollen is established, the probability for symptoms within the next 3 years increases (odds ratio, 13.6) (Figure 5).

Simple detection of preclinical sensitization to grass pollen may therefore allow prediction of the onset of hay fever in an allergen-specific manner.

Frequently, chronic rhinitis symptoms in children are associated with sensitization to house dust mites. In these cases, a causal relationship between allergen exposure and symptoms is not easy to demonstrate.

Early predictors

Parental airway allergies, particularly parental AR, are the strongest predictor for AR in childhood and adolescence (at least one parent with AR versus a negative AR family history; hazard ratio, 2.52). This increased risk is proportional up to 20 years. In addition, early sensitization to food allergens (eggs and milk) and early eczema have been shown to be strong independent predictors of AR.[20,27]

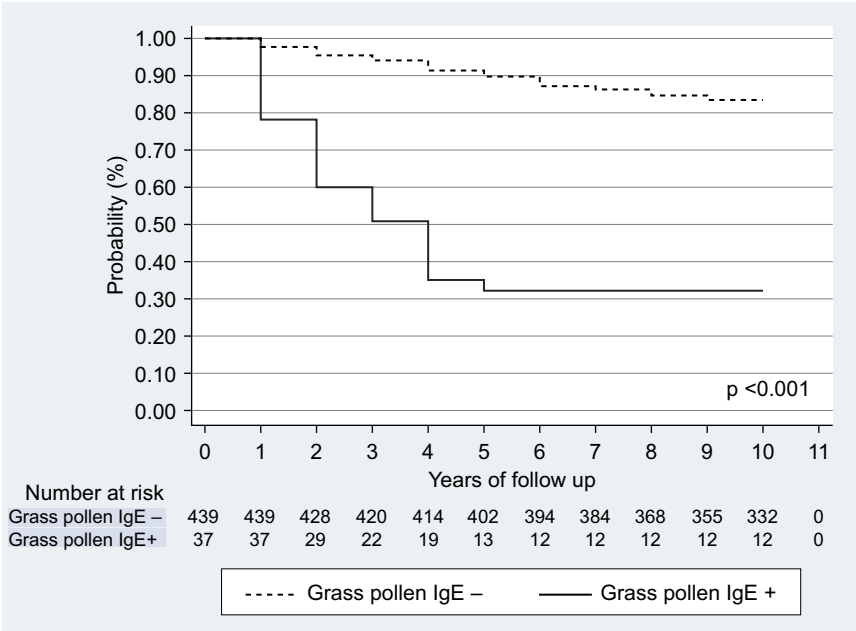


Figure 5 Probability of remaining free of seasonal AR to grass pollen after 3 years by sensitization to *Phleum pratense* (grass pollen) extract.[11]

Other parental manifestations as well as biomarkers (parental IgE or cord blood IgE) are not independently linked to AR in childhood. Food avoidance during pregnancy, smoking, the use of thyroxin or corticosteroids, and the mode of delivery have not been found to be linked to AR.[20]

Protective factors

Unfortunately, potentially modifiable exposures and behaviors during pregnancy, birth, and infancy (breast-feeding, pollution, tobacco smoke, exposure to pets, vaccination, and other diseases) have not been confirmed to be linked to AR; only in children who develop AR with concomitant asthma does avoiding tobacco smoke exposure—particularly during pregnancy—and completing vaccination for life vaccines seem to have a preventive effect. The impact of the season at birth on sensitization and subsequent AR has been studied in the literature[28] but was not confirmed in longitudinal studies to be relevant.[20]

Principles of therapy

Allergic rhinitis related to outdoor or indoor aeroallergens may not be controlled by allergen avoidance, which often is unrealistic in children. Current guidelines recommend symptomatic pharmacotherapy and allergen-specific immunotherapy.[28]

First-line treatment of AR is composed of nonsedating antihistamines such as cetirizine, levocetirizine, loratadine, desloratadine, and fexofenadine. They have been shown to be effective and safe in young children; most of them are approved for treatment after the first year of life. Because the effect of antihistamines is relatively weak, symptoms may require atopical corticosteroids, which by themselves have a stronger capacity to control symptoms than H1-blockers alone and demonstrate an additive effect together with antihistamines. The application of systemic corticosteroids (intramuscular depot injections) is not recommended but is still widely practiced in some countries despite side effects and safety concerns, particularly for children.[28]

After registration of the first anti-IgE antibody to treat severe uncontrolled asthma, a variety of studies on seasonal AR have been published, which suggests that in severe persistent AR this pathogenesis-oriented intervention may be of additional benefit for children who were not sufficiently controlled by standard therapy.[29]

Allergic rhinitis is a key indication for specific immunotherapy, during which the relevant allergenic proteins are applied to the patient in a standardized way. In many countries children from age 5 years are eligible for immunotherapy, which is applied commonly via the subcutaneous route, as has been proposed for more than a century.

More recently, immunotherapy protocols in which either aqueous extracts or rapidly dissolving tablets containing pollen (grass, birch, and ragweed) are applied (SLIT) have been carefully evaluated in clinical development programs and found to be equally effective as classical subcutaneous allergen treatment. Particularly for children, this nontraumatic mode of application via the sublingual route is an attractive alternative; however, local side effects of this treatment, which primarily are observed in the first days of immunotherapy, have stimulated discussions regarding whether the prescription of an adrenaline autoinjector for patients who practice this mode of home treatment may be meaningful. The United States Food and Drug Administrative requires adrenaline autoinjectors for SLIT with grass pollen tablets.

Options for prevention

Whereas some preventive interventions seem to be promising in atopic dermatitis, food allergy, or asthma, the options for AR seem to be limited. In the German prospective MAS birth cohort, no single modifiable risk factor was linked to AR. Interventions aiming at primary prevention of AR are currently not justified.

Specific immunotherapy is advised in patients with a protracted disease. Only in recent years has the disease-modifying intervention been investigated early in the disease process. Currently, protocols are applied in a number of clinical trials looking at a potential preventive capacity for sublingual and subcutaneous allergen application. Target groups for preventive treatment could be children with an obvious susceptibility for atopic diseases (positive family history), manifest atopic eczema, and IgE sensitization at a preclinical or early clinical stage of the disease. It remains to be demonstrated in future studies just how much the incidence of AR can be reduced.

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Genes and Atopic Phenotypes

8

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Introduction

Allergic disease, or atopy, and its associated clinical traits (especially asthma) have long been recognized as being heritable, with family-based twin and migration studies providing the earliest and most compelling evidence for genetic contributions.[1–5] The many studies demonstrating incremental increases in risk of atopy with the presence of one or both atopic parents are further testimony to a significant heritable component.[6–10] Heritability refers to the fraction of phenotype variability attributable to genetic variance, and heritability estimates for atopy and associated traits including asthma, allergic rhinitis, atopic dermatitis ([AD] or eczema), and food allergy vary widely, from 33% to 95% (described extensively in Refs 11–13). The considerable range of heritability for atopic traits reflects the complexity of gene–environment interactions, complexities of the environment itself, and, importantly, the fact that heritability is not constant; for example, heritabilities can differ according to the variance contributed by the environment over time.[14]

Another challenge in disentangling the genetic basis for atopy is the heterogeneity of the atopic phenotype. Consider, for example, the long-held appreciation for asthma, AD, allergic rhinitis, and food allergy as "phenomena of hypersensitiveness."[15] For asthma, the phenomenon of immunoglobulin E (IgE)-mediated airway responsiveness (or bronchial hyperreactivity) to environmental stimuli is only one domain in a complicated syndrome that includes physiological (airway obstruction), pathological (development of irreversible airway remodeling and airflow limitation), and clinical (characteristics symptoms of wheeze, cough, shortness of breath, and airway obstruction reversed in response to treatment) components.[16–18] Hierarchical cluster analyses have been used to subdivide asthma (and allergic rhinitis[18]) even further, identifying distinct clinical phenotypes of asthma associated with severity.[19,20] Early studies examining genetic and environmental components of variance of quantitative traits associated with asthma demonstrated independent inheritance of total and specific IgE levels, blood eosinophil counts, and airway hyperresponsiveness, implicating multiple genetic determinants contributing to this complex disease.[2,21,22] To this end, identifying the genetic factors contributing to asthma (and by proxy, all atopic diseases) has been likened to trying to find a needle in a haystack.[23]

Tools for disentangling the genetics of atopy

To appreciate how far the field of genetic epidemiology has come in identifying genes associated with risk of early atopic phenotypes—and how far the discipline has yet to go—it is useful to consider the technological tools used in the past and present (summarized

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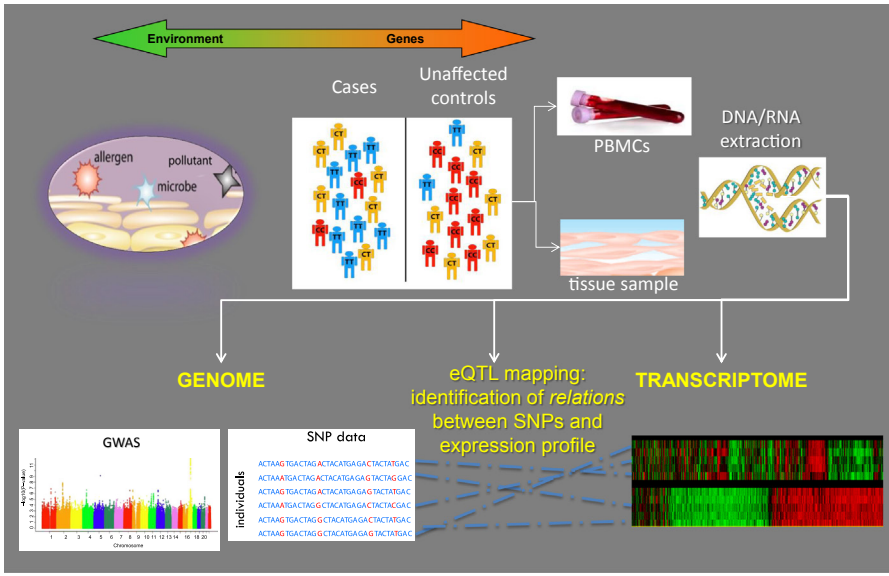


Figure 1 Schematic overview of approaches used to disentangle the genetic basis for complex traits.

in Figure 1). After the introduction of the polymerase chain reaction in the 1980s, genetic fingerprinting could be performed by amplifying deoxyribonucleic acid (DNA) fragments in the human genome, examining them for variable fragment lengths of repeats (i.e., restriction fragment length polymorphisms). These microsatellite markers spanning the human genome-enabled family-based, genome-wide linkage studies, a robust approach to identifying genetic hot spots associated with complex traits. This technology led to nearly a dozen genome-wide linkage studies on asthma and its associated phenotypes,[24–34] and multiple chromosomal regions were identified as candidate loci, from which six novel asthma genes were identified using positional cloning.[34–39] Genome-wide linkage studies were similarly performed for allergic rhinitis,[40–44] AD (summarized in Ref. 45), and associated traits, with the occasional observation of linkage peaks overlapping with associated quantitative traits (i.e., total serum IgE, skin test index, eosinophil counts[24]).

After the sequencing of the approximately 20,000–25,000 genes across the three billion chemical base pairs that make up human DNA and publication of the human genome,[46,47] the technology shifted to genotyping biallelic markers (e.g., single nucleotide polymorphisms [SNPs]) and relatively simple structural variants, such as insertions/deletions and repeats, directly in genes of interest. This technological shift allowed researchers to expand genetic studies beyond linkage toward genetic association studies; using this approach, literally hundreds of candidate genes have been identified for the atopic phenotypes.[13,48–51] The International HapMap Project[52–54] greatly expanded the Human Genome Project by cataloging genomes representing four biogeographical groups (continental populations from Europe, Africa, and Asia), which further pushed the technological capacity to rapidly (and relatively cheaply) genotype hundreds of thousands of common (>5%) SNPs on thousands of DNA samples at a time. The content of these commercially available SNP array panels grew exponentially with expansion

of the human genome catalog through the 1000 Genomes Project,[55] as has the capacity for discovery of genetic associations with the development of more efficient methods for analysis and SNP genotype imputation methodologies,[56,57] in which genotyped content can be combined with ungenotyped (i.e., inferred) content derived from the public genome catalog. Since the first genome-wide association studies (GWAS) published in 2005 on macular degeneration,[58] almost 2000 studies summarizing more than 14,000 SNPs associated with various complex and quantitative traits have been published,[59,60] including 43 GWAS on asthma, five GWAS on AD, five GWAS on allergic rhinitis, five GWAS on atopy/allergy, and nine GWAS on IgE (discussed in detail below).

Genome-wide association studies have been hugely successful in confirming suspected pathways and elucidating novel ones for a plethora of complex traits, which has led to a greatly expanded drug target list.[61] However, as a consequence of their design, GWAS rarely identify the causal variant(s) associated with a disease (because GWAS SNP panels are composed of haplotype tagging SNPs in linkage disequilibrium with the true unobserved disease-causing SNPs), and to date the cumulative genetic risk of the variants identified asthma alone is under 15%.[50] An unexpected outcome of the many GWAS performed so far is that the vast proportion (>85%) involves variants in intergenic or intronic regions.[62] Again, this observation is not necessarily surprising because the tag SNPs selected for the GWAS panels are common variants, and coding/exonic variation tends to be rare and therefore poorly tagged by a common variant, whereas intronic and intergenic regions have a spectrum of variation that is common.

In addition to measuring the genome, interrogation of the transcriptome, or the collection of all the transcripts present in a given cell, can tell us something about which genes are turned on or off in the cells and tissues of an individual, and how changes in the normal level of gene activity are associated with disease.[63] The transcriptome includes all ribonucleic acid (RNA) molecules, but the major type that has a critical role in making proteins is messenger RNA (mRNA). Unlike the exome, which comprises all of the genome's exons (or the 1.5% of the genome that gets translated into proteins), the transcriptome only includes RNA molecules that are found in a specified cell population. Whole genome gene expression profiling, or *transcriptomics*, is a robust approach used to measure RNA expression in a biological system, and until recently it has relied primarily on synthetic oligonucleotide microarray platforms.[64] Transcriptomics has been widely applied in allergic disease, and dozens of studies focusing on whole blood and target cells of the immune system and tissue from the upper and lower airways have been performed to date (reviewed in Ref. 65). As the genomic and transcriptomic databases have grown, an innovative approach for mapping functional noncoding variation has been increasingly used, called *expression quantitative trait locus (eQTL) mapping*. Expression quantitative trait locus mapping is based on the idea that the abundance of a gene transcript (a quantitative trait) is directly modified by genetic polymorphisms in regulatory elements, and it can be used to find disease markers identified in GWAS that are also associated with gene transcripts. To date, several studies have integrated findings from asthma GWAS with cataloged genome-wide gene expression data[66–71] and many others are under way.

Massively parallel, second-generation DNA sequencing, or next-generation sequencing (NGS)[72] is the most recent technological advance in the field of genomics, and as the cost of NGS continues to drop, its application in elucidating the pathophysiological underpinnings of complex diseases such as atopy is increasing. Resequencing

genes empowers investigators to assess the contribution that *both* rare and common variants make to disease and overcome the limitations of GWAS, which focuses on common variation, and NGS is a major advance over conventional Sanger termination sequencing,[73] which is costly and cannot be done on a large scale. Next-generation sequencing has wide applicability and can be used as a targeted approach to follow-up on specific genetic regions identified by GWAS, or as an unbiased approach to discovering coding variants in the entire exome (approximately 30 Mb in total; called exome sequencing) or genome (called whole genome sequencing [WGS]).[74] The same robust NGS technology has similarly transformed transcriptomics and is called RNA sequencing. This is a more powerful approach than array-based gene expression because of its higher correlation with protein expression[75] and smaller technical variation.[76] To date, there are relatively few publications leveraging NGS in allergic disease, but numerous NGS studies are under way and it is anticipated that novel discoveries are forthcoming. The limited reports thus far include a study in which WGS performed on 16 founders implicated a 6-kb-pair deletion in an intron of *NEDD4L* and increased risk of asthma, which was replicated by targeted resequencing and genotyping in independent samples.[77] Elsewhere, whole exome sequencing was performed on 60 self-reported African American children with AD, with a focus on loss-of-function mutations in the S100-fused-like proteins (*SFTP*) genes (*FLG*, *HRNR*, *FLG2*, *RPTN*, *CRNN*, *TCHH*, and *TCHHL1*), but the study failed to identify significant associations with risk of disease.[78] Ribonucleic acid sequencing has been used to measure transcriptomic changes in human airway smooth muscle in asthmatic patients compared with nonasthmatic subjects[79] to identify genes differentially expressed in response to glucocorticosteroid exposure (*CRISPLD2*,[80] *FAM129A*, and *SYNPO2*[81]), and to characterize the gene signature of a severe complication of AD, eczema herpeticum.[82]

Do genes co-associated with multiple childhood atopy phenotypes represent atopic march candidate genes?

Disentangling the genetic basis for the sequential presentation of AD, food allergy, allergic rhinitis, and asthma—the hallmark feature of the atopic march[83]—presents a unique set of challenges. In addition to the heterogeneity of each individual clinical trait (i.e., childhood- versus adult-onset asthma, intrinsic versus extrinsic AD), comorbidities are common and vary temporally, and the development of one phenotype can predict the development of another. For example, AD and allergic rhinitis predict the development of atopic asthma,[84–88] and *severity* of AD predicts the development of allergic sensitization[89] and airway disease in general.[90,91] Although it remains unclear whether the sequential pattern of development of AD, food allergy, allergic rhinitis, and asthma is a result of a causal relationship or a shared cause, there are considerable genetic data supporting both common and unique pathways.

Atopic dermatitis. A defect in epithelial barrier integrity has been proposed as the underlying cause in the onset of AD and progression of the atopic march[92]; to this end, the extensive genetic epidemiological studies performed on AD over the past 2 decades have elucidated a set of adaptive and innate immune response genes and skin barrier dysfunction genes that are consistently associated with risk of AD, but also the

other clinical traits representing childhood atopy (i.e., food allergy, allergic rhinitis, asthma) (Table 1). In one of the first of half a dozen genome-wide linkage studies performed on AD, the strongest signal was in the epidermal differentiation complex locus on chromosome 1q21,[93] which contains a large and diverse family of genes associated

Table 1 Genes associated with childhood atopy

Stage of the atopic march	Associated gene(s)
Eczema	
Candidate gene studies	*ADAM33*,[163] *CD14*,[152,155] ***FLG***,[96–102,104,146,164] *IL1RL1*,[165] *IL10*,[139,166] *IRF2*,[118] *ORAI1*,[124] *RANTES*,[167–169] *SPINK5*,[132,170–172] *TSLP*[119]
GWAS	*ACTL9*,[109] *ADO-EGR2-ZNF365*,[110] *BAT1*,[109] *CARD11*,[110] *CCDC80*,[110] *CLEC16A*,[110] *Corf10*,[110] *C11orf30*,[107,110,173] *CYP24A1-PFDN4*,[110] ***FLG***,[108,173] *GLB1*,[110] *GPSM3*,[110] *HLA-C*,[110] *IL1RL1-IL18R1-IL18RAP*,[110] *IL6R*,[122] *IL13*,[109,110,173] *LOC100505634*,[110] *MIR1208*,[110] *NCF4*,[109] *OR10A3-NLRP10*,[110] *OVOL1*,[109,110] *TNXB*,[173] *ZBTB10*[109]
Food allergy	
Candidate gene studies	*CD14*,[130] ***FLG***[100,134–138], *HLA-DQB1*,[151] *SPINK5*,[132] *STAT6*[131] *(No GWAS performed to date)*
Allergic rhinitis	
Candidate gene studies	*ADAM33*,[68,141–143] *CD14*,[144,145] ***FLG***,[146] *IL13*,[174,175] *MRPL4*,[176] *TSLP*[147]
GWAS	*ADAD1*,[133] *AKR1E2*,[68] *CLEC16A*,[125] *CROCC*,[125] *C11orf30-DLG1*,[68] *LINC0048*,[68] *LRRC32*,[125,133,134] *ENTPD6*,[125] *ETS1*,[133] *FERD3L*,[68] *FOXA1-TTC6*,[133] *GLI3*,[125] *IL1RL2-IL1RL1*,[133] *IL33*,[133] *HLA-C-MICA*,[133] *HLA-DQA1-HLA-DQB1*,[133] *LPP*,[133] *MRPL4*,[135] *NFATC2*,[133] *PPM1A-DHRS7*,[125] *PLCL1*,[133] *PTGER4*,[133] *SEMA6A*,[125] *SMAD3*,[133] *TLR1-TLR6-TLR10*,[133] *TMEM232-SLCA25A46-TSLP*,[125,133] *ZNF776*[68]
Childhood asthma	
Candidate gene studies	*ADAM33*,[136,137,140,148] *CD14*,[160] ***FLG***,[105,106,121,146,177] *RANTES*,[84,85] *SPINK5*,[101,178] *TSLP*[101,147]
GWAS	*AB13BP*,[179] *ATG3*,[179] *CHCHD9*,[180] *IGSF3*,[179] *ORMDL3-GSDMA-GSDMB*,[181] *HLA-DPB1*,[182] *HLA-DQB1*,[181] *HPSE2*,[179] *IL1RL1-IL18R1-IL18RAP*,[181] *IL13*,[181] *IL2RB*,[181] *IL33*,[181] *KLHL5*,[179] *MKLN1*,[179] *PDE4D*,[183] *PSAP*,[179] *RORA*,[181] *SLC22A5*,[181] *SLC30A8*,[182] *SMAD3*,[181] *TLE4*,[180] *XPR1*[179]
Severe asthma	
Candidate gene studies	*CD14*,[158,176,184] ***FLG***,[156] *IL6R*[175]
GWAS	*AC01*,[12] *C5orf56*,[12] *EMX2*,[185] *IL1RL1-IL18R1*,[12,181] *IL12RB1*,[185] *KIAA1271*,[186] *NDFIP1*,[12] *ORMDL3-GSDMA-GSDMB*,[12] *RAD50*,[186] *SCG3*,[186] *UPK3A*,[185] *YES1*,[185] *ZNF665*[12]

with skin barrier dysfunction, including loricrin (*LOR*), involucrin (*IVL*), members of the *S100* gene family, a large group of the late cornified envelope gene family, many of the small proline-rich proteins, peptidoglycan recognition proteins (i.e., *PGLYRP3, PGLYRP4*), and most notably, filaggrin (*FLG*). Linkage was also reported in 17q21,[94] where the gene encoding one of the keratins (*KRT16*) is localized. Additional loci implicated by genome-wide linkage included 3p26-p22, 5q13, 11p14, 3q21, and 4q22, but evidence for linkage extended over large portions of the chromosome and pinpointing the candidate gene proved challenging.[45]

After the family-based linkage era, multiple candidate gene studies of AD ensued. Perhaps the best known candidate gene for AD emerging from the candidate gene approach is *FLG*, also known as filament-aggregating protein. Through cloning the gene encoding *FLG*, Irvine and colleagues[95] found numerous tandem *FLG* repeats, because of its tight regulation at the transcriptional level in terminally differentiating epidermis, they proposed *FLG* to be an important candidate for disorders of keratinization. Within the gene encoding *FLG*, multiple variants associated with risk of AD have been identified, but two null mutations (R501X and 2282del4) account for most genetic associations to date, with a combined allele frequency among AD patients of 18% and 48% for the R501X and 2282del4 mutations, respectively, and an effect size (e.g., odds ratio) exceeding 3.[96–101] The effect size is even greater for subphenotypes including early-onset, severe, and persistent AD[102,103] and the severe complication/viral dissemination, eczema herpeticum.[104] Alternatively, it has been suggested that the *FLG* loss-of-function mutations are not associated with late-onset AD,[105] which further supports the role of this candidate gene in the atopic march. Notably, the R501X and 2282del4 are ancestral European variants carried on conserved haplotypes[78] and are far less common in African ancestry populations.[104,106]

Not surprisingly, an association between *FLG* and the risk of AD was replicated in one of the first AD GWAS.[107] In addition to the *FLG* gene, at least 18 additional AD risk loci achieving genome-wide significance ($P < 5 \times 10^{-8}$) have been identified through the Esparza-Gordillo and colleagues GWAS plus two other GWAS[107–110] and a study of more than 30,000 AD patients genotyped on the Immunochip.[111] In addition to innate and adaptive immunity and epidermal barrier function genes, whole genome approaches have implicated interleukin-1 (IL1) and nerve growth factor signaling, regulatory T cells, the inflammatory response, and the vitamin D pathway,[112] thereby expanding our understanding of the underlying pathogenesis of AD. Genes in these pathways have been implicated in limited studies focusing on disease severity[113–115] and viral dissemination (e.g., eczema herpeticum).[104,116–119]

Food allergy. Although a role for genetics in the risk of food allergy has long been assumed because family history and twin studies have consistently shown a significantly higher concordance rate of food allergy (e.g., peanut allergy) among monozygotic twins compared with dizygotic twins and siblings,[120–122] relatively little is known about specific genetic determinants for this and its associated trait, food sensitization. Unlike AD, asthma, and allergic rhinitis, no genome-wide linkage studies or GWAS have been performed specifically on food allergy or food sensitization (Table 1). A major challenge in identifying genetic determinants uniquely associated with risk of food allergy is the considerable coexistence of food allergy with the other

IgE-mediated traits, especially AD, in which the prevalence of food allergy among children with AD is approximately 35%,[123,124] and there is an even higher proportion of children with food allergy who also have a history of AD[125] and severe AD.[126]

Limited candidate gene studies include associations in the HLA-D locus,[127–129] *CD14*,[130] *STAT6*,[131] *SPINK5*,[132] and *FOXP3*,[133] but not surprisingly, the *FLG* loss-of-function mutations have been the most extensively examined candidate variants for risk of food allergy.[100,134–138] Some studies have suggested that the association is driven by a co-association with AD,[138] whereas others have demonstrated that the association is not accounted for by coexistent AD[126,139] (or asthma[137]), suggesting, in the case of food allergy, that the barrier defect resulting from the loss-of-function variant(s) leads to enhanced exposure of the food-borne allergen (e.g., peanut allergen) to antigen-presenting cells.[126,136,140]

Allergic rhinitis. Similar to AD, multiple independent genome-wide linkage,[40–44] candidate gene,[68,141–147] and GWAS[68,125,133,134] research has been performed on allergic rhinitis (Table 1). With regard to the confounding of food allergy by a history of AD, as discussed above, it has proved challenging to disentangle uniquely allergic rhinitis genes from asthma genes, and from atopic march genes because allergic rhinitis is common (affecting 30% of adults and 40% of children[148]) and frequently coexists with asthma.[142] A study integrating GWAS data and gene expression data from CD4+ T cells isolated from patients with allergic rhinitis and a coexpression network analysis replicated four of the 17 allergic rhinitis loci previously reported and those that appear to be specific to allergic rhinitis, as opposed to being shared with other allergic phenotypes.[68] Nevertheless, as summarized in Table 1, several candidates identified through the biased candidate gene approach as well as the unbiased GWAS approach overlap with other childhood atopy traits (e.g., *ADAM33*, *CD14*, *FLG*, *TSLP*, *IL33*, and *IL1RL2*). The association between *FLG* mutations and allergic rhinitis has been reported in several studies,[101,102,146] and similar to *FLG* associations in risk of food allergy, associations appear to be independent of coexistence of other atopic traits, such as asthma.[102]

Asthma. Of the atopic diseases, the largest proportion of linkage and candidate gene studies and GWAS has been performed for risk of asthma (see Appendix A in Ref. 143). The most strongly associated and replicated genes include the *ORMDL3-GSDMA-GSDMB* locus on chromosome 17, as well as *DENND1B, GAB1*, *HLA, IKZF4*, *IL1RL1*, *IL33*, *IL2RB*, *IL6R*, *LRRC32*, *PDE4D*, *RORA*, *SLC30A8*, *SMAD3*, *TLR1*, and *TSLP*.[59] However, the discovery populations from which these reports were published were not limited to childhood-onset asthma. Accepting a threshold of significance at $P < 10^{-5}$ and restricting studies to those focusing on childhood-onset asthma, nine of these 16 asthma candidate genes remain (*ORMDL3-GSDMA-GSDMB*, *HLA-DPB1, HLA-DQB1, IL1RL1-IL18R1-IL18RAP*, *IL2RB*, *IL33*, *PDE4D*, *RORA*, *SLC30A8*, and *SMAD3*); additional genes associated with childhood asthma include *AB13BP*, *ATG3*, *CHCHD9*, *IGSF3*, *HPSE2*, *IL13*, *KLHL5*, *MKLN1*, *PSAP*, *SLC22A5*, *TLE4*, and *XPR1* (Table 1). Upon closer examination, genes common to the atopic phenotypes emerge; note that independent asthma GWAS have identified SNPs in the *HLA-DQB1* locus also associated with food allergy and allergic rhinitis, the *IL13* locus is associated with AD, and the same *IL33* and *IL1RL1* loci are associated with AD and allergic rhinitis.

Because *ADAM33*, one of the asthma candidate genes to have been identified by positional cloning,[34] is involved in airway remodeling, it was previously suggested that *ADAM33* might be more relevant in adult or nonatopic asthma versus atopic asthma, which could explain the disparate results from multiple replication studies. Several childhood asthma candidate gene studies have replicated the association between *ADAM33* SNPs and risk of asthma (Table 1), but other, more robustly powered childhood asthma studies have not.[138,149,150] The role of *ADAM33* in asthma is further complicated by the idea that a common environmental factor, passive smoke exposure, interacts with *ADAM33* to exert its lung remodeling function, a hypothesis that potentially explains the involvement of ADAM33 in chronic obstructive pulmonary disease.[151,152] On the other hand, several candidate gene-based studies have implicated *ADAM33* SNPs and risk of allergic rhinitis, primarily in adult populations (Table 1).

Similar to AD, food allergy, and allergic rhinitis, common *FLG* mutations have been implicated in risk of asthma, and similar to the other traits, uncertainties remain whether the association between *FLG* mutations and asthma exists without the coexistence of AD.[146,153,154] Allergic disease associated with *FLG* mutations has been suggested to be a causal risk factor for asthma.[155] For example, Henderson and colleagues observed that AD patients with *FLG* mutations have a greater risk of asthma compared with AD patients without the *FLG* mutations,[153] and others have shown that asthmatic patients with *FLG* mutations are more severe with more frequent exacerbations compared with asthmatic patients without *FLG* mutations.[156] In summary, there is compelling evidence that *FLG* is a unifying gene in the atopic march.

A greater challenge in asthma genetics has been to adjust for allergic rhinitis, which is especially common in childhood asthma. In fact, few studies have performed tests for association for risk of asthma while adjusting for the others. In one of the few examples, Ferreira and colleagues identified 11 SNPs in 10 chromosomal regions that were significantly associated ($P < 3 \times 10^{-8}$) with asthma plus allergic rhinitis, which remained significant but was weaker when the two phenotypes were considered separately.[134] Moreover, the group observed that an SNP in *LRRC32* was specifically associated with an increased risk of allergic rhinitis, whereas associations with *GSDMA* and *TSLP* SNPs were stronger for asthma without allergic rhinitis compared with rhinitis without asthma. In contrast, SNPs in or near *ZBTB10* and *CLEC16A* were only associated with risk of disease with the combined asthma-allergic rhinitis phenotype, which led the authors to conclude that these genes are "bona fide genetic risk factors for allergic disease."[134]

Summary and conclusions

Taken together, review of the most consistently associated genes among the four atopic phenotypes suggests a set of candidates that are common to each trait (Figure 2). Moreover, these shared candidates can be generally categorized as those that have an inherent role in the adaptive and innate immune response and skin barrier dysfunction, a hallmark feature of the atopic march. Importantly (but not covered in this review), the environmental context as well as countless additional genetic factors (i.e., race/ethnicity, comorbidities) no doubt contribute to modify both expression of the atopic phenotypes as well as the contribution of the candidate genes. Consider, for

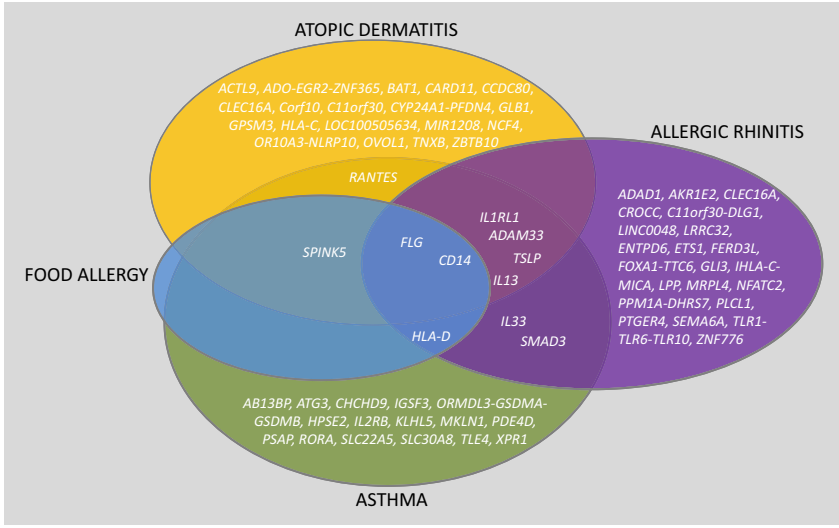


Figure 2 Candidate genes that are common and specific to clinical manifestations of the atopic march.

example, that the sequential progression of clinical phenotypes in a tropical environment where exposure to parasites and poor living conditions are common is different from that observed in temperate, industrialized countries.[145] Indeed, the extent to which major differences in the macroenvironment confound genetic associations with risk of AD—as in the case of a functional promoter polymorphism in the gene encoding *CD14* associated with a *lower* risk of atopy among rural farm children in Bavaria,[157] as opposed to a *greater* risk of atopy among children living in an urban tropical environment[158]—remains poorly understood. Finally, an increasing appreciation for the capacity of the respiratory tract and skin microbiota to educate the host's immune system[159–161] and the application of NGS to measure the microbiome[162] have collectively led to an explosion of activity related to understanding the impact of commensal and pathogenic microbial exposure on atopic phenotypes. Consideration of these nongenetic factors, in addition to further unraveling of the causal variants and characterization of their contributions to the atopic march, is under way.

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Epigenetic Factors Before and After Birth

9

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Introduction

Beginning with the proliferation of the zygote, cell maturation and differentiation represent the basic processes that promote and orchestrate the ontogenesis of each living being. The underlying molecular interactions were long thought to be exclusively driven and programmed by the genetic repertoire imprinted in the genomic deoxyribonucleic acid (DNA) sequence. In the 1940s the "dictate of the genes" was disproved by the Waddington concept of "epigenetics."[1] Initially restricted to mechanisms that regulate the maturation of stem cells toward fully differentiated cells, this concept was later revised by including the phenomenon of nonsequence inheritance and a broad spectrum of gene-by-environment interactions that shape and consistently modify the phenotype throughout the whole lifespan. Nowadays, epigenetics is a well-recognized concept that explains how the environment influences ontogenetic development to adapt the phenotype optimally to exogenous conditions. However, epigenetics might also explain how pathogenetic conditions are established when this interplay is disturbed by detrimental exogenous factors and is imbalanced toward, for example, inflammatory conditions.[2,3]

In contrast to the genetic repertoire of an individual, which is mostly the same in all cells of the body, the epigenetic signature might strongly differ between specific cell types and tissues. It has become apparent that exogenous hits shape the epigenetic signature on the cell type level throughout the whole life and from the first ontogenetic development in utero. This developmental period is particularly susceptible to gene-by-environment interactions because the replication machinery is highly active in promoting cell proliferation in the growing fetus.[4] Immune maturation is initiated in early pregnancy when hematopoietic stem cells start to differentiate into progenitor cell lines that later develop into naïve and mature immune cells in the last trimester. Even in the well-protected compartment of the maternal uterus, environmental stimuli transferred through the placenta might affect fetal immune development. Targeting the physiological traffic in the cell nucleus during replication, these external impacts are necessary to program fetal gene expression toward a competent postnatal immune response to defend the newborn against dangerous impacts.[5] Depending on the mother's lifestyle and living conditions, some maternal impacts might decelerate

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immune maturation, hamper the development of immune-competent cell lineages, and might thereby contribute to later immune deviation. An array of nutritional, xenobiotic, and lifestyle factors were recognized to interact with the genetic makeup and contribute to the establishment, progression, and chronology of inflammatory processes.[6,7] Other stimuli such as the microbial environment might foster a tolerogenic immune system that is resilient against disturbance and capable of resolving inflammation. However, it is still unsolved how immune functions are mechanistically affected by environmental stimuli. Epigenetic mechanisms might explain the last step of gene regulation and expression in cells that are directly involved in immune regulation. The preceding steps that might explain how environmental stimuli are translated to cell-communicative mechanisms in the nucleic compartment are largely unknown. Recently conducted studies provide elegant approaches to unraveling this mystery.[8] It is becoming evident that there is no universal "factor X" acting as an omnipotent translator between the environment and genes. Epigenetic modifications are closely bound to a genetic program and the resulting physiological cascades. As a consequence, it is a Sisyphean challenge to identify the numerous loci affected out of the myriad of putative targets along the immunological pathways that shape the immune response and the respective clinical phenotype. By applying novel techniques to investigate the missing links that initiate epigenetic imprinting, we get closer to elucidating the complex chain that is called gene-by-environment interaction.[9]

In this chapter, we aim to provide a comprehensive overview of the pieces of this puzzle that have been identified. This chapter will give a short introduction in the principles of epigenetic regulation with a main focus on DNA methylation and histone modification. Based on recently conducted studies and experimental approaches, we aim to explain how selected environmental factors such as lifestyle act on immune functions via epigenetic mechanisms and how these impacts might contribute to promotion and prevention of allergies and asthma.

Principles of epigenetic mechanisms

In principle, epigenetic mechanisms are responsible for gene silencing and activation to differentially regulate gene transcription and expression. As described above, some of these modifications might be transferred during cell replication to next-cell generation. Others are of a transient character, implemented to regulate transcriptional traffic within the cell metabolism. These transient modifications could be affected by enzymatic machinery that reverses the silenced status of a certain gene into an active form.[10]

Historically, DNA methylation was the first nucleic mechanism to be recognized as an epigenetic process. This molecular modification of DNA represents a basic step in regulating gene activity as a molecular response to a broad spectrum of cellular stressors. Although it acts directly on the genomic DNA, methylation does not modify the DNA sequence. Deoxyribonucleic acid methylation chemically addresses cytosine residues within the DNA sequence by adding a methyl group to position 5 at the cytosine-guanine-diphosphoester (CpG) site (Figure 1). CpG sites are dinucleotides built from cytosine and guanine, linked by the phosphodiester configuration. These

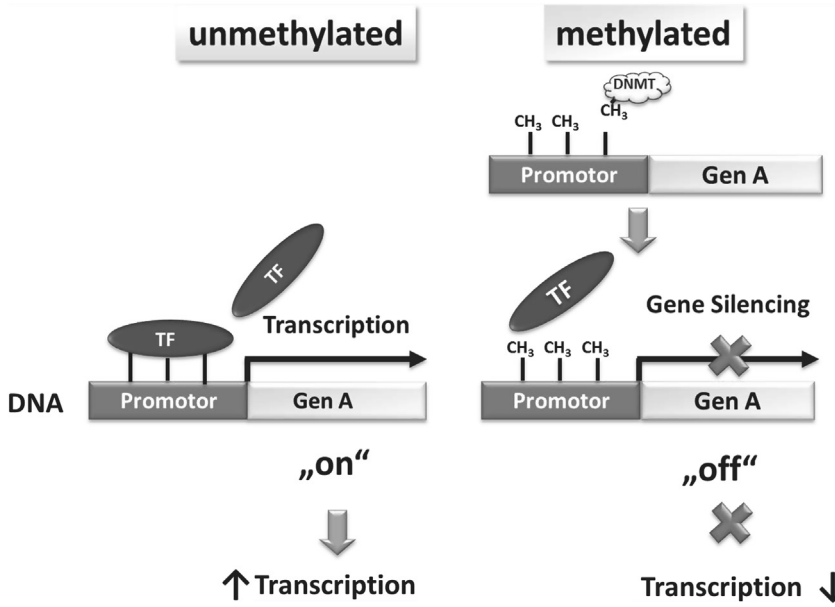


Figure 1 DNA methylation: unmethylated CpG sites at the promoter region are open for transcription machinery (right). Methylation at CpG sites hampers transcription factor binding at the promoter region of genes, switching transcription off. DNA methyltransferase (DNMT) catalyzes this reaction (left).

CpG sites are distributed throughout the whole genome and accumulate in regulatory sequences of genes such as promoters or enhancer regions. These clusters are called CpG islands when they contain more than 60% of genomic CpGs.[11] Following observations that genomes of fully differentiated and resting cells are methylated up to 90%, and that in contrast, functionally active and proliferating cells were predominantly unmethylated in the CpG islands, the concept was developed that DNA methylation might have a regulatory effect on gene expression.[10] It was found that some of these chemical modifications are stable even after cell replication, and therefore are heritable.[12] If transient or permanent, CpG methylation mostly results in gene silencing, but until now it has not been clear how CpG methylation leads to silencing of gene expression. The methyl group might act in a stereochemical mode because it might limit access of transcription factors to the promoter binding site. In binding studies, it was also shown that methylated CpG is able to attract specific proteins that act competitively with the binding transcription factors.[13] CpG islands represent the main target regions for DNA methyltransferases (DNMT) that catalyze methyl group transfer from methyl donors. DNMT1 is the major DNMT important for maintaining the DNA methylation status of genes in a resting state. DNMT1 retains gene silencing and might thus contribute to heritable transfer of CpG methylation.[14,15] DNMT3a and DNMT3b are the main enzymes for de novo methylation of promoter regions.[16,17] In addition, these enzymes interact with histone modification, the other important mechanism to establish epigenetic signatures. DNMT3a is able to

bind to methyl-CpG–binding protein 2 (MeCP2), which is competitive with the binding of transcription factors.[18] The DNMT3a/MeCP2 complex is described to initiate the recruitment of histone-modifying enzymes and thereby interact with chromatin modification.[19]

Chromatin or histone modification is another principal mechanism to imprint epigenetic marks. Chromatin modifications might render gene transcription or gene silencing and might also facilitate DNA repair mechanisms. Whereas DNA methylation might regulate gene transcription in the ontogenetic program, chromatin remodeling seems to be the basic principle for maintaining memory of gene expression pattern in somatic cells.[20]

Chromatin represents the superior structure of DNA and nucleic proteins, the histones, in the cell nucleus. Built from cationic histone octamers and wound DNA segments, the nucleosomes are the main targets for chromatin remodeling. Histone modifications act on the histone subunits of the histone octamer, which is organized into two dimers of histones, H2A and B, and two dimers of histones, H3 and H4. Acylation and restructure by deacylation of N-terminal peptide tails that stick out the core protein are the basic mechanisms that modulate nucleosomes.[21] Ubiquitination, phosphorylation, and sumoylation might also occur at these structures. Acetylation exclusively occurs at lysine residues and decreases the affinity of histones to the anionic DNA by neutralizing the cationic NH_3^+-group at the ε-position. In consequence, the condensed package of the heterochromatin becomes scattered and the DNA filament within this segment becomes assessable for transcription factors and subsequently to the transcription machinery.[22,23] Two enzymes catalyze the opening and closing of the nucleosome via acetylation and deacetylation: the histone acetyltransferase (HAT) and the histone deacetylase (HDAC). Histone acetyltransferases are assigned to two enzyme families. Type A HATs are closely involved in activating gene expression as located in the cell nucleus. Type B HATs have a crucial role in the de novo synthesis of histones to assemble already acetylated histones before translocation into the nucleus.[24,25]

Histone methylation is the other main mechanism that modifies nucleosome structures.[26] Beside lysine, arginine is a target for methylation catalyzed by histone methytransferases. In contrast to acetylation, methylation might occur on multiple lysine and arginine sites of histone tails. In any case, histone methylation controls gene expression by regulating the accessibility of nucleosomal DNA to the RNA polymerase II complex and promoting specific transcription factors. This Janus-faced methylation might promote gene transcription and repression as well, depending on where and how methyl groups are chemically added. For example, trimethylation at the histone 3K4 locus (H3K4me3) activates the transcriptional machinery, whereas trimethylation of the same histone but at the K27 locus is described to repress gene transcription.[27] Mono-, di-, and tri-methylation is involved in stem cell development, as shown for myeloid and lymphoid lineage development originating from the hematopoietic stem cell[28] (Figure 2).

Furthermore, acylation might occur near phosphorylation of serine residues. Phosphorylation at histone H3 is an expressive example of the variability of gene regulation subsequent to histone modification. Depending on the specific combinatorial pattern, H3 phosphorylation marks either an open chromatin during gene activation or a highly condensed chromatin during mitotic replication.[28]

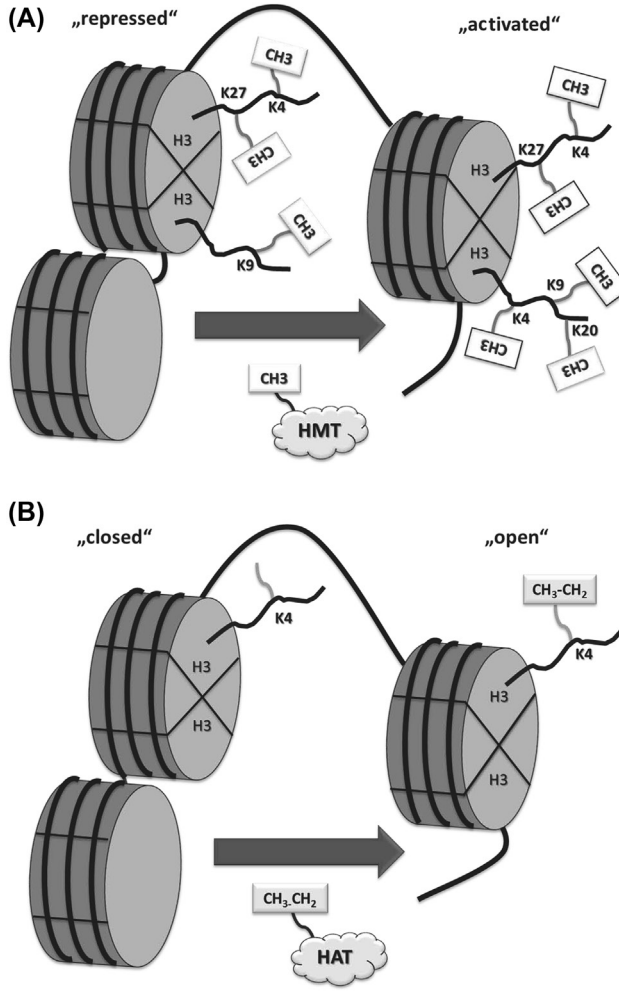


Figure 2 Histone modification: Both histone methylation (A) and histone acetylation (B) act on lysine residues located in tails of the histones in the promoter region of target genes. This example depicts gene activation and silencing in hematopoietic stem cells during lymphoid or myeloid lineage development. Genes activated by transcription factors are characterized by acetylation at H2K4ac, H3K2me1, H3K4me1, and H3K9me1 and become activated in downstream cells in either the lymphoid or myeloid lineages. Loci marked with nucleosomes containing H3K9me3 are silenced in these cells.[28]

Epigenetics in T-cell development as a decisive mechanism for allergy development

Developing from the hematopoietic stem and progenitor cell, naïve T cells keep a minimum level of pluripotency to further differentiate into distinct T-cell subsets.[5] The development of the CD4+ T-helper-cell lineage in favor of CD8+ T cells is regulated by the expression of Zinc Finger Protein T-helper–inducing POZ/Krüppel-like factor, also known

as cKrox (Th-POK), which skews progenitor cells toward CD4+ differentiation. Interestingly, in progenitor cells, epigenetic marks are set at CD8+-associated genes, which are methylated in favor of fostering Th-POK expression followed by CD4+ differentiation.[29]

T-helper-cell subsets are distinguished by a characteristic cytokine profile to evolve effector functions to protect against extracellular and intracellular pathogens and against cancer cells (Th1, Th2, and Th17) or to regulate T-cell subset development to maintain well-balanced homeostasis of the immune system (for details, see other chapters). T-cell lineage is based on activation of intracellular signaling pathways to induce lineage-specific transcription factors, which themselves induce the expression of distinct cytokine profiles. Binding of these T-cell lineage-specific transcription factors to the promoter regions of cytokine encoding genes or regulatory gene regions seems to be the next step in T-cell development. When naïve cells are bound to polarization into Th1 or Th2 subsets, accessibility of either the Th1-favoring interferon *(IFN)G* locus or the Th2-driving Conserved Noncoding Sequence (*CNS*) *1* region near interleukin (IL)-4, IL-5, and IL-13 coding genes must be given. Deoxyribonucleic acid hypermethylation at the conserved regulatory elements near the *IFNG* locus fosters Th2-cell lineage development locus by silencing the *IFNG* locus, whereas a permissive histone mark at the *CNS 1* locus enhances Th2 cytokine expression. To promote appropriate T-cell development in both directions, permissive histone modifications and DNA hypomethylation are established at the *IFNG* locus as well as in *CNS 1* enhancer region.[30–33] In turn, repressive H3K27 trimethylation in regulatory regions of cytokine genes might have a strong impact on T-cell lineage development. These effects are meditated by Enhancer of Zeste Homologue (Ezh)2 or Ezh1 methyltransferase components of polycomb repressive complex (PRC)2. In vitro experiments have shown that silencing of the *IFNG*-, the trans-acting T-cell–specific transcription factor (*GATA3*), and the *IL-10* locus in naïve Th cells depends on Ezh. It was shown in detail that Ezh2 facilitates correct expression of Th1- and Th2-specific transcription factors in the respective Th subset development, and therefore supports appropriate Th-cell differentiation.[34]

Th17 cells represent a distinct T-cell lineage that has been suggested to be linked to allergic diseases including asthma.[35] Epigenetic mechanisms are thought to be involved in Th17 differentiation, because permissive histone modifications are observed for *IL17A* and *IL17F* in Th17 differentiation.[3] Ikaros, a hematopoietic transcription factor, seems to have a strong impact on early lymphocyte development in the bone marrow and thymus. It has a critical role in shaping naïve T cells in the periphery in response to acute activation signals. Ikaros is also involved in epigenetically regulated transcription of a plethora of Th17 determining genes such as *AHR*, *RUNX1*, *RORC*, *IL17A*, and *IL22* because it integrates Th17 polarizing signals. To promote and maintain Th17 lineage development, Ikaros sets a limit to histone modifications that repress transcription of Th17 determining genes.[36]

The development of regulatory T-cell (Treg) subsets is essential to maintaining immune homeostasis. In turn, the Forkhead Box Protein (FOXP)3, the Treg lineage-specifying transcription factor, is crucial for maintaining Treg-cell–specific suppressive function. Demethylation of the regulatory elements of the Treg-specific transcription factor FOXP3 is a precondition for Treg development and thus maintenance of a tolerogenic adaptive response. As described for Th17 lineage commitment,

regulation of the *FOXP3* gene locus is orchestrated by a complex interplay of transcriptional and epigenetic control mechanisms.[37] In the thymus, Treg development is under control of phosphoinositide 3-kinase activation (PI3K–Akt) signaling downstream of the T-cell receptor (TCR). This complex negatively regulates thymus Treg-cell differentiation. Studies have shown that in thymus-derived Treg, expression of FOXP3 highly depends on factors downstream of the TCR signaling cascade according to the strength and duration of TCR stimulation by antigens. These downstream factors include nuclear factor (NF) κB and members of the Forkhead Box O (FOXO) family. T-cell receptor signaling under strong antigen activation enhances NFκB activity and inactivates FOXO proteins. A lack of antigen interaction instructs FOXO proteins, which are negatively regulated by TCR signaling, to translocate back to the nucleus and to induce Foxp3 transcription jointly with NFκB and other factors. A hit and run model proposes that thymocytes that differentially and infrequently come into contact with high-affinity antigens are selected to differentiate to Treg cells when the epigenetic marks at the regulating regions of the *FOXP3* gene are permissive to FOXO and NFκB.[38,39]

Differentiation of naïve CD4+ T cells toward Treg development in the peripheral epithelial tissues (pTreg) is predominantly under the control of exogenous stimuli. Adaptation to environmental niches near diverse microbiota correlates with a broad spectrum of different TCR–antigen interactions of specific TCR subsets. Treg function in mature Treg cells of the periphery depends on the micromilieu, which includes TCR-activating and costimulatory factors, cytokines, and the complement. These signals are translated downstream via activation of the (PI3K)–Akt signaling pathway to repress FOXO1 translocation and nuclear transcriptional activity. As a result, repression of FOXO1 promotes expression of chemokine receptor 7, which might promote Treg-cell function in vivo.[39]

Epigenetic control in fetal immune development

Epigenetic and transcriptional control of T-cell differentiation is important for establishing a well-balanced T-cell repertoire. In utero, these processes are influenced by endogenous and exogenous stimuli. Activation of naïve CD4+-helper T cells via the TCR and major histocompatibility complex II peptide complex and subsequent signal cascades is crucial to induce T-cell activation and differentiation. A well-balanced and functional T-cell subset repertoire at birth is essential to defend hazardous pathogens and prevent autoimmune and allergic reactions. As described, epigenetic mechanisms regulate imprinted ontogenetic programs, but also act as mediators of upstream signals coming from the micro- and macroenvironment. Gene-by-environment interactions meditated by epigenetic modifications are the driving force shaping a tolerogenic or imbalanced T-cell response, and therefore favor homeostatic or pathogenic traits. Initially, epigenetic modifications act on naïve noncommitted precursors to pave the way to T-cell subset development.[40,41] Epigenetics in both Th1 and Th2 differentiation have been extensively studied.[42] Deoxyribonucleic acid methylation is considered to be the main epigenetic mechanism controlling Th1 expression. During pregnancy and early infancy, fetal/neonatal interferon-gamma (IFN-gamma) production is low in CD4+ T cells and is associated with hypermethylation of the *IFNG* promoter.[43]

Furthermore, DNA methylation at Th1 cytokine genes supports the development of Th2 cells.[44] These epigenetic marks are helpful in establishing a Th2-skewed environment in utero to maintain pregnancy and protect the allograft from abortion. Nevertheless, the molecular tools for differentiation of naïve T cells are implemented because the apparatus for histone acetylation, which provides accessibility to the *IFNG* and *IL4* loci, is fully functional in late pregnancy.[45] Moreover, induction of IFN-gamma expression by H2.0-like homeobox protein (Hlx)1 is enabled in immature T cells by a permissive epigenetic mark at the promoter region of the *IFNG* gene.[46]

Environmental factors and epigenetic modifications in early allergy development

The vast majority of T-cell studies were conducted in isolated human cells, cell lines, or in vitro-induced or polarized T cells. An overwhelming body of evidence on epigenetic interactions in T-cell differentiation derived from those investigations has accumulated in the past decade. Undoubtedly, these studies are highly important for understanding the fundamental mechanisms of T-cell–mediated inflammatory and particularly allergic disease, but they mostly fail to link T-cell development to environmental impacts that might cause epigenetic modifications in T cells.

Compared with in vitro observations, epigenetic in vivo studies on allergy and asthma in animal models or observational studies in human populations are rare. A number of studies were conducted to investigate associations between exogenous impacts and allergy development suggesting that epigenetic modulation might be the mediating mechanism to explain the observed effects on the phenotype. These studies clearly outlined that Waddington's postulate of a lifelong interplay between the genotype and the environment is evident. Birth cohorts have impressively demonstrated that these interactions occur in the womb mediated by the mother. Nonetheless, clear evidence for epigenetic modification by direct measurement of epigenetic marks is missing in most of the studies.[47] The following paragraphs give an overview on the state-of-the-art knowledge derived from in vivo studies providing evidence about how epigenetic signatures contribute to allergy development and prevention.

Lifestyle factors such as dietary habits, psychosocial stress, tobacco use, and ambient air pollution by chemical compounds are associated with early allergy development. Microbes and their specific components might trigger or prevent allergic diseases based on gene-by-environment interactions. Even allergens as the causative agents for sensitization might be seen as agents that foster Th2 conditions by epigenetic programming.[40,41]

Allergens as triggers for Th2 responses are associated with epigenetic modifications in target cells

Epigenetic mechanisms in the context of allergic sensitization were mainly studied in mouse models of allergic airway inflammation. Transgenerational models were employed to investigate transmaternal gene-by-environment interactions during

pregnancy and their impacts on the allergic phenotype of the offspring.[48] Such experiments clearly support transmaternally submitted changes in the epigenetic signature of the progeny. When female mice were subjected to a sensitization protocol and subsequently mated, allergen-naïve offspring of asthmatic mothers showed substantial changes in DNA methylation in their splenic CD11c (+) dendritic cells (DCs) compared with controls. When DCs from those animals were adoptively transferred to nonallergic recipients, the animals developed an increased susceptibility to multiple allergens. Maternal allergy might direct the offspring's DCs repertoire in a proallergic direction by establishing respective epigenetic marks.[49] Changes in global DNA methylation were also reported when animals were treated with house dust mites (HDM). Asthma candidate genes of the Akt-signaling cascade, cAMP-pathway, and fatty acid metabolism were targeted by epigenetic modification when HDM was applied intranasally.[50] Epigenetic marks seem to be inherited to the F2 generation, as shown by Niedzwiecki et al. Grand-offspring during of pregnancy *Aspergillus fumigatus*-sensitized mice showed decreased DNA methylation at the *IL4* promoter compared with mice exposed after birth or sham-treated.[51] In our laboratory, T cells were subjected to epigenetic programming. Treatment of female mice with ovalbumin (OVA) within a protocol of allergic airway inflammation was associated with a significant increase in DNA methylation at the *IFNG* promoter in CD4+ T cells and decreased IFN-gamma expression, whereas only minor changes were observed at the *CNS* 1 region of the Th2 cytokine coding gene locus. Application of the DNMT inhibitor 5-Aza-2′-deoxycytidine (5-Aza) after sensitization resulted in an abrogation of the allergic phenotype.[52]

Microbes in the human environment are capable to protect against allergy development via epigenetic mechanisms

A plethora of studies conducted at traditional farming sites have strengthen the hypothesis that environments enriched with higher numbers of diverse microbial communities protect against early sensitization and development of allergic diseases.[53] Detailed analysis of epigenetic marks in farm children compared with children from nonfarm families elucidated a variety of epigenetic effects associated with a decreased allergy and asthma prevalence in farm children. In a sample of pregnant farm and nonfarm mothers recruited for the PAULCHEN Study, neonates of traditional farm families were found to have an enhanced neonatal Treg function in cord blood. In addition, increased *FOXP3* expression and higher levels of DNA demethylation were found in cord blood from farm neonates compared with infants born to nonfarm women. Maternal farm milk consumption during pregnancy was associated with demethylation of the *FOXP3* promoter in the farmers' offspring.[54] Epigenetic effects in Tregs associated with farm milk consumption in early childhood were also reported from the Protection Against Allergy Study in Rural Environments (PASTURE) birth cohort. Farm milk exposure at age 4.5 years was associated with increased Treg-cell numbers and higher *FOXP3* demethylation upon stimulation of those cells. Farm milk consumption might

induce a regulatory phenotype early in life that is resilient against T-cell deviation and protect against the development of infant allergies.[55] Additional data from the PASTURE cohort support epigenetic control of asthma-related genes conveyed by the farming environment. Deoxyribonucleic acid methylation analysis was carried out in asthma gene candidates isolated from cord blood and peripheral blood cells at age 4.5 years. The Orm1-like protein *(ORMDL)1* and Signal Transducer and Activator of Transcription *6* loci were hypomethylated in DNA from farm children compared with nonfarm children, whereas the DNA repair protein (*RAD*)50-gene and the *IL13* locus were hypermethylated. Changes in DNA methylation between birth and age 4.5 years clustered in the genes highly associated with asthma (*ORMDL* family) and IgE regulation (*RAD50*, *IL13*, and *IL4* loci), but not in the Treg genes (*FOXP3* and Runt-related Transcription Factor 3 *[RUNX3]*).[56]

Prenatal epigenetic modulation observed in placentas from farm women recruited for the Assessment of Lifestyle and Allergic Disease During Infancy study indicates the crucial role of microbial stimuli in shaping the immune system. Deoxyribonucleic acid methylation of the promoter region of CD14, an essential cofactor to sense microbial compounds by Toll-like receptor (TLR)4, was decreased in placentas obtained from farm women. In line with this finding, CD14 mRNA expression was upregulated in these placentas, indicating that microbial exposure during pregnancy fosters innate immune functions to establish an adequate early immune response in the offspring.[57]

In an experimental murine setup, we could show that the prototypic farm-derived bacterium *Acetinobacter lwoffii* F78 is capable of protecting against an allergic phenotype via changes in the epigenetic signature of T cells. In a prenatal administration, *A. lwoffii* F78 prevented the development of an experimental asthmatic phenotype in the progeny in an IFN-gamma–dependent manner. Chromatin immunoprecipitation in T cells of prenatally exposed and later OVA-sensitized and challenged offspring revealed an elevated H4 acetylation at the *IFNG* promoter whereas promoter regions of Th2 cytokine coding genes were not significantly modified at the chromatin level. We proved this finding by treating offspring with Garcinol, a pharmaceutical that inhibits de novo acetylation at histone sites, and found lowered H4 acetylation at the *IFNG* promoter. In addition, offspring were no longer protected against asthma development.[58] Gram-negative bacteria such as *A. lwoffii* F78 might induce allergoprotection via lipopolysaccharide (LPS)-dependent TLR4 signaling.[59] Permanent early low-dose exposure to bacterial LPS as occurs in natural environments might cause persistent chromatin modifications at genes coding for factors that are involved in signaling cascades downstream the TLR complex.[60] When it comes to interactions between innate and adaptive immune cells upon *A. lwoffii* F78 exposure, the notch signaling pathway seems to be involved in programming T cells toward Th1 differentiation.[61] These processes are initiated by upregulation of Notch ligands Delta 1 and Delta 4 in DC via a myeloid differentiation primary response gene 88(Myd88)-dependent pathway. The underlying epigenetic mechanisms are still unknown, whereas for Th2 differentiation via the Jagged/Notch complex GATA3 induction was associated with epigenetic remodeling of the *GATA3* promoter toward an open euchromatin structure.[62]

Airborne, xenobiotic exposures act though epigenetic modulation and enhance early allergic development

Xenobiotics are defined as chemical substances that are exogenous to the human body. Inhaled xenobiotics have a major role as risk factors in allergy, and particularly asthma development, by stressing both the immune system and the functionality of the lung. Traffic-related air pollution is the prominent source of diesel exhaust or polycyclic aromatic hydrocarbons (PAH). Also, indoor environments are polluted with some of these substances owing to tobacco smoke or indoor chemicals. Depending on living conditions during pregnancy and early childhood, these exposures contribute to allergy and asthma development later in life.[63,64]

A number of studies found traffic-related air pollution to be positively associated with wheezing, asthma development, and asthma exacerbations among children.[65–68] Epigenetic impacts from fine particle matter (PM) were exclusively investigated in adult subjects and provided evidence that the inducible nitric oxide synthase gene complex is transiently epigenetically modified upon PM_{10} exposure.[69] Treatment of murine macrophages with PM in vitro caused rearrangement in the global DNA methylation pattern.[70]

Maternal exposure to high levels of PAH during pregnancy was shown to directly affect DNA methylation in the *IFNG* promoter of cells from cord blood, which indicates that there is a direct link between ambient PAH exposure and immune functions by silencing the *IFNG* gene.[71] This assumption is strengthened by the finding that infant Treg development seems to be modified by PAH exposure, because the promoter of the *FOXP3* gene was hypermethylated in children with higher PAH amounts in the environment.[72] Diesel exhaust particles are thought to modify epigenetic pattern in cells and gene loci relevant to immune and asthma development. Similar to PAH, diesel exhaust particles are capable of increasing DNA methylation at multiple CpG sites of the *IFNG* promoter. Accordingly, some CpG in the *IL4* promoter were hypomethylated in mice subjected to diesel exhaust within a sensitization protocol using *Aspergillus fumigatus* to establish allergic airway inflammation.[73] Parallel to PAH, *FOXP3* expression seems to be affected by diesel exhaust, as measured in a United States inner city cohort.[74]

In the 1930s, Emil Bogen was the first to recognize the detrimental impact of tobacco smoke on health.[75] He allocated a plethora of pathologic bronchial outcomes observed in smokers to the main irritant components of tobacco smoke. In 1950, a case report by Rosen and Levy was the first to associate allergic childhood asthma with environmental tobacco smoke (ETS).[76] Nowadays, there is growing body of evidence that prenatal and early childhood exposure to tobacco smoke represents the prominent environmental risk factor for the development of childhood asthma.[77] Investigations into epigenetic modulations that seem to be involved in the damaging process that occurs in the respiratory tract of smokers point to a crucial role of chronic inflammation. In a study that compared lung biopsies from healthy nonsmokers with those obtained from matched cigarette smokers, smoking had a decreasing impact on the expression of HDAC2 and HDAC. Significant induction of IL-β and tumor necrosis

factor expression observed in this study pointed to activation of the inflammasome in smokers.[78] In addition, the adaptive immune response seems to be affected by ETS exposure in childhood. In a study population of school-aged children, increased multiple DNA methylation of the *IFNG* promoter and, concordantly, decreased expression of IFN-gamma was observed in T cells isolated from children exposed to ETS and ambient air pollution as well. Expression of *FOXP3* was lower in these children.[79] Data published by the Asthma Biorepository for Integrative Genomic Exploration consortium underline the importance of prenatal tobacco exposure. Prenatal smoking by the mother was reported to set a number of DNA methylation marks in the offspring genes that were found to be persistent until school age.[80] These data suggest that prenatal exposure to tobacco smoke is associated with epigenetic modifications in the fetus that persist into childhood. However, the biological significance of these altered loci remains unknown. Epigenetic modifications associated with ETS exposure were mainly found in detoxifying pathways altering the capacity of a biochemical response to xenobiotics (reviewed in Refs 63,81). This might lead to accumulation of toxic intermediates in the lung tissue, and thus to favored inflammatory conditions in the airways.

Dietary and metabolic factors are capable to modify epigenetic programs

Deoxyribonucleic acid methylation is based on a preceding methyl metabolism providing a sufficient amount of methylating compounds. These pathways are closely linked to the methionine/homocysteine metabolism. The essential amino acid methionine acts as an important methyl-donor in its activated form, s-adenosyl-methionine (SAM). Upstream of SAM, methyl residues are introduced into the metabolism via tetrahydrofolate, which transfers a methyl residue to homocysteine to form methionine. Tetrahydrofolate acts as a central carrier of methyl residues that might be limited in the metabolism if not supplemented by nutrition.[82] Limited supplementation of methyl donors in pregnancy as caused by maternal starvation or malnutrition is associated with severe consequences for later disease development in the offspring. Health outcomes observed in offspring conceived during the Dutch hunger winter of 1944–1945 underpin these assumption. The German blockade of 1944–1945 led to starvation and malnutrition (e.g., limitation of folate) in the Dutch population. Offspring born to starving mothers were shown to develop obesity and subsequently diabetes and cardiovascular disease more often than siblings conceived at other times. In addition, these subjects were characterized by lower levels of DNA methylation at the promoter of the insulin-like growth factor (*IGF*) 2 gene, a locus that is crucially involved in orchestrating embryonic and fetal growth.[83] A prenatal model of experimental allergic airway inflammation pointed to similar effects during allergy development. Mice dams were fed with a diet rich in methyl donors, such as folate and methionine, during pregnancy and weaning. Ovalbumin-sensitized and -challenged offspring of those mice employed an enhanced asthmatic phenotype compared with sham-treated controls. These observations were associated with elevated levels of

DNA methylation and decreased protein expression of the asthma-relevant transcription factor *RUNX3*.[84] On the other hand, we could show an abrogation of experimental asthma in OVA-sensitized mice that were postnatally treated with the demethylating agent 5-Aza, associated with an increased Th1 response in those mice.[52]

Human cohort studies provide a more inconsistent result. Whereas an Australian birth cohort reported an increased risk for asthma in the progeny at preschool age when the mother was supplemented with folate in second and third trimesters of gestation,[85] no significant associations were reported for the *Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg* (KOALA) birth study in a comparable study design.[86]

Obesity is currently thought to promote asthma.[87] Obese pregnant mothers more often give birth to children with a lower birth weight, who later catch up in growth during infancy.[88] This exponential growth during childhood and infancy is associated with a higher risk for central obesity, diabetes type 2, and cardiovascular disease later in life. The so-called "thrifty phenotype" is prone to developing epigenetically regulated, low-grade inflammation in adipose tissue associated with the secretion of adipokines that foster an inflammatory condition in other tissues and organs.[89–91]

Alternatively, hormonal imprinting was suggested to cause low birth weight and retarded organ development in newborns.[92] Caused by maternal persistent stress or metabolic imbalance, intrauterine growth retardation is associated with reduced activation of placental 11β-hydroxy steroid dehydrogenase (HSDH). Persistent inactivation of 11β-HSDH putatively caused by epigenetic modifications at the respective gene is followed by elevated cortisol levels in the fetal circulation and activation of the hypothalamic–pituitary–adrenal (HPA) axis as degradation of cortisol and cortisone is enabled.[93,94] This might cause epigenetic modification at the glucocorticoid receptor genes not only in the brain, but also in organs such as the lung and placenta.[95,96] Lung development was shown to be negatively affected by an imbalanced HPA axis associated with an elevated risk for lung diseases later in life.[97]

Conclusion

Epigenetic research performed within the past decade underlined the concept that chronic pathologies develop as a result of complex gene-by-environment interactions. Investigations have clearly outlined that the epigenome is a highly dynamic system that cooperates with the transcriptional network to adapt the genetic repertoire to the challenging environmental conditions around us. Lifestyles and exogenous challenges were altered within one century; compared with former times, current postmodern human habitats are characterized by a less diverse composition of commensal microbial communities, a higher burden of various xenobiotic agents, high-caloric nutrition, and multiple psychosocial stressors. Nevertheless, Westernized living conditions have significantly prolonged the individual lifespan. Starting in pregnancy, these environmental factors as a whole are capable of modifying our genetic program with risks for later allergic and autoimmune inflammation, as well as chronic conditions (Figure 3). In this context, epigenetic modifications serve as a physiological tool to transmit environmental stimuli; they serve as a scientific tool to analyze mechanisms of disease

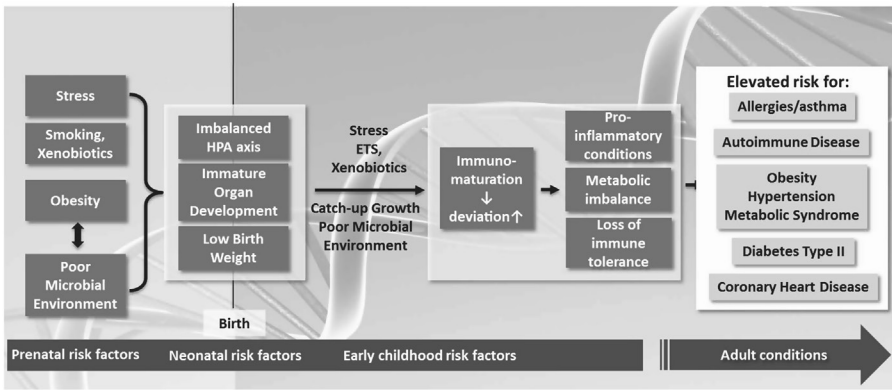


Figure 3 Environmental factors that drive allergic conditions and chronic inflammation from prenatal life to adulthood by epigenetic interactions. Maternal and postnatal detrimental factors foster an imbalanced immune response in infancy and promote chronic conditions such as allergic and autoimmune disease in adulthood.

development; and finally, they will be a future therapeutic tool to control and intervene in pathologic processes. However, before it comes to clinical intervention strategies based on epigenetic mechanisms, the currently putative connected between environmental exposures and the establishment of epigenetic marks has be understood and has to verified as causative for disease development.

Abbreviations

AHR Aryl-hydrocarbon-receptor
ALADDIN Assessment of Lifestyle and Allergic Disease during Infancy
Asthma BRIDGE The Asthma Biorepository For Integrative Genomic Exploration
5-AZA 5-Aza-2′-deoxycytidine
CCR7 chemokine receptor type 7
CD Cell determinant
CHiP Chromatin-Immunoprecopitation
CNS Intergenic Th2 regulatory region conserved noncoding sequence 1
CpG Cytosine-guanine-diphosphoester
DC Dendritic cell
DNA Deoxyribonucleic acid
DNMT DNA methyltransferase
ETS Environmental tobacco smoke
EzH Enhancer of Zeste Homolog
FOXP3 Forkhead-Box-Protein P3
GATA3 Trans-acting T-cell–specific transcription factor
HAT Histone acetyltransferase
HDAC Histone deacetylase
HDM House dust mite
HMT Histone Methyltranferase

HLX-1 H2.0-like Homeobox Protein-1
11ß HSDH 11β-hydroxy steroid dehydrogenase
IFN Interferon
IL Interleukin
IGF-2 Insulin-like Growth Factor 2
iNOS inducible Nitric Oxid Synthase
KOALA Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg
LPS Lipopolysaccharide
MHC Major Histocompatibility
MeCP2 Methyl-CpG binding protein 2
mRNA Messenger ribonucleic acid
MyD88 Myeloid differentiation primary response protein 88
NFκB Nuclear factor κB
ORMDL Orm1-like Protein
OVA Ovalbumin
PAH Polyaromatic hydrocarbon
PASTURE Protection Against Allergy Study in Rural Environments
PI3K-Akt Phosphoinositide 3-kinase activation
PM Particular matter
RAD50 DNA repair protein 50
RUNX Runt-related transcription factor
RORC RAR-related orphan receptor C
SAM s-Adenoyl-methionin
STAT6 signal transducer and activator of transcription 6
TCR T-cell receptor
Th T-helper
THF Tetrahyrdofolate
Th-POK Th-inducing POZ-Krüppel factor
TNF Tumor necrosis factor
TLR Toll-like receptor
Treg T-regulatory

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The Physiological Induction of Tolerance to Allergens

10

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Introduction

Tolerance is defined as a specific state of nonresponsiveness to a previously encountered antigen. Maintenance of a healthy immunological state requires a delicate balance between the establishment of tolerance to self or harmless exogenous antigens and the ability to mount protective immune responses against pathogens. This constant balance between tolerance and inflammation is referred to as immune homeostasis and requires strict regulation of immune responses by innate and adaptive components of the immune system. In healthy nonallergic individuals, the default immune response to innocuous exogenous antigens (such as inhaled or ingested allergens and commensal bacteria) is the induction of peripheral tolerance. Disruption or failure of peripheral tolerance results in sensitization and allergy.

The intestinal tract and lung represent the two major mucosal sites in the body in which there is direct interaction with the environment and consequently, constant exposure to a vast array of antigens and commensal bacteria. The specialized functions of these organs in facilitating absorption of nutrients and gas exchange necessitate that the mucosal lining be composed of a single layer of specialized epithelial cells, which increases vulnerability to antigen ingress and encounter with the immune system. It is therefore crucial for robust mechanisms that control inappropriate inflammatory responses to be in place in these tissues.

In both the intestine and the lung, tolerance to harmless antigens may be induced by repeated low-dose antigen exposure or by a single exposure to a high dose of antigen.[1–4] In the case of oral tolerance, repeated ingestion of low doses of antigen leads to induction of antigen-specific regulatory T cells (Treg), whereas ingestion of a high dose of antigen typically results in anergy or clonal deletion of antigen-specific T helper cells[3] (although Treg may also have a role). In airway tolerance, repeated low-dose exposure to inhaled allergen results in development of antigen-specific Treg that express cell surface membrane-bound transforming growth factor-β (TGF-β),[5] whereas high-dose exposure to inhaled allergen induces interleukin 10 (IL-10) secreting $CD4^+$ Treg.[2]

The establishment of oral tolerance and airway tolerance depends on an elaborate interplay between cells of the innate and adaptive immune systems, with both shared and unique mechanisms involved at each mucosal site. In this chapter, we will discuss the physiological induction of tolerance to allergens in the healthy nonallergic

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individual, focusing on allergens that gain entry via the intestinal or respiratory tracts, where the usual immune response is induction of tolerance.

Mechanisms of oral tolerance

The intestinal mucosa is the largest surface area of the body exposed to the external environment. The principal function of the small intestine is the absorption of dietary nutrients, which necessitates the intestinal epithelium to exist as a single monolayer of cells to facilitate the transfer of nutrients and precludes the presence of an absolute barrier to luminal contents. This characteristic also allows a vast array of environmental antigens, including dietary antigens, commensal bacteria, and pathogenic microorganisms, to interact continuously with the mucosal surface. Although it is important to respond rapidly to invading pathogens, immune responses to the myriad of dietary antigens that reach the small intestine would be inappropriate. Moreover, the intestinal tract is specifically adapted to colonization by trillions of commensal microorganisms that provide critical functions for the host, including assisting the normal development of host gut-associated lymphoid tissues (GALT), regulating oral tolerance and intestinal mucosal immune responses, and aiding in digestion of selected nutrients. Averting immune activation in response to the microbiota is therefore crucial to maintain beneficial symbiosis and avoid excessive unwarranted inflammation. Consequently, the underlying intestinal mucosal immune system must maintain immune homeostasis by appropriately guiding tolerogenic responses to dietary antigens and commensal bacteria while mounting protective inflammatory responses to pathogens. Disruption of homeostatic mechanisms could result in infection as a result of invading pathogens, uncontrolled immune activation leading to inflammatory bowel disease, or inappropriate responses to food antigens leading to food allergy.

The induction of antigen-specific tolerance within the GALT is known as oral tolerance. Wells and Osborne first described oral tolerance in 1911, when they observed that guinea pigs failed to develop anaphylaxis to ingested dietary antigens.[6] It was later shown that oral tolerance is an active immunological process that can be conferred by populations of T cells, and not simply the absence of an immune response to orally administered antigens. Adoptive transfer of $CD4^+$ or $CD8^+$ T cells harvested from mice previously tolerized to an antigen through oral feeding conferred antigen-specific tolerance in recipient naïve mice.[7,8] In the 20 years since, significant progress has been made in understanding the immune mechanisms that regulate physiological tolerance to ingested antigens and commensal organisms. It is now recognized that a wide range of cells within the intestine contribute to induction and maintenance of oral tolerance, including epithelial cells, macrophages, dendritic cells, and induced Treg (iTreg).

Intestinal epithelial cells

The intestinal epithelium forms a dynamic barrier that protects the underlying intestinal immune system from encounter with luminal antigens and microorganisms. Exclusion of antigens and microorganisms represents an important mechanism for

maintenance of immune homeostasis in the intestine. In addition, epithelial cells possess immunoregulatory functions that can direct mucosal immune responses toward tolerance or inflammation.

Although most cells lining the intestinal lumen are absorptive enterocytes adapted for metabolic and digestive function, other specialized intestinal epithelial cells, including goblet cells and Paneth cells, contribute to frontline barrier function. Goblet cells synthesize secretory mucin glycoproteins (MUC2) and bioactive molecules such as epithelial membrane-bound mucins (MUC1, MUC3, and MUC17), trefoil factor peptides (TFF), resistin-like molecule β, and Fc-gamma binding protein (Fcγbp).[9] The most abundant of the mucins secreted by goblet cells is MUC2, which plays an important role in organizing the intestinal mucous layers at the epithelial surface. MUC2 forms trimers that crosslink with TFF3 and Fcγbp resulting in a highly viscous extracellular layer. This mucus layer forms the frontline of innate host defense by excluding intestinal antigens from encounter with host immune cells. Moreover, colonization by commensal intestinal microbiota is limited to an outer loose mucus layer, whereas an inner adherent mucus layer is largely devoid of bacteria. The finding that MUC2-deficient mice develop spontaneous colitis and have increased susceptibility to inflammation-induced colorectal cancers supports the crucial role of goblet cell mucins in maintaining physiological oral tolerance.[10] Epithelial barrier function is further reinforced by Paneth cell (and to a lesser extent, enterocyte) production of antimicrobial compounds, which have a role in maintaining tolerance to commensal microorganisms. Paneth cells are uniquely capable of secreting compounds such as defensins, cathelicidins, and lysozyme that disrupt pathogenic properties of bacteria.[11] In addition, Paneth cells and enterocytes produce C-type lectin regenerating islet-derived protein IIIγ, which has a function similar to MUC2 in excluding bacteria from the epithelium.[12] Another important function of intestinal epithelial cells relevant to intestinal immune homeostasis is the transport of secretory immunoglobulin A (IgA) across the epithelial barrier. Secretory IgA antibodies offer a first line of antigen-specific immune defense by binding to luminal antigens, thereby limiting access of antigens to the mucosal surface. Dimeric IgA complexes produced by plasma cells in the lamina propria are bound by the polymeric immunoglobulin receptor expressed on the basolateral membrane of intestinal epithelial cells and are actively transcytosed to the intestinal lumen.[13]

In addition to barrier actions, intestinal epithelial cells exert immune regulatory functions that guide responses toward tolerance or inflammation. There is close crosstalk between intestinal epithelial cells and antigen-presenting mononuclear phagocytes and dendritic cells (DCs), allowing these populations to coordinately direct appropriate homeostatic responses. Intestinal epithelial cells produce immunoregulatory cytokines and growth factors (such as thymic stromal lymphopoietin, TGF-β, and retinoic acid) in response to pattern recognition receptor (PRR) signaling by commensal bacteria that promote the development of DCs and macrophages with tolerogenic properties.[14,15] Specialized intestinal epithelial cells (microfold cells [M cells] and goblet cells) also can directly sample luminal antigens for presentation to underlying mucosal immune cells; these pathways of antigen encounter represent major avenues for induction of tolerogenic responses to ingested antigens and are discussed in the next section.

Antigen sampling from the intestinal lumen

Luminal antigens can be sampled without disrupting the epithelial barrier via a number of pathways (Figure 1(A–D)). Recent studies suggest that each of these pathways may preferentially sample antigens with different characteristics and selectively direct immune responses toward either inflammation or tolerance.

Transcytosis of antigen by M cells is the best-studied pathway of antigen sampling (Figure 1(A)). M cells are specialized epithelial cells most commonly found in the follicle-associated epithelium overlying Peyer's patches (PP) and isolated lymphoid follicles (ILF).[16] Scattered M cells may also be present in the villous epithelium.[17] The localized development of M cells overlying PP and ILF is determined by interaction between receptor activator of nuclear factor-κB (RANK) ligand, which is selectively expressed by stromal cells within PP and ILF, and RANK, which is expressed by all epithelial cells in the small intestine.[18] M cells continuously transcytose antigens across the small intestinal epithelium by pinocytosis, macropinocytosis, and receptor-mediated endocytosis.[19,20] Particulate antigens such as commensal bacteria are preferentially sampled by M cells and delivered to CD103$^+$ DCs within PP and ILF, which in turn promote IgA responses via both T-cell-dependent and T-cell-independent mechanisms.[21] CD103$^+$ DCs induce B-cell IgA class-switch and expression of gut-homing receptors on B cells through the production of cytokines and retinoic acid, respectively, resulting in the migration of IgA-producing B cells to the intestinal tissues.[22] Hence, under homeostatic conditions, M cells primarily mediate transport of commensal bacteria across the intestinal epithelium and facilitate the induction of antigen-specific IgA responses that support tolerance.

Recently, a novel mechanism of *antigen sampling by goblet cells* that preferentially promotes the induction of tolerogenic responses to soluble antigens has been described[23] (Figure 1(B)). Small soluble antigens can enter goblet cells and pass across the epithelium to DC in the underlying lamina propria, through so-called goblet cell-associated passages (GAPs).[23] These GAPs favor the transport of low-molecular-weight soluble antigens such as dietary proteins and selectively deliver antigens to lamina propria CD103$^+$ DC.[23] Mutant mice that lack goblet cells, and therefore GAPs, have markedly reduced luminal antigen delivery to lamina propria DC, which indicates that GAPs are a major pathway for passage of luminal antigens to lamina propria DC.[23] Because CD103$^+$ DCs are known to induce tolerogenic iTreg and IgA-producing B cells with gut-homing properties (see next section), it is likely that antigen sampling through GAPs represents an important pathway for induction of homeostatic tolerance to dietary antigens.

CX3CR1$^+$ DC within the lamina propria are capable of directly sampling luminal antigens by extending dendrites across the epithelium, so-called transepithelial dendrites[24] (Figure 1(C)). Expression of tight junction proteins such as occludin, claudin 1, and zonula occludens 1 allow dendrites to extend between epithelial cells into the intestinal lumen while preserving the integrity of the epithelial barrier.[25] Dendritic cell transepithelial dendrites are formed in response to Toll-like receptor (TLR) signaling, and this process is regulated by CX3CL1/fractalkine expressed on epithelial cells.[24] Studies suggest that transepithelial dendrite antigen sampling is primarily

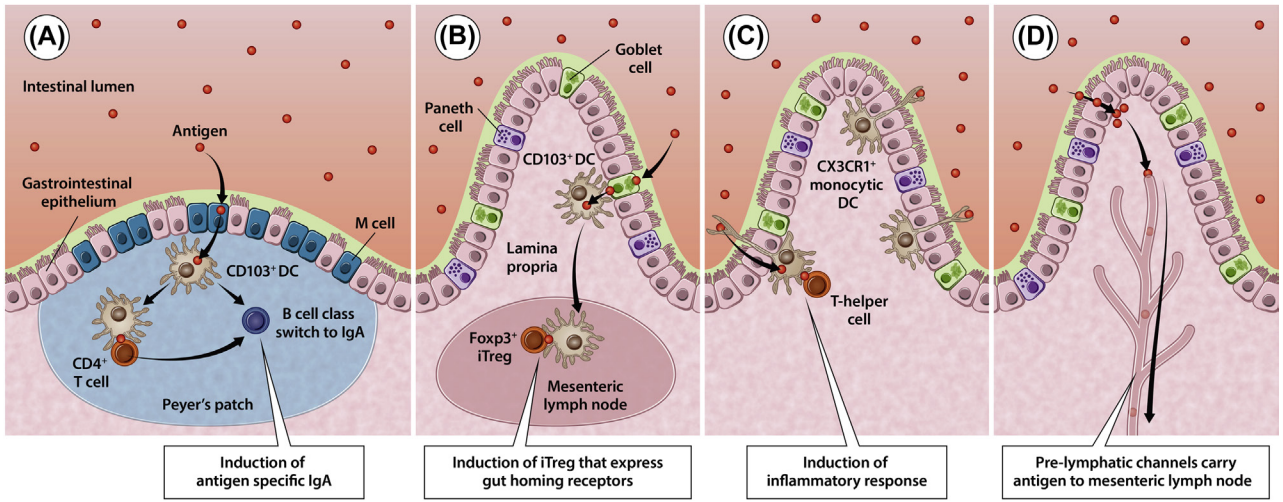


Figure 1 Pathways of antigen sampling from the intestinal lumen. (A) Specialized M cells. M cells situated in the follicle-associated epithelium overlying Peyer's patches and isolated lymphoid follicles preferentially sample particulate antigens and commensal bacteria. Antigen is delivered to CD103⁺ DCs that induce antigen-specific IgA responses. (B) Goblet cell-associated pathways. Goblet cells preferentially sample soluble antigens such as dietary antigens and deliver these to lamina propria CD103⁺ DC. CD103⁺ DCs migrate to the mesenteric lymph nodes, where they induce differentiation of iTreg with gut-homing properties. (C) Transepithelial dendrites. CX3CR1⁺ monocytic DCs extend transepithelial dendrites between epithelial cells into the intestinal lumen that preferentially sample pathogenic bacteria and induce differentiation of T-helper cells within the lamina propria. (D) Paracellular leak. Small soluble molecules can pass through pores in tight junctions between epithelial cells. Antigen accumulates briefly beneath the epithelium before being cleared through lymphatic channels to the mesenteric lymph nodes.

activated in response to pathogens and predominantly promotes protective inflammatory responses. For example, DC transepithelial dendrite formation is virtually absent in the healthy intestine,[23] with numbers increasing after infection or TLR signaling.[26] Furthermore, CX3CR1$^+$ DCs preferentially induce TH17 inflammatory responses.[27] Dendritic cells within Peyer's patches are also capable of forming transepithelial dendrites that extend between M cells to capture pathogenic bacteria or particulate antigens.[28] Taken together, these observations suggest that the principal functions of CX3CR1$^+$ DCs are to capture pathogenic bacteria from within the intestinal lumen and initiate protective inflammatory responses before pathogens breach the epithelial barrier.

A fourth mechanism of antigen sampling in the intestine is *paracellular leak around epithelial cells* (Figure 1(D)). The epithelial layer maintains its barrier properties by forming tight junctions between cells. Most tight junctions are tightly sealed so that only small molecules less than 4 Å are able to pass through the pores in claudin proteins.[29,30] Paracellular leak can occur if larger pores form in the tight junctions as a result of expression of altered claudin proteins.[30] Paracellular leakage of low-molecular-weight molecules has been observed in healthy intestine,[23] whereas intestinal inflammation is associated with transit of larger macromolecules such as proteins and carbohydrates.[30] In the healthy homeostatic state, paracellular leakage of low-molecular-weight soluble antigens leads to transient collection of antigen just beneath the epithelium, followed by rapid clearance through prelymphatic channels and lacteals to the draining mesenteric lymph nodes, where antigen processing by resident DCs proceeds. In contrast to M cells and GAPs, paracellular leakage preferentially allows passage of smaller soluble antigens (less than 10 kD in size), which are not efficiently captured by lamina propria DC.[23] It is currently not known whether antigens sampled by this pathway lead to inflammatory or tolerogenic immune responses within the mesenteric lymph nodes.

Antigen-presenting cells

There are two distinct populations of antigen-presenting cells (APCs) within the intestinal lamina propria, the CD11c$^+$CD103$^+$ DC and the CD11c^{lo}CX3CR1$^+$ monocyte-derived DCs (more recently referred to as CX3CR1$^+$ resident intestinal macrophages).[31–33] CD103$^+$ DCs are migratory cells that traffic to mesenteric lymph nodes, where they present soluble antigens to naïve T cells and induce differentiation into Foxp3$^+$ iTreg through a TGF-β and retinoic acid-dependent mechanism.[34,35] The retinoic acid produced by CD103$^+$ DCs is also responsible for imprinting gut-homing properties on iTreg, inducing expression of CCR9 and α4β7, which mediate recirculation of iTreg back to the small intestine lamina propria.[36–38] CD103$^+$ DCs are also found in Peyer's patches and intestinal lymphoid follicles, where they induce B cell IgA class-switching and imprint gut-homing receptor expression on B cells through the production of cytokines and retinoic acid.[22] Therefore, CD103$^+$ DC play a critical role in inducing tolerogenic responses under homeostatic conditions, directing the development of iTreg and IgA-producing B cells that home to the intestinal lamina propria where they exert their tolerogenic effects (Figure 2).

In contrast, CX3CR1$^+$ monocytic DC have a dominant role in initiating immune responses to pathogens while supporting tolerogenic responses within the lamina propria. CX3CR1$^+$ monocytic DC lie in close physical contact with intestinal epithelial cells and lack migratory capacity in the steady state. These cells express tight junction proteins that allow the formation of transepithelial dendrites that protrude into the lumen to sample exogenous antigens[24] (Figure 1(C)). In addition, CX3CR1$^+$ monocytic DC phagocytose

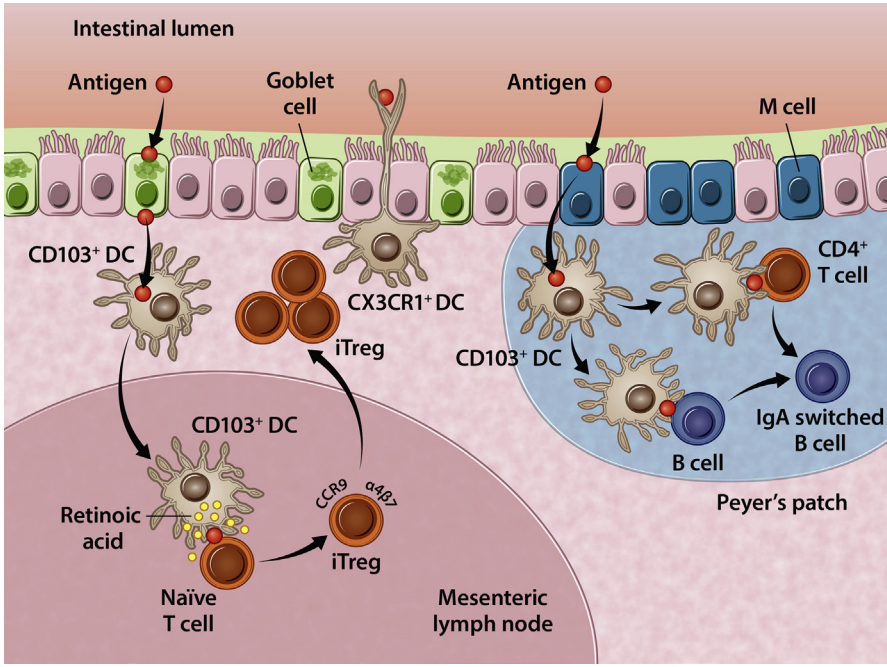


Figure 2 Mechanisms of physiological tolerance to ingested antigens. The intestinal epithelium and mucus barrier form the frontline of defence in supporting physiological tolerance to ingested antigens by excluding antigen encounter with underlying mucosal immune cells. The major pathway for induction of physiological tolerance to ingested antigens involves the coordinated actions of goblet cells, migratory CD103$^+$ DC, and iTreg. Soluble dietary antigens are preferentially sampled by goblet cell-associated passages and delivered to lamina propria CD103$^+$ DC. CD103$^+$ DC traffic to mesenteric lymph nodes by a CCR7-dependent mechanism, where they induce differentiation of FoxP3$^+$ iTreg and imprint expression of gut-homing receptors (CCR9 and α4β7) on iTreg. Induced Treg migrate back to the intestinal lamina propria, where they exert their suppressive effects. CX3CR1$^+$ monocytic DCs that are resident in the lamina propria provide additional signals that lead to expansion of iTreg populations within the lamina propria. Dietary antigens may also be sampled by M cells overlying Peyer's patches and delivered to Peyer's patch CD103$^+$ DC that induce IgA class switch of B cells (through T-dependent and T-independent mechanisms) and expression of gut-homing receptors (through production of retinoic acid), allowing IgA-producing B cells to migrate back to the lamina propria. Because food-specific IgA antibodies are generally not detected in healthy nonallergic individuals, this pathway is likely to be less relevant for induction of physiological tolerance to dietary antigens.

pathogens that traverse the epithelial barrier.[24] Upon capture and processing of pathogens, CX3CR1$^+$ DC present antigen to lamina propria T cells inducing T helper inflammatory responses.[27] Conversely, CX3CR1$^+$ monocytic DCs contribute to the induction of physiologic tolerance by producing IL10 and promoting the survival and expansion of iTreg that have homed to the intestinal lamina propria from mesenteric lymph nodes[39] (Figure 2).

Induced regulatory T cells

CD103$^+$ DCs that arrive in the mesenteric lymph node provide signals to naïve T cells that induce FoxP3 expression and differentiation to iTreg. At the same time, CD103$^+$ DC imprint expression of gut-homing receptors on iTreg that mediate migration of iTreg to the intestinal lamina propria where suppressor functions are exerted (see previous section). Intestinal FoxP3$^+$ iTreg are critical for inducing and maintaining oral tolerance. It was shown that mice previously tolerized to ovalbumin by oral feeding experienced breakdown of tolerance after depletion of FoxP3$^+$ iTreg.[39] Consistent with this, iTreg are the predominant Treg population within the intestinal lamina propria, with only a minority being nTreg. Moreover, nTreg do not appear to be required or sufficient for successful oral tolerance induction.[40] Nevertheless, a study suggests that nTreg within the intestine may have an important role in tolerance induction to commensal microorganisms.[41]

Migration of iTreg back to intestinal tissues is necessary for inducing and maintaining tolerogenic effects. Absence of CCR9 and α4β7 expression (which mediate iTreg migration to the intestinal lamina propria) results in failure of oral tolerance to ingested antigen.[42] A GPR15-dependent pathway (independent of retinoic acid induced α4β7 and CCR9 expression) was reported to regulate homing of iTreg to the colon.[31] The absence of GPR15 was associated with reduced iTreg migration to the colon but did not affect entry into the small intestine.[31] This pathway may primarily regulate colonic migration of iTreg involved with tolerance to commensal microorganisms. Other major classes of Treg have been identified to contribute to regulation of oral tolerance in various disease models, including TGF-β–secreting (LAP$^+$) Th3 cells, IL10-secreting Tr1 cells, and CD8$^+$ Treg[43]; however, their role in the induction of physiological oral tolerance to allergens remains to be determined.

Mechanisms of airway tolerance

Similar to the intestinal tract, the lung has a large surface area that is in contact with the external environment, and hence is constantly exposed to a diverse range of antigens. Although all inhaled particles are potentially immunogenic, most do not represent a threat to the individual. Mechanisms that promote tolerance are therefore essential to avoid constant induction of de novo immune responses that would lead to chronic inflammation. Control of unwarranted immune responses is especially critical in the lung because any degree of inflammation affecting the alveolar epithelium can compromise gas exchange. At the same time, tolerance mechanisms must be tightly regulated to allow effective immune responses to pathogens. A complex network

composed of the pulmonary epithelium, resident lung macrophages, pulmonary DCs, and Treg cells controls the balance between induction of tolerance to environmental antigens and protection against aeropathogens. The key events that mediate airway tolerance are shown in Figure 3 and are explained below.

The pulmonary epithelium and overlying mucociliary layer provide a physical barrier between the internal tissues and external environment. The epithelium

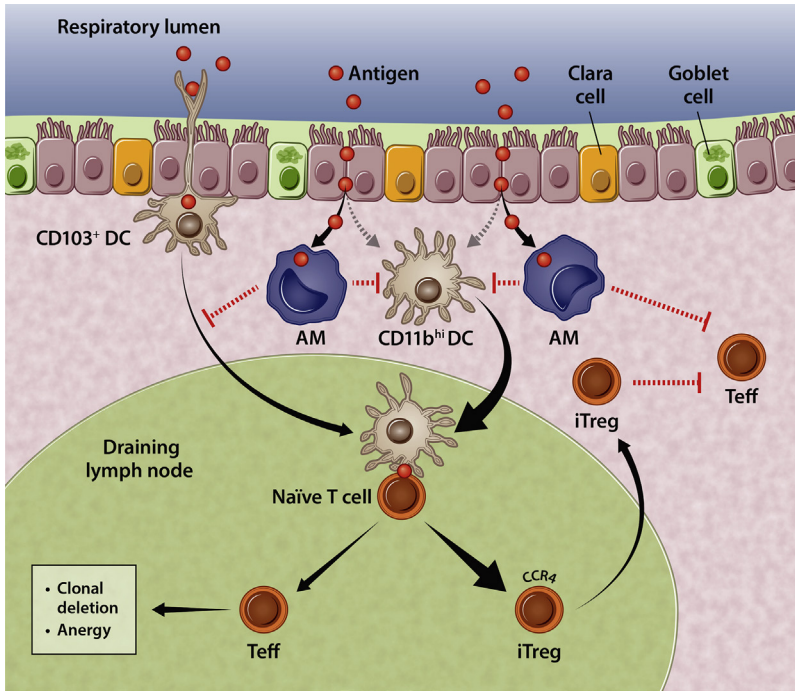


Figure 3 Mechanisms of physiological tolerance to inhaled antigens. The mucociliary layer and epithelium provide a barrier against entry of antigens into the airway and lung tissue. Alveolar macrophages (AM) rapidly phagocytose antigens that traverse the airway epithelium, thereby limiting antigen encounter with airway DC. Two major populations of migratory DCs can be identified in the lung: $CD103^+$ DCs that lie close to the epithelium and $CD11b^{hi}$ DC that are found within the interstitial tissue. $CD103^+$ DCs extend dendrites between epithelial cells into the airway lumen and directly sample inhaled antigens. $CD11b^{hi}$ DC capture antigens that have entered the lamina propria by passage between epithelial cells. $CD11b^{hi}$ DCs have a greater capacity to migrate to draining lymph nodes than $CD103^+$ DCs. Within the draining lymph nodes, DCs induce differentiation of $FoxP3^+$ iTreg that express CCR4. Induced Treg migrate back to the lung (by a CCR4-dependent mechanism), where they exert their suppressive effects. This represents the major pathway for induction of physiological tolerance to inhaled allergens. Dendritic cells may also induce T-helper/T-effector cells (Teff), leading to a transient T-helper 2 response; however, under homeostatic conditions, Teff rapidly undergo clonal deletion and/or anergy. Alveolar macrophages have additional actions that support homeostasis, including inhibition of antigen processing by interstitial DC, inhibition of DC migration to draining lymph nodes, and suppression of T-effector cells within the lung.

is composed of ciliated columnar, mucus-secreting goblet cells, and Clara cells that secrete surfactant, which adhere together via tight junctions localized at the apical cell surface. Antigens that overcome the mucociliary barrier can be sampled by epithelial cells through PRR, including the TLR, nucleotide-binding oligomerization domain–like receptors and protease-activated receptors, and C-type lectins, which recognize pathogen-associated molecular patterns from viruses, bacteria, fungi, protozoa, and multicellular parasites. Upon activation of PRR, pulmonary epithelial cells produce cytokines and chemokines that lead to recruitment of cells of the innate and adaptive immune system resulting in inflammation. In a mouse model, it was shown that epithelial cells can present self-antigen to naive T cells and induce Foxp3$^+$ Treg cells via a TGF-β–dependent pathway,[44] although it is not known whether a similar capacity for induction of Treg exists for presentation of exogenous antigens.

Macrophages resident in the conducting airways and lung alveolar spaces have an important role in regulating steady-state homeostasis. Alveolar macrophages are found immediately below the epithelium in both the conducting airways and alveoli as well as within the airway spaces. Their primary role is to phagocytose and sequester antigens, thereby limiting antigen capture (and processing) by DC.[45] In the absence of danger signals, alveolar macrophages are kept in a hyporesponsive state by close tethering to epithelial cells,[46] binding of surfactant proteins to the inhibitory receptor signal regulatory protein-α,[47] and ligation of surface CD200 receptors by CD200 expressed on the luminal surface of airway epithelium.[48] With ensuing inflammatory signals from epithelial cells, macrophage tethering is reversed, allowing phagocytosis and secretion of proinflammatory cytokines. Studies suggest that different subpopulations of lung macrophages may regulate progression to either a proinflammatory response or tolerance, with the lung interstitial macrophages having a unique capacity to inhibit DC maturation after TLR stimulation, thereby promoting tolerance and preventing sensitization to allergens.[49] Alveolar macrophages can also suppress DC antigen presentation[50] and migration to regional lymph nodes,[51] as well as T-cell responses.[52] In coculture experiments, purified human alveolar macrophages were shown to actively induce antigen-specific CD4$^+$ T-cell unresponsiveness (functional inactivation) and inhibit CD4$^+$ T-cell response to secondary stimulation.[52] This effect was independent of cytotoxic T-lymphocyte–associated protein 4 triggering or Fas-induced apoptosis.[52] Hence, macrophages can minimize unwarranted lung tissue inflammation by limiting unnecessary immune responses from DC and T cells (Figure 3).

The conducting airways of the lung contain a dense network of resident and migratory dendritic cells within and below the epithelium. Pulmonary DC sample antigens in the lung and then migrate to local draining lymph nodes, where they present antigen to naïve T cells and direct the induction of either tolerogenic or helper/effector immune responses. Most inhaled antigens that reach the lung-draining lymph nodes have been trafficked there by migratory pulmonary DC.[51,53] There are two migratory DC subsets in the lung, CD11c$^+$CD11b^{lo}CD103$^+$ DC and CD11c$^+$CD11b^{hi}CD103$^-$ DC. CD103$^+$ DC primarily reside in the conducting airways closely associated to the epithelium and have a greater capacity to sample luminal antigens than CD11b^{hi} DC. The subepithelial location of CD103$^+$ DC is mediated by interaction of CD103 (αε integrin) and β7 integrin with E-cadherin

expressed basally by epithelial cells. The more efficient sampling of antigens by $CD103^+$ DC results from a greater expression of several tight junction proteins including claudin-1, claudin-7, and Z0-2 that allow cellular protrusions to extend through the epithelial barrier into the airway lumen. In contrast, $CD11b^{hi}$ DC are predominantly located in the lung parenchyma, within the peribronchial and interstitial tissues, and have a greater capacity to migrate to regional draining lymph nodes than $CD103^+$ DC.[51]

Depending on the nature of the antigen (innocuous versus pathogen) and the environment in which an antigen is sampled and presented by migratory DC to naïve T cells in the draining lymph nodes, DC may promote either tolerogenic or proinflammatory/T-helper responses. Under steady-state conditions, sampling of innocuous antigens leads to induction of iTreg that facilitates development of antigen-specific tolerance (Figure 3). There may also be some limited induction of T-helper responses, but this is followed by rapid progression to clonal deletion and anergy. In contrast, the presence of pathogen-derived substances, tissue damage, or adjuvants leads to activation of migratory DC with expression of costimulatory molecules and cytokines that promote the development of antigen-specific T-helper or T-effector cells, resulting in inflammation. Although $CD103^+$ DCs are the major tolerogenic DCs in the gut, it is unclear whether the same is true for the induction of airway tolerance.

The induction of $Foxp3^+$ iTreg in the local draining lymph nodes and their migration back to the lung represent an important mechanism for airway tolerance to inhaled allergens. After DC-induced differentiation and activation of iTreg within the lymph nodes, iTreg acquire expression of CC chemokine receptor 4 (CCR4), which mediates migration back to the lung.[54] Recruitment of iTreg to lung tissues occurs rapidly (within 24 h) after allergen challenge.[55] The suppressive effects of iTreg are exerted only once they return to the lung, and migration depends on CCR4.[56] CCR4 deficiency results in failure of iTreg accumulation within the lung after inhaled allergen exposure and severe lymphocytic infiltration of the lungs.[54] Similarly, adoptive transfer studies in mice support the role of CCR4 ligands, CCL17 and CCL22, in the recruitment and retention of Treg cells within the lung during chronic allergen challenge.[57] Retention of iTreg within the lung and maintenance of airway tolerance requires continued exposure to airborne antigens. In a mouse model of allergic airways disease, antigen withdrawal resulted in decreased numbers of Treg within the lung and reemergence of inappropriate Th2-dependent immune responses to respiratory antigens.[55]

Other cells with regulatory potential may have a role in maintaining immune homeostasis within the lung. T-helper 1–like $Foxp3^+$ regulatory cells[58] and $CD8^+$ T cells[59] have suppressor effects in animal models, likely mediated by production of IL10. A population of B cells within local draining hilar lymph nodes has been shown to migrate to sites of inflammation within the lung and induce accumulation of $Foxp3^+$ Treg, possibly through TGF-β–mediated differentiation of naïve $CD4^+CD25^-$ effector T cells into $Foxp3^+$ Treg.[60] Nevertheless, the roles of these cells in the induction of physiological airway tolerance to inhaled antigens remain to be determined.

Mechanisms of action of iTreg in mediating oral and airway tolerance

Peripheral induction of antigen-specific Treg cells represents the fundamental mechanism leading to both oral and airway tolerance. Induced Treg have broad-ranging effects including inhibition of T-helper cell differentiation and inhibition of effector cell, APC, and innate cell function; however, the specific mechanisms by which they control immune responses are still not completely understood. Induced Treg use multiple pathways to control inflammatory responses, and it is likely that no single mechanism is responsible for the full range of biological functions. Moreover, the suppressive mechanisms that are operational in a particular tissue environment will depend on the diverse biological context in which tolerance functions are activated.

Several mechanisms for iTreg suppressor function have been demonstrated. Induced Treg can mediate their effects via both cell–cell contact and secretion of cytokines. Cell surface-bound TGF-β and secreted IL10 are key factors mediating suppressor activity, whereas expression of inhibitory molecules such as CTLA-4 and PD1 is also involved.[61] Antigen specificity does not appear to be essential for in vivo suppression by airway iTreg cells,[62] and potent nonspecific inhibitory effects on APCs and effector cells may be mediated by secreted IL10.[62,63] Induced Tregs can engage costimulatory receptors on the surface of APCs, resulting in weak or negative signals to naïve and effector T cells. Induced Tregs have also been demonstrated to exert direct cytotoxic actions via production of granzyme B/perforin and induction of apoptosis in effector cells and DCs under certain conditions.[64,65] Induced Tregs may also compete directly with effector cells for APC cytokines such as IL-2, thus limiting the effect of these signals on effector cells.[66]

Early-life exposures can influence immune programming of tolerance

In keeping with the theme of the first 1000 days of life, studies suggest that early-life (prenatal and postnatal) environmental exposures can influence immune programming and affect the individual's capacity to achieve and maintain immune homeostasis.

Exposure of mothers during pregnancy (but not after delivery) to a farming environment rich in microbial compounds was associated with increased gene expression of pathogen recognition receptors, TLR2, TLR4, and CD14 in peripheral blood cells and reduced allergic sensitization at school age in offspring,[67] which suggests that prenatal environmental exposures can modify the subsequent capacity for appropriate tolerance responses to allergens. In addition, reduced maternal intake of vitamin D was associated with increased risk of asthma in children age 3–5 years.[68] Infants who later develop egg allergy were reported to have impaired Treg function at birth,[69] which

suggests that impaired tolerance responses at birth (which may reflect prenatal events and/or genetic factors) can result in disruption of immune homeostasis and susceptibility to failure of oral tolerance. In mice, exposure of pregnant mothers to aerosolized endotoxin and continued postnatal exposure of offspring pups (but not prenatal exposure on its own) prevented subsequent allergen sensitization in pups.[70] Taken together, these studies support the suggestion that prenatal and early postnatal exposures can influence immune tolerance responses at the time of birth and in later infancy.

The establishment of a healthy intestinal microbiota is essential for the normal development of GALT and programming of appropriate mucosal tolerance responses in the lung and intestine. Acquisition of the intestinal microbiota commences at the time of birth and evolves rapidly over the first 6 months to stabilize around the second year of life.[71] Absence of an intestinal microbiota in germ-free mice is associated with abnormal anatomical development of the GALT,[72–75] reduced number and function of intestinal iTreg,[76,77] immune dysregulation with Th1/Th2 imbalance,[74,78] and failure of oral tolerance induction.[78] Seeding of a single bacteria species (e.g., *Bacteroides fragilis*, *Bifidobacterium infantis*, or segmented filamentous bacteria) into the colon can reverse these abnormalities, but only if this occurs in the early postnatal period.[72–75,78] In line with these observations in mice, alterations in infant intestinal microbiota composition in the first weeks of life have been associated with later development of sensitization and allergic disease (eczema, asthma, and food allergy) in the first years of life.[79–81] These findings highlight the important role of the intestinal microbiota in regulating mucosal tolerance and immune homeostasis in the lung and intestine. The critical role of the microbiota in regulating immune responses is discussed in more detail in the following chapter.

Summary

The mucosal lining of the intestinal tract and lung represent major sites of interaction with the external environment, and an immense array of ingested or inhaled antigens as well as commensal microorganisms are continually in contact with the intestinal and airway epithelium. The induction of physiological tolerance to harmless antigens and commensal microorganisms is vital to avoid unwarranted inflammation; yet, the ability to mount efficient inflammatory responses to potential pathogens remains important. This dynamic balance between physiological tolerance to allergens and commensal microorganisms versus inflammatory immune responses to pathogens is coordinately regulated by innate and adaptive components of the mucosal immune system. Interactions among epithelial cells, antigen-presenting cells and peripheral iTreg promote the development of physiological tolerance, with iTreg playing a critical role. Although the past decade has revealed significant insights into the mechanisms of oral and airway tolerance, many aspects remain poorly understood. Improved understanding of the function, regulation, and cross-talk between cell populations involved in peripheral tolerance induction will undoubtedly facilitate the development of novel strategies for treating or preventing allergic disease.

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Microbiome and the Effect on Immune Response

11

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Introduction

Allergic diseases have become a major public health problem in affluent societies.[1,2] Because changes in the genotype cannot explain such a rapid increase in the prevalence of allergy, the loss of protective factors or the appearance of risk factors in the environment must contribute to the increased prevalence of these diseases since the middle of the last century.[3] In 1976, the Canadian pediatrician John Gerrard and coworkers suggested that allergic disease is the price paid for relative freedom from diseases caused by viruses, bacteria, and helminths.[4] This hypothesis was based on the observation of low-allergy prevalence in indigenous populations in northern Canada, where helminth infection and untreated viral and bacterial diseases were common, compared with Caucasian Canadians living in urban environments. It was not until 1989 that the notion that infections early in life might prevent allergy development raised general interest, when David Strachan demonstrated an inverse relationship between the risk of hay fever and the number of older siblings.[5] The term "hygiene hypothesis" was coined to describe the association between a high-allergy prevalence and reduced infection incidence due to hygienic conditions. To emphasize the role of exposure to nonpathogenic beneficial microbes, "the microbial deprivation hypothesis" is a more appropriate name, however.[6] Because other immune-mediated diseases such as multiple sclerosis, type 1 diabetes, and Crohn disease also show rising incidences in economically developed countries,[7–9] a theory of microbial deprivation syndromes of affluence has been proposed.[6] This chapter will review microbiome and immune development and show how a reduced intensity and diversity of microbial stimulation may result in an abnormal immune maturation during childhood.

Coevolutionary interdependency of the microbiome and the immune system

No animal has evolved independently of microbial symbionts.[10,11] Most symbiotic relationships display various degrees of mutualism, in which both organisms benefit, but the

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associations can also be commensal, in which one organism benefits while the other is unaffected.[11,12] When the symbiont displays pathogenic properties and benefits while the host is harmed, the relationship is termed parasitic.[11,12] Microbes were the only life form on earth for most of its history, with multicellular life arising in a world dominated by prokaryotes.[10,11] Intimate, complex, and dynamic interactions between animal hosts and microbes have profoundly influenced animal evolution, and still do.[10,11] Nearly half a billion years of coevolution with vertebrates have reciprocally shaped the repertoires of the microbial symbionts and the immune system.[12–14] According to the hologenome concept, the holobiont (the host plus all of its symbiotic microorganisms) with its hologenome (the sum of the genetic information of the host and its symbiotic microorganisms) is considered a unique biological entity and a unit of selection in evolution.[11] The microbiota is the aggregate of all microorganisms in a holobiont,[11] whereas the microbiome is defined as the sum of genetic information of the microbiota.[15] The microbiome can change more rapidly and by more processes than the host organism alone, thus influencing the adaptation and evolution of the holobiont.[11] Our modern and affluent lifestyle reduces exposure to microbes with which we have coevolved, however, leading to establishment of microbial communities differing substantially from those of our ancestors.[16–18]

The immune system shapes homeostasis within the holobiont, allowing a mutualistic partnership, with the microbiota providing digestive and protective advantages to the host in a sheltered environment with abundant nutrition, whereas the pathogenic and invasive behavior of microbes evokes eliminatory measures.[11,12] It is likely that our immune system has evolved as much to preserve and protect beneficial microbes as to fend off pathogens.[13,14,19,20] In vertebrates, the evolution of an adaptive immune system, with the capacity to recognize and remember both beneficial and pathogenic microbes, provided opportunities for more sophisticated relationships with increasingly complex microbial communities, because innate inflammatory mechanisms could be both suppressed and promoted.[13,14,19–21] The number of bacteria in the human intestine is estimated to be around 100 trillion (10^{14}), outnumbering the host cells by a factor of 10 or more.[22,23] The mammalian intestine is the ecological site on earth with the highest density of bacteria.[22] Humans and other vertebrates rely on gut symbionts to perform crucial physiological functions such as digestion of complex dietary fibers and production of certain vitamins and metabolites, thus increasing the ability of the host to extract nutrients from food by providing the metabolic benefit of millions of additional genes and activities.[24] By establishing these metabolic networks, the microbiota also increases the infection resilience of the host by limiting the resources and physical niche space available to potential pathogens.[11,12,14] Synergistic interactions and cooperation between the microbiota and the host thus lead to improved fitness of the holobiont.[11,12]

Microbiome acquisition and development

The first interactions between the microbiota and the host may occur *in utero*.[25,26] Thus, evidence contradicts the sterile womb paradigm, proposing that the sterile fetus first acquires bacteria through passing the birth canal.[25,26] Any microbial presence *in utero* has been assumed to be dangerous for the fetus, and intrauterine infections can

lead to preterm birth.[27] Microbial deoxyribonucleic acid (DNA) from Lactobacilli was demonstrated by quantitative polymerase chain reaction in all 29 placental samples collected during elective cesarean section after normal-term pregnancies, however, whereas DNA from *Bifidobacterium* and *Bacteroides* was detected in more than one-third of samples.[28] Intracellular bacteria were also histologically demonstrated in the basal plate, the peripheral region of the placenta on the maternal side in contact with the uterine wall, at a similar rate in preterm and term pregnancies without overt infection.[29] Furthermore, extensive deep sequencing studies identified a low abundance but metabolically rich placental microbiome in normal healthy pregnancies at term.[26] This microbiome was primarily composed of nonpathogenic commensal microbiota from the Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, Actinobacteria, and Fusobacteria phyla.[26] Compared with the microbiomes from the vagina, gut, skin, nose, and mouth at the phylum level, the placental microbiome was characterized by a higher abundance of Proteobacteria (with *Escherichia coli* the most commonly detected species) and the unique presence of Tenericutes, including *Mycoplasma* and *Ureaplasma*.[26] Unexpectedly, the placental microbiome was more closely related to the mouth microbiome than the vaginal, gut, or other body microbiomes, which suggests that the placental microbiome could be partially established by hematogenous spread of oral microbiota.[26,27]

Although less extensively characterized, microbial DNA has also been detected during normal healthy pregnancy in amniotic fluid[28,30] and fetal membranes obtained after elective cesarean section,[31] as well as in umbilical cord blood[32] and meconium[33–36] (the neonate's first intestinal discharge, representing material ingested or secreted *in utero*, including amniotic fluid and fetal gastrointestinal epithelial cells and mucus). Potential bacterial contamination from contact between skin and the meconium was controlled for in two studies by sampling an internal portion of the meconium.[33,34] Furthermore, the meconium microbiota composition was distinct from the microbiota of vagina, feces, or skin of pregnant women.[34] Samples from amniotic fluid shared a larger relative abundance of genera with the meconium than oral and vaginal samples.[36] Some of these studies also cultured bacteria, reporting viable bacteria in umbilical cord blood[32] and meconium[33] of healthy infants. Interestingly, the meconium microbiota composition may be affected by maternal health status[34,35] and may influence offspring disease development.[34,35]

The potential mechanisms of intrauterine colonization are elusive, but the entry could take place by ascension of vaginal microbes or hematogenously through the placenta.[25,27] It has thus been suggested that maternal bacteria may travel to the placenta via the bloodstream after translocation over the gut and oral epithelium.[25,27] Dendritic cells may facilitate transport of maternal bacteria by taking up luminal bacteria intracellularly and then migrating to lymphoid organs.[25] In murine studies, transmission of orally administered labeled bacterial strains from the mother to the fetus can be demonstrated,[32] and pregnancy and lactation are associated with enhanced translocation of gut bacteria to mesenteric lymph nodes.[37] Evidence for microbial maternal transmission is becoming increasingly widespread across the animal kingdom.[25] This may provide the offspring with important microbes at birth, imprinting the offspring microbiota and immune system in preparation for the much larger inoculum

transferred during vaginal delivery[38–47] and breastfeeding[37,47–50] (Figure 1), and may have shaped the microbiome composition in animal species over evolutionary time. "Heirloom" microbes received from the mother may be uniquely evolved to the offspring's genotype, and vertical transmission compared with horizontal transmission may increase the chance for optimal mutualism.[25] If the holobiont is to be considered a unique biological entity and a unit of selection in evolution, both host and symbiont genomes must be transmitted with accuracy from one generation to the next.[11] A study among great apes demonstrated that the gut microbiota composition was phylogenetically conserved among species over evolutionary time scales and had diverged in a manner consistent with vertical inheritance.[51] Furthermore, the microbiota is shared to a greater extent between related humans than unrelated humans who cohabit, which emphasizes the impact of early colonization events on the microbiota in later life.[52,53]

During normal vaginal delivery, the neonate is inoculated with large numbers of bacteria from the mother's vaginal and gut microbiota.[38,40,54] In contrast, bacteria originating from the hospital environment and skin predominantly colonize neonates born by cesarean section.[40] Vaginally delivered infants, but not infants born by cesarean section, shared a significantly higher proportion of gut microbiota 16S rRNA gene sequences with their own mother than with other mothers for up to 2 years of age,

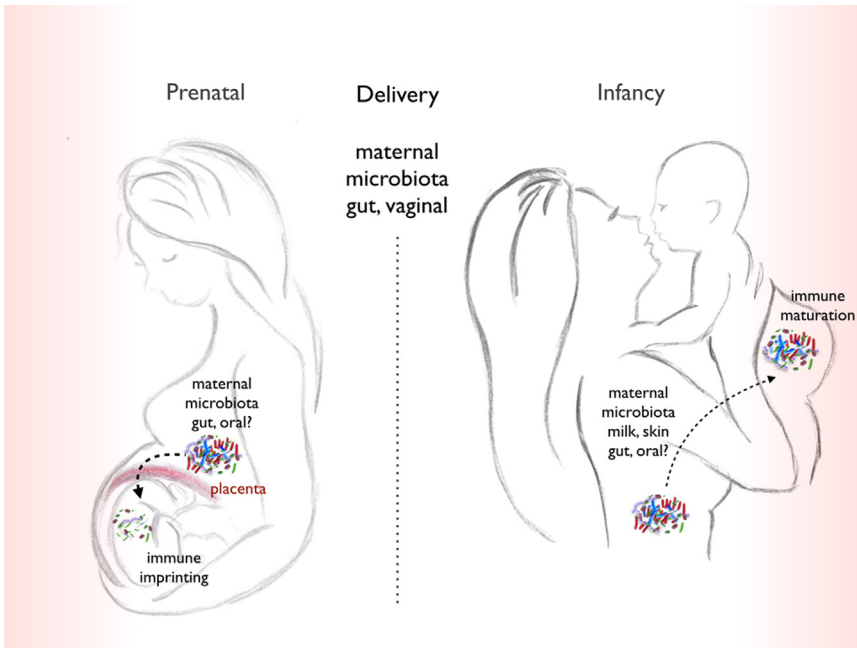


Figure 1 Transmission of microbes from the mother to the fetus during pregnancy may provide the offspring with important microbes at birth, imprinting the microbiota and immune system in preparation for the much larger inoculum transferred during vaginal delivery and breastfeeding. Heirloom microbes received from the mother may be uniquely evolved to the offspring's genotype, increasing the chance for optimal mutualism.

particularly within the Bacteroidetes and Firmicutes phyla.[38] These anaerobic phyla do not appear to grow outside the gut, and thus need to be transmitted between human hosts.[22] In another study, the vertical transmission of *Bifidobacterium* strains from maternal to infant gut during the first 3 months of life depended on normal vaginal birth.[54] Thus, cesarean section delivery, which is performed with increasing rates worldwide and may increase the risk for development of allergy, asthma, type 1 diabetes, and celiac disease,[55,56] may disrupt the opportunities for the microbiota to be transferred from a mother to the baby to provide a genetically tailored microbiota and optimal mutualism.[17,25] The potential for vertical transmission of ancestral microorganisms to the next generation is also decreased, particularly when horizontal transmission is also diminished by antibiotics, antibacterial agents, decreased family size, etc.[16–18] Delivery by cesarean section has been associated with persistent changes in the gut microbiota of children observed up to age 1,[41,42] 2,[38] and 7[57] years. The disruptions in infant gut microbial ecology caused by cesarean section delivery include a reduced abundance of the genus *Bacteroides*[38,41–46] and a decreased diversity of the Bacteroidetes phylum.[38] Increased occurrence of colonization with *Clostridium difficile*, an opportunistic pathogen, expanding when gut microbiota niches are vacant,[58] has also been reported in several studies among infants born by cesarean section.[41–43,45]

After birth, the early colonizers are accompanied by externally acquired species as the infant gut microbiota diversity increases with age.[38] During this early phase, the gut microbiota composition is highly dynamic within and between infants.[59,60] An adult-like gut microbiome may be obtained after about age 3 years,[61] although differences have been demonstrated in gut microbiota composition between adolescents and adults.[62] Because the neonatal gut initially contains oxygen, a large proportion of the early colonizers are aerotolerant facultative bacteria, including *Enterobacter*, *Lactobacillus*, *Staphylococcus*, and *Streptococcus* genera.[59,60,63] When the bacteria consume the oxygen in the intestine, strict anaerobes, including Bifidobacteria, *Bacteroides*, Eubacteria, and Clostridia, begin to dominate at age 1 week.[59,60,63]

Apart from the delivery mode, another strong influence shaping the developing infant gut microbiota relates to nutritional factors in early life. Breastfeeding provides a secondary route of maternal microbial transmission.[25,37,48–50] Thus, *Staphylococcus, Streptococcus, Lactococcus, Enterococcus, Lactobacillus*, and *Bifidobacterium* genera can be cultured from aseptically collected human breast milk, whereas culture-independent molecular techniques have also demonstrated the presence of, for example, *Bacteroides, Prevotella, Clostridium*, and *Veillonella*.[25,37,48–50] Although some of the bacteria may originate from maternal skin and infant oral cavity, the presence of anaerobic gut bacteria suggests that maternal bacteria may travel to the mammary gland via the bloodstream after gut translocation.[25,37,50] Although enteromammary transfer has not been formally proven, identical bacterial strains were demonstrated in milk cells, blood cells, and fecal samples from lactating women,[37] and gut-associated obligate anaerobic genera were shared between maternal feces, breast milk, and neonatal feces.[49] The gut microbiota of exclusively breast-fed infants is dominated by Bifidobacteria,[43] which thrive on human milk oligosaccharides.[64] Gut microbiota diversity increases after weaning and introduction of solid food, with enhanced colonization of butyrate producers, including *Bacteroides* and certain *Clostridium* species.[44,65–67] The maternal oral microbiota may have

represented another source of vertical microbial transmission during evolution. Thus, complementary feeding during lactation to provide the infant with adequate nutrition commonly involved premastication, that is, chewing up solid foods and then feeding the resulting mash to the infant.[68] Further diversification of the diet gradually increases Bacteroidetes and Firmicutes diversity and abundance toward adult levels, whereas interindividual differences decrease.[60,61] The diversity reached is less in affluent societies compared with unindustrialized rural[61,69] and hunter-gatherer[70,71] communities, however. Infection with helminths, with potent immunoregulatory and potential allergy and autoimmune protective properties, is also common in these non-Western communities, as reviewed in detail elsewhere.[72,73]

Relatively little is known about microbiome development at extraintestinal sites. Several studies employing culture-independent molecular techniques have demonstrated that the lower respiratory tract, previously believed to be sterile, harbors a diverse microbiota.[74] It would thus be highly interesting, although technically challenging, to characterize airway microbiota development during infancy and childhood. Few studies have investigated development of the skin microbiota, which may shape cutaneous innate and adaptive immune responses.[75–77] The initial skin microbiome has a low diversity across the body; vaginally delivered neonates are colonized by microbes present in the mother's vagina (e.g., *Lactobacillus* and *Prevotella*), whereas babies born through cesarean section predominantly are colonized with bacteria originating from the hospital environment and skin (e.g., *Propionibacterium*, *Staphylococcus*, and *Corynebacterium* genera).[40] The Firmicutes phylum dominates the infant skin microbiota, with diversity and site specificity increasing during the first year of life.[78] Upon sexual maturation and enhanced hormone-stimulated sebaceous gland activity, the skin becomes colonized by increased amounts of lipophilic *Corynebacterium* and *Propionibacterium* genera.[79]

Culture-independent molecular techniques have revealed that the oral microbiome represents one of the most diverse microbial habitats in humans,[80] but no longitudinal studies have characterized oral microbiota development during infancy and childhood with such methodology. Because the oral cavity is the first site of encounter between the most foreign antigens and the immune system, the composition of the developing oral microbiota deserves further detailed assessment. Facultative anaerobic genera such as *Streptococci* and *Staphylococci* initially colonize mucosal surfaces such as the tongue and the buccal and palatal epithelium in the neonate, and then open up for subsequent colonizers by environmental modifications and species-to-species interactions.[81] These subsequent colonizers include the anaerobic genera *Veillonella*, *Prevotella*, *Fusobacterium*, and *Leptotrichia*, and the aerotolerant facultative genera *Haemophilus* and *Neisseria*.[81,82] The infant oral microbiota is affected by birth mode,[47] because cesarean section delivery is associated with reduced diversity at 3 months[83] and increased colonization of certain *Lactobacillus* genera at 8 months.[84] At tooth eruption, saliva-coated hard tissues offer new epitopes for bacteria attachment, and the relative abundance of Bacteroidetes (mainly genus *Prevotella*), Veillonellaceae family, and Spirochetes increases with age.[81,85] Further detailed assessment of the developing infant gut, airway, oral, and skin microbiome in relation to immune maturation represents important future research areas.

Effects of the microbiome on immune development

The microbiota has a fundamental role in normal immune development and regulation, and interactions occurring during critical time windows of developmental plasticity may exert particularly long-lasting effects.[7,14,23,56,86,87] Germ-free mice born and raised under sterile conditions show extensive defects in the development of gut-associated lymphoid tissues, with fewer and smaller Peyer's patches and mesenteric lymph nodes than animals housed under specific pathogen-free conditions, as well as impaired development and maturation of isolated lymphoid follicles and few immunoglobulin A (IgA)-secreting plasma cells in the lamina propria.[14,23,86,87] Systemic immunological disturbances are also observed in germ-free mice, including fewer germinal centers in the spleen,[86–89] decreased numbers of splenic T-helper (Th) cells, and overproduction of Th2 cytokines.[76,87,89,90]

Recovery of IgA production, intestinal lymphoid structures, and mesenteric lymph node size and cellularity is observed after microbial colonization of the germ-free animals.[14,23,86,87,91] Furthermore, monocolonization of germ-free mice with *Clostridium*-related gram-positive-segmented filamentous bacteria, adhering tightly to the surface of epithelial cells in the terminal ileum, induces the development of intestinal isolated lymphoid follicles and tertiary lymphoid tissue[92] and profoundly enhances the number of Th17 cells in the lamina propria.[93–95] Moreover, whereas induction of Th17 cells was not observed after transfer of rat or human microbiota to germ-free mice, addition of murine-segmented filamentous bacteria expanded Th17 cells.[96] Although the rat microbiota contained segmented filamentous bacteria, they failed to colonize mice, which demonstrates the importance of host specificity.[96] The presence of segmented filamentous bacteria in the human microbiota remains to be proven.[95]

Colonic regulatory T cell numbers and function are enhanced in germ-free mice after colonization with murine microbiota,[97,98] altered Schaedler flora,[99] a consortium of 46 murine[97] or 17 human[100] *Clostridia* strains, and various *Bacteroides* strains.[101] The gut microbiota-derived short-chain fatty acids butyrate and proprionate can also stimulate peripheral regulatory T cell generation via epigenetically mediated histone deacetylase inhibition, enhancing acetylation of the murine Foxp3 locus.[102] Moreover, monocolonization of germ-free animals with polysaccharide A (PSA) producing *Bacteroides fragilis* increases the suppressive and interleukin 10 (IL-10)-producing capacity of colonic regulatory T cells and induces IL-10 production.[103] The zwitterionic PSA molecule has an unusual combination of positive and negative charges within each repeating unit and signals through TLR2 directly on $Foxp3^+$ regulatory T cells to promote immune tolerance.[104]

The neonatal immune system has thus evolved to require diverse developmental signals from microbes to mature normally.[7,23,56,105] Appropriate timing of these microbial developmental signals seems to be required for an optimal development.[7,23,56,105] Thus, several immunological abnormalities observed in germ-free mice can be reversed only if the animals are colonized with symbiotic microbes as neonates, whereas later colonization fails to reverse homeostasis. Normalization of excessive

allergen-induced Th2 and IgE responses in germ-free mice seems to require a critical colonization time window after both oral[106–108] and airway[109] exposure to allergens. Furthermore, reversal of Th2 cytokine-dependent oxazolone-induced colitis via regulation of invariant natural killer T cell homeostasis is obtained only after neonatal colonization,[77,109,110] with similar findings observed for intestinal proinflammatory responses[111,112] and lipopolysaccharide-induced proinflammatory responses in mesenteric lymph node cells.[113]

It is not clear whether a similar critical colonization time window exists in humans, but early life events occurring during critical periods of immune development can have a long-term impact on the development of immune-mediated diseases.[3,7,56,105,114–117] Even prenatal exposures may shape immune developmental trajectories. For example, maternal exposure to a traditional farm environment during pregnancy protects against allergic sensitization and disease, whereas exposures during infancy alone have weaker or no effect at all.[118–120] Prenatal pet exposure may associate with decreased IgE levels at birth and during infancy.[121,122] Furthermore, maternal antibiotic use during pregnancy has been associated with an increased risk for development of asthma in childhood[123–127] and allergic disease.[124,128] Moreover, prenatal supplementation seems to be crucial for the preventive effect of probiotics on infant eczema.[56,115–117,129–136] It is thus becoming increasingly evident that the maternal microbial environment during pregnancy is important in childhood immune programming.[3,7,56,105,115–117] The close immunological interaction between the mother and the offspring during pregnancy[7,137–139] provides enormous opportunities for the maternal microbial environment to influence the immune development of offspring, potentially via epigenetic effe cts.[7,56,105,115,116,140,141] For example, increased DNA demethylation of the Foxp3 locus in cord blood cells and enhanced neonatal regulatory T cell function was associated with maternal farm exposure during pregnancy.[142] Furthermore, the promoter regions of the Th2-associated genes *RAD50* and *IL-13* were hypermethylated, indicating decreased transcription, in neonates from farming compared with nonfarming families.[143]

The Th2-skewed state of the neonatal immune system[7,144] is likely a consequence of the intrauterine immune milieu during pregnancy.[105] The fetal–maternal interface is characterized by high levels of Th2-like[145,146] and anti-inflammatory[147] cytokines, as well as enrichment of T regulatory cells,[148] most likely functioning to divert the maternal immune response away from damaging Th1-mediated immunity toward fetal alloantigens.[149,150] This neonatal Th2-skewing is even more marked in infants later developing allergic disease,[138,144,151] which supports the idea that the prenatal immune environment can influence allergy development.[105,139] The neonatal Th2 bias should then develop toward a more balanced immune phenotype, including maturation of Th1-like responses[144,152] as well as appropriate development of regulatory T cell[7] and protective mucosal IgA responses.[153,154] Failure of Th2-silencing during maturation of the immune system may underlie development of Th2-mediated allergic disease.[7,144,155] Appropriate microbial stimulation, both prenatally and postnatally, may be required to avoid this pathophysiological process.[116,119] In this respect, the gut microbiota is

quantitatively the most important source of microbial stimulation and may provide a primary signal for the maturation of a balanced postnatal innate and adaptive immune system.[7,13,56,156–160]

Influence of the microbiome on allergy development

Childhood allergy development is associated with an altered gut microbiota during the first months of life.[45,161–170] The results from the different studies are inconsistent, however, and the microbiological techniques employed vary.[42,45,161–170] Also, assessments of stool samples reflect luminal colonic microbiota, whereas the major part of the gut immune system is situated in the ileum.[13,14,23,76,171] The proportion of facultative anaerobes such as lactobacilli is higher in the ileum than in the colon.[70,172] No specific microbes with consistently harmful or allergy protective roles have yet been identified. Thus, some but not all studies have associated later allergy development with low rates of bifidobacteria[161,162,166,167] or lactobacilli[166,167] colonization during infancy, whereas some studies have found evidence for early *Clostridium difficile* colonization as a risk factor for later allergy development.[161,163,173,174] The increased detection rate of *C. difficile* in infants later developing allergy suggests that disruptions in infant gut microbial ecology precede disease development, because this opportunistic pathogen expands when gut microbiota niches are vacant.[58]

Early establishment of a diverse gut microbiota may be more important than the distribution of specific microbial species in shaping a normal immune maturation.[7,56,58,175] Increased diversity enhances resiliency to ecological disturbances and may obviate the need for so-called keystone species, that is, species with a central role in the system whose loss causes dramatic changes.[22,58,176] Furthermore, repeated exposure to new bacterial antigens may be required for the development of proper immune function and regulation.[7,56,58,175] In support of this theory, a low gut microbiota diversity during infancy was demonstrated in children developing eczema[164,165,170] or sensitization[168] in studies using cultivation-independent molecular techniques such as denaturing gradient gel electrophoresis[165,168] or terminal restriction fragment-length polymorphism[164,170] of bacterial 16S rRNA genotypes. The diversity within specific microbial phyla or genera cannot be identified with these types of molecular methods, however, and the sensitivity appears to be low because the median number of peaks/bands was much lower than the expected number of bacterial species.[164,165,168,170] We employed more sensitive and powerful next-generation high-throughput bar-coded 16S rRNA 454-pyrosequencing, demonstrating that atopic eczema development was preceded by a low diversity of the total microbiota at age 1 month.[169] This reduced diversity was found within the bacterial phylum Bacteroidetes and the genus *Bacteroides*,[169] and *Bacteroides* colonization at 1 month was also associated with high levels of the Th1-associated chemokines CXCL10 and CXCL11 during infancy.[38] Interestingly, a Western lifestyle with a low dietary fiber intake has been associated with a reduced Bacteriodetes abundance in the gut microbiota in children[69] and adults.[70,71] Mice fed a high-fiber diet also had increased fecal Bacteroidetes abundance, leading to enhanced circulating short-chain fatty acid levels and protection against allergic lung

inflammation.[177] Furthermore, delivery by cesarean section, a risk factor for development of allergy and other immune-mediated disorders,[55,56] was associated with decreased relative abundance and diversity within the Bacteroidetes phylum during the first year of life and less frequent detection of the major genus *Bacteroides*.[38] Several other studies have found that birth mode affects colonization with *Bacteroides*,[41–46] anaerobes that do not appear to grow outside the gut and thus may need to be transmitted between human hosts.[22]

Our cohort was not large enough to address sources other than the mode of delivery contributing to gut microbiota diversity.[38,169] Although all infants were breast-fed at age 1 month, when we observed that a low diversity was associated with allergy development, some infants were not exclusively breast-fed.[178] Partial breastfeeding was associated with an increased diversity, but the differences in diversity of the total microbiota, Bacteroidetes, and *Bacteroides* genera between infants developing allergy or not remained among exclusively breast-fed children.[178] Thus, other microbial sources, possibly from older siblings[42,43,45,166,179] and pets,[179–182] must contribute to the higher diversity among infants who remained healthy. Hygienic practices may also influence microbial diversity and allergy development.[183] Interestingly, children whose parents cleaned their pacifier by sucking it, thereby providing oral microbiota, were less likely to have asthma, eczema, and sensitization at age 18 months than were children whose parents cleaned the pacifier by boiling or cold water.[184] Like premastication of solid foods,[68] this represents another potential source of maternal vertical microbial transmission. Maternal diet during pregnancy,[115,116,185,186] prenatal and postnatal exposure to antibiotics,[42,43,45,179] and household antibacterial agents[187,188] are other important factors deserving further study with powerful culture-independent molecular techniques in large cohorts.

Further supporting the concept of the importance of early gut microbiota diversity in driving normal immune maturation, we found that children with asthma at school age had a lower diversity of the total microbiota at age 1 week and 1 month than nonasthmatic children.[189] A similar association was not found for allergic rhinoconjunctivitis development, although these findings should be confirmed in larger studies. Because viral lower respiratory tract infections are linked to asthma development among atopic children,[3,190,191] it is tempting to speculate that a reduced mucosal barrier function could be a consequence of a less diverse microbial stimulation. This may be linked to high airway viral infection susceptibility, amplification of Th2 responses, and subsequent asthma development.[3,190,191] Low salivary secretory IgA levels, a possible risk factor for lower respiratory tract infection susceptibility, are associated with late-onset wheeze development in sensitized infants[154] and, interestingly, also with low intestinal microbial diversity.[156]

Environmental microbial exposures in the home have also been associated with allergy development. We have evolved immunoregulatory responses to environmental microbial symbionts as well as to our own microbiome, because microbes present in our surroundings are inevitably taken into the body daily in large quantities.[192] Exposure to high levels of house dust endotoxin, derived from the cell wall of gram-negative bacteria, has shown protective associations with eczema[193–196] and asthma[197–201] in several longitudinal[193–196,198,200] and cross-sectional[197,199,201] studies. In infants and

toddlers, high endotoxin exposure may be a risk factor for infection-related wheeze, however.[193,196] High house dust endotoxin levels have also been reported in environments with a low-allergy prevalence, such as in farms[197] and in Estonia at the end of the past century.[194] Furthermore, we found that low endotoxin levels correlated with a slower maturation of the salivary secretory IgA system.[153]

More recent evidence implies that the diversity of microbial exposures in the environment is critically important for allergy protection, and suggests that high endotoxin levels may be a marker for an increased microbial load. Thus, a strong inverse association between microbial diversity in house dust and asthma at school age was found, as assessed in mattress dust by single-strand conformational polymorphism gel electrophoresis of bacterial 16S rRNA genotypes in one cohort and in airborne dust samples by culturing bacterial and fungal taxa in another cohort.[202] Increased microbial diversity also tended to associate with less atopy, but this was not statistically significant.[202] Living on a farm was associated with a high microbial diversity in the environment.[202] Among inner-city children, a reduced bacterial richness, particularly within the Bacteroidetes and Firmicutes phyla, was associated with recurrent wheeze and allergic sensitization at age 3 years, as analyzed by a 16S rRNA-based phylogenetic microarray in living room dust collected at age 3 months.[203]

It is not easy to disentangle the effects of the environmental microbial exposures on allergy development from the gut microbiome-mediated effects, however. Not only were lower endotoxin levels found in Estonian compared with those in Swedish homes,[194] the infant gut microbiota composition also differed in these two countries with a low and high-allergy prevalence at the end of the past century.[204] Thus, *Lactobacilli* and *Eubacteria* were more commonly detected in the gut microbiota of Estonian than Swedish infants, whereas the reverse was true for *C. difficile*.[204] We also noted that high house dust endotoxin levels were associated with an enhanced diversity of bifidobacteria in the gut microbiota of Swedish infants.[166] A more recent detailed characterization of the house dust and infant gut microbiota with high-throughput 16S rRNA gene sequence analysis indicated an association between these communities, because 14 bacterial operational taxonomic units representing the classes Clostridia, Actinobacteria, Bacilli, and Gammaproteobacteria co-occurred at a significantly higher frequency in matched dust–stool pairs than in randomly permuted pairs.[205] Interestingly, gavage exposure of mice to dust collected from houses with dogs attenuated airway hyperreactivity and allergen sensitization, whereas dust from houses without pets, with a low microbial richness and diversity,[206,207] had no effect.[208] After dog-associated dust exposure, the cecal microbiome of the mice was enriched in Clostridia and Bacilli classes, including *Lactobacillus johnsonii*, and gavage with this strain could also confer the allergy-protective effect.[208] To add to the complex associations between environmental exposures and our microbiome, it has been proposed that biodiversity loss at the level of macrobiota, including plants and animals, may reduce microbiota diversity,[209] with decreased opportunities for interactions between environmental and human microbiomes.[209,210] In support of this theory, a high environmental biodiversity in home surroundings, defined as high species richness of native flowering plants and in forest and agricultural land-use type, was associated with less allergic sensitization among adolescents and increased generic diversity of skin Gammaproteobacteria.[211]

Future perspectives

The continuously decreasing cost–throughput ratio of current sequencing platforms will allow analysis of the microbiome in larger pediatric cohorts with increased sensitivity and depth to further establish how microbial exposure shapes immune and allergy development.[56,212] Novel next-generation probiotic candidates may be identified in such studies, for example, indigenous gut bacteria producing regulatory T cell promoting short-chain fatty acids[102,213,214] and immunomodulatory *Bacteroides* strains.[101] Probiotic interventions have so far failed to prevent asthma,[130,134,215,216] although immunomodulation in infants has been demonstrated[159,217] and promising preventive effects have been observed for atopic eczema.[56,129,131,133] The preventive effects on eczema have primarily been observed in studies with combined prenatal and postnatal administration, although prenatal probiotic supplementation has not been given until the last trimester of pregnancy.[56,115,116,129,131,133] If prenatal microbial exposure is vital for the preventive effect, starting probiotic supplementation from the second trimester of pregnancy, when circulating fetal T cells have developed, may have a more powerful allergy- and asthma-preventive effect.[115,116] Such studies using traditional probiotics are ongoing and similar interventions with next-generation probiotics may be tested in the future.

In conclusion, the infant microbiome seems to shape immune maturation and to have major influences on allergy development. Further studies on the appropriate timing of interventions and the complex interactions between the infant immune system and the microbiome are required to translate these findings into preventive strategies required to combat the asthma and allergy epidemic.

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Indoor Allergen Exposure

12

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Allergy is an immediate hypersensitivity reaction caused by a skewed immune response lacking tolerance. Immunoglobulin E-mediated immune responses are directed against antigens that are usually environmental protein-containing allergens that normally are not harmful to the human body. Certain antigens seem to be more likely to become an allergen. Among these, indoor allergens have been focused on, because children and adults in industrialized countries often spend more time indoors than outdoors. Pet and house dust mite allergens have a major role in the development of specific sensitization and to a certain extent favor the development of allergic airway disease, especially asthma. However, in this complex process factors such as the type of allergen, timing, dose, and route of exposure are modulators. Furthermore, cofactors of exposure such as bacterial compounds may interact with allergen exposure. It is likely that the same allergen exposure may have a different impact depending on the genetic background of an individual. There are examples of filaggrin loss-of-function mutations and the effect of early cat ownership and the development of atopic eczema early in life,[1] CD14 polymorphisms in the endotoxin signaling pathway,[2] and variants in the 17q21 chromosomal region (ORMLD3) and the interaction of pet ownership and the significance for asthma in Croatian children.[3]

Sensitization may occur early, preceding the clinical onset of disease. An impaired barrier function of the skin such as in atopic eczema may facilitate sensitization via the skin during early infancy.[4] The second route of sensitization is via inhalation. The oral route has not been investigated for indoor allergens.

Numerous studies and publications have investigated the relationship between indoor allergen exposure, sensitization, and allergic airway disease.[5–9] In Scandinavian countries, because of climatic conditions, pet exposure is of major significance. In Central Europe, Australia, the United States (US), and the United Kingdom, house dust mite exposure and pet exposure are of similar importance. Moreover, cockroach allergen can be found in South and Central America and parts of the US. There are few data on mold spore exposure, which derives mainly from the outdoor environment.

Information has been gained by measuring exposure of pet and house dust mite allergens in dust from households, such as carpet and mattress dust; however, these sites do not reflect the direct inhaled exposure of an individual. Furthermore, there are only a few studies with repeated measurements of exposure. It remains unknown whether high exposure to indoor allergens for a short period has the same effect as exposure to a similar concentration for a longer period. Another problem with respect to measuring exposure is that exposure to indoor allergens such as mite and pet also occurs outside the home environment.[10,11]

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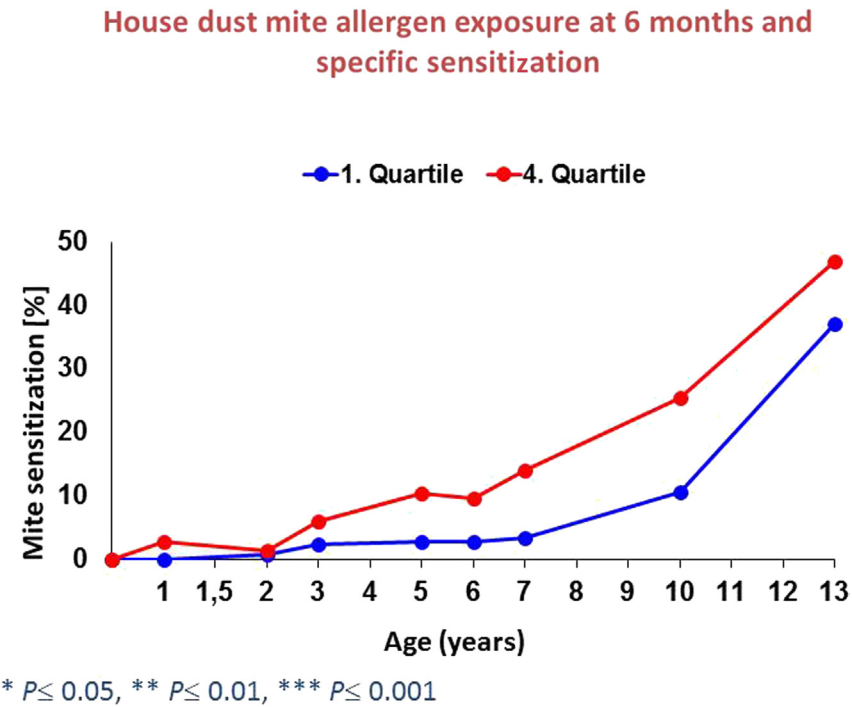


Figure 1 Association between house dust mite allergen exposure (Der p 1 plus Der f 1/g of dust) at age 6 months in dust samples of the German Multicentre Allergy Study (MAS) and sensitization to house dust mite.[6]

There is an assumption that early exposure during a certain window of opportunity is crucial for initiating the sensitization process, and there is evidence that this period is early in life rather than in utero.[12] Recent data from the US and the Urban Environment and Childhood Asthma study (URECA) indicate that sensitization to indoor allergens was mainly determined by cumulative exposure to cockroaches, mice, and dust mites at age 3 years rather than at age 1 year.[13] However, environmental assessment was performed only in a nested case–control study of 104 children. In the German Multicenter Allergy Study (MAS), which included over 1000 participants, exposure over a year of life was often a good marker for later sensitization[5,6] (Figure 1).

Pet exposure

Cat and dog allergens can be found in homes and in public places such as schools.[11,14] Allergic sensitization can occur in high- and low-exposure areas; therefore, allergen reduction with air cleaners and such seem to be not effective in terms of primary and secondary prevention.[15,16] However, exposure in sensitized asthmatic individuals worsens symptoms.[17] A Norwegian study and reports from the European Community Respiratory Health Survey presented findings indicating that cat exposure early in life

is a risk factor for later allergy and asthma,[18,19] whereas others suggested a protective effect of early-life pet exposure.[20,21] Because allergic children often have allergic parents, which caused avoidance behavior toward pets, reverse causation has to be taken in account. Sensitization was strongly related to asthma in German and British longitudinal studies.[6,22] In the Isle of Wight Study, 50% of mite-sensitized children showed asthmatic symptoms at age 4 years but 44% of cat-sensitized children were asthmatics.[22] The major dog lipocalin allergen Can f 1 may be less allergenic than cat allergen, which mainly is Fel d 1, because of the similarity of the lipocalin protein family to human serum proteins. Some authors suggested that exposure to dogs is associated with increased exposure to bacterial compounds such as endotoxin, thus modulating the immune response and decreasing the risk for allergy and asthma.[23,24] In a Canadian study, dog ownership during the first year of life was associated with an increased risk for asthma at age 7 years but not for specific sensitization.[25] Nevertheless, in a pooled analysis of 11 European birth cohorts, no association was found between asthma and allergy at school age early in life[26] and pet ownership.

In another publication, increased vaginal *Escherichia coli* colonization in women living with a cat or a dog was associated with an increased rate of urinary tract infections and an increased rate of oral antibiotics use. Thus, living with a cat or a dog can affect commensal vaginal flora, possibly interfering with immune responses.[27]

Preventive measures reducing pet allergen exposure

Reduction of pet allergens in the domestic environment is difficult. Estimates of the total Fel d 1 in cats, based on shaving the whole cat, ranged from 3 to 142 mg. Only a small proportion of Fel d 1 (0.002%/hour) becomes airborne.[28] Washing of pets was not well accepted by the animals and only temporarily reduced allergen concentration shed by animals.[28] Other chemicals turned out to be inefficient in reducing cat allergen exposure.[29] There have been discussions about different breeds and gender (males produce more allergen than females[30]), but female animals can produce enough pet allergen to induce sensitization and allergic reactions. Filter systems installed in households also were not found to sufficiently reduce domestic exposure to pet allergens[16] and therefore had no significant impact on clinical symptoms.

House dust mite

House dust mite exposure depends on the regional climate and the microclimate in homes. *Dermatophagoides* ssp. requires a higher indoor humidity (above 70% relative humidity) and constant temperature (24 °C). These conditions can usually be found in mattresses. However, in dry and hot or cold areas such as deserts or the north European countries (Sweden and Norway), exposure to house dust mites is rarely reported to be a major problem. Numerous allergens have been described: Der p 1 and Der p 2 from *D. pteronyssinus* and Der f 1 and Der f 2 are thought to be the major allergens eliciting immune responses in most mite-allergic patients (Table 1).

Table 1 Mite allergens

Der p 1	Cysteine proteases	24K
Der p 2	NPC2 family (lipid binding protein)	14K
Der p 3	Trypsin	25K
Der p 4	Amylase	56–63K
Der p 5	Unknown function	15K
Der p 6	Chymotrypsin	25K
Der p 7	Unknown function	22K
Der p 8	Glutathione S transferases	25K
Der p 9	Serine protease with collagenolytic activity	24K
Der p 10	Tropomyosin	37K
Der p 11	Paramyosin	102K
Der p 13	Fatty acid binding protein	15K
Der p 14	Apolipophorin	190K
Der p 15	Chitinases	60K
Der p 18	Chitinases	52K
Der p 20	Argininkinase	40K
Der p 21	Unknown function	16K
Der p 23	Peritrophin-like proteins	10K
Der p 30	Oxidoreductases	20K

According to www.Allergome.org.

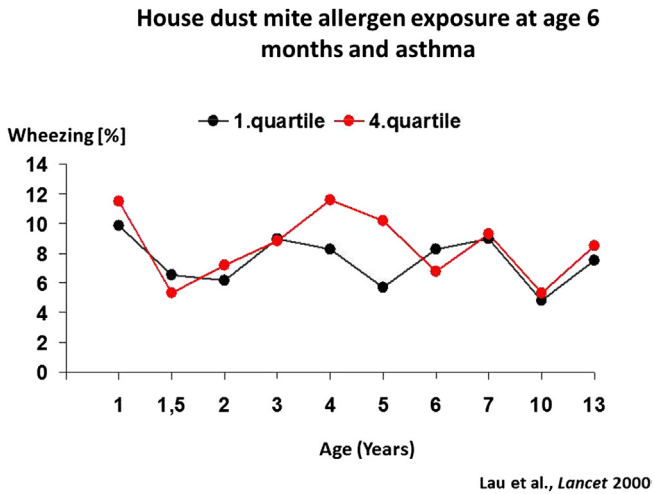


Figure 2 Association between exposure to mite allergens at age 6 months and asthma.[6]

Most published studies failed to prove a direct relationship between allergen exposure and the development of allergic asthma (Figure 2); however, sensitization to indoor allergens is clearly related to allergic asthma[6,31–33] (Figure 3). Furthermore, continuous exposure to indoor allergens is a risk factor for declined lung function in already sensitized schoolchildren[7] (Figure 4) and for severe pediatric asthma.[34]

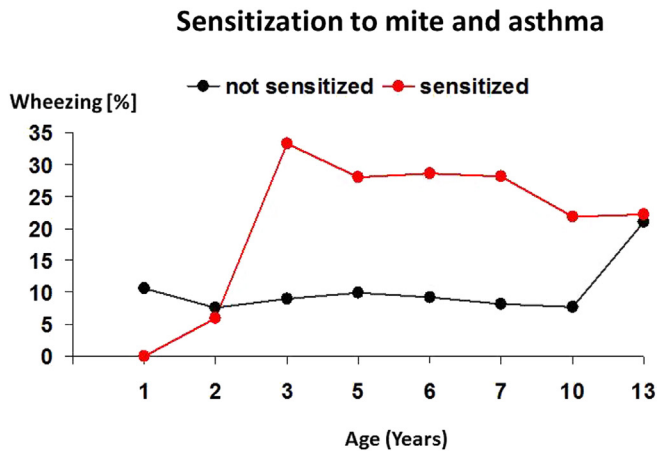


Figure 3 Association between sensitization to house dust mite and asthma.[6]

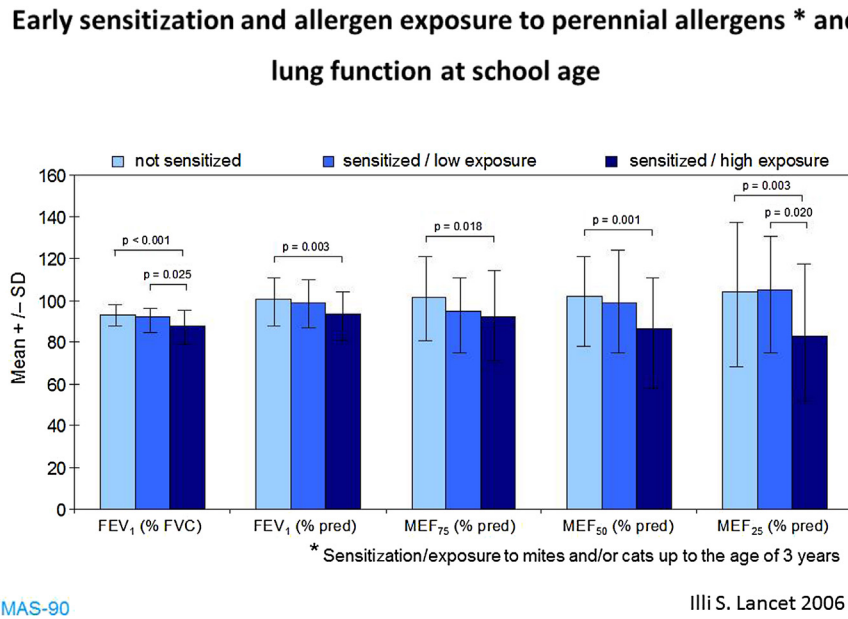


Figure 4 Association between sensitization and exposure to perennial allergens during the first 3 years of life in the German MAS and lung function at early school age.[7]

For asthma at age 13 years, sensitization to indoor allergens increases the risk threefold for those who wheeze before age 3 years and sevenfold for those who start wheezing after their third birthday, according to the German MAS.[35]

In the Melbourne Atopy Cohort Study, a birth cohort study composed of 620 babies enrolled before birth by recruiting pregnant women, a positive skin prick test for house dust mite at age 1 or 2 years predicted wheeze at age 12 years (adjusted odds ratio,

3.31 (95% confidence interval [CI], 1.59–6.91) at 1 year of age and 6.37 (95% CI, 3.48–11.66) at 2 years.[36] Among eczematous 1-year-olds, those who were sensitized to house dust mite had a 67% probability (95% CI, 45–84%) of wheeze at age 12 years compared with a 35% probability (95% CI, 25–45%) among those not sensitized to house dust mite.

In the population-based Manchester Asthma and Allergy Study (MAAS), atopic phenotypes were redefined using a five-latent-class model for timing and type of sensitization to specific allergens.[9] The highest risk for asthma at age 8 years and early hospital admission for wheeze/asthma was found in children with "multiple early atopic vulnerability," that is, sensitization to many allergens early in life, but house dust mite sensitization alone also turned out to be a risk for hospital admission for asthma.

For early childhood (preschool age), sensitization to *Alternaria*, cats, house dust mites, and grass pollen seems to be strongly linked to an asthmatic phenotype in the British Isle of Wight study.[22] In another birth cohort study from Boston, exposure to more than 10 μg mite allergen/g dust in infancy was related to the development of childhood asthma at age 7 years; the risk for late-onset asthma especially increased 5-fold. High exposure to endotoxin decreased the risk for asthma.[37]

As part of the Global Allergy and Asthma European Network (Ga^{2}len, www.ga2len.net), five population-based European birth cohort studies with a focus on asthma and allergy with information on house dust mite allergen exposure early in life (INMA, Spain; BAMSE, Sweden; LISAplus and MAS, Germany; and PIAMA-NHS, The Netherlands) were included in a pooled analysis assessing the impact of early-life exposure to major house dust mite allergens Der p 1 and Der f 1 on the development of wheezing, asthma, and allergic sensitization from infancy to age 8–10 years.[38]

The rates of exposure to Der p 1 and Der f 1 were clearly related to sensitization to house dust mite, with the lowest prevalence in the Swedish BAMSE cohort. One-third of all participating children were found to have high exposure to Der p1 and Der f 1 at home. In the MAS and the PIAMA cohort, the relative ratio increased for Der p 1 exposure and asthma after age 6 years.

The Childhood Asthma Management Program in the US reported allergen sensitization and exposure to indoor allergens to be risk factors that decreased the likelihood of remitting asthma in adolescence.[39]

House dust mite avoidance

Unfortunately, primary prevention procedures reducing domestic mite allergen exposure have not achieved decreased sensitization rates; in contrast, whereas 3-year-old children were found to have less airway resistance, they had higher sensitization rates than the control group.[15] In the Dutch PIAMA study, at age 1 and 2 years, mite allergen levels were lower than those found in previous Dutch studies. The intervention measure had a significant effect on mite allergen levels, without important clinical benefits up to age 2 years. The prevalence of night cough without a cold in the second year of life was lower in the group with active covers compared with the group with

placebo covers (adjusted odds ratio, 0.65; 95% CI, 0.4–1.0). No effect of the intervention was seen on other respiratory symptoms, atopic dermatitis, and total and specific IgE.[40–42] Using a controlled multifaceted approach including house dust mite allergen avoidance after birth, the British Isle of Wight study observed a reduction in sensitization in the active group[43] and a lower prevalence of asthma at age 18 years[44] with an odds ratio of 0.51; however, atopy was not different at that time. A Canadian multifaceted interventional trial included 545 high-risk infants who were identified before birth. Avoidance of house dust mites (including encasement of mattresses, duvets, and pillows); hot water washing of bedding; avoidance of environmental tobacco smoke; and encouragement of breast feeding or supplementation with a hypoallergenic formula resulted in a significant decrease in asthma and rhinitis without colds in the intervention group at age 1 year.[45] The mite allergen concentration was significantly reduced[46] and cat allergen levels decreased after cats were removed. However, the use of an acaricide on carpets and upholstered furniture did not reduce mite allergen levels. The Australian Children Asthma Prevention Study, which also introduced fatty acid supplementation, failed because the intervention did not achieve significant allergen reduction.[47]

However, mite allergen reduction as a tertiary approach can reduce symptoms and bronchial hyperresponsiveness,[48–50] although a meta-analysis doubted this.[51] When analyzing effect sizes, it should be clear that the only studies that can be included successfully achieve allergen reduction with similar methods, addressing mite avoidance measures in a clearly defined mite-allergic population.[52] This was not always the case in the Cochrane review by Gotzsche et al.[51]

Cockroaches

Cockroach allergen exposure does not have a major role in northern European countries, Germany, Austria, and Switzerland. It is a common problem in many areas of the US, South America, and Mediterranean countries. Exposure and sensitization are associated with inner-city asthma and lower social status.[8,53] In the US, the inner-city population with asthma is often sensitized to cockroaches (60–80%[54]). Poorer outcome and increased asthma-related health care use (emergency rooms) in inner-city children with asthma and cockroach sensitization may also be because of social status, exposure to other risk factors such as fast food, obesity, and tobacco smoke exposure, and to less access to pharmacotherapy (inhaled corticosteroids).

Molds

There is not much direct evidence for an association between mold growing indoors and asthma. Of course, indoor mold reflects bad housing conditions such as poor insulation and a humidity problem. Sensitization to molds (*Penicillium*, for example) increases the risk of asthma[55] but such sensitization is usually a result of outdoor

exposure to mold spores. On the other hand, in another study, exposure to beta (1,2)-glucans from molds seems to be beneficial in preventing allergic sensitization.[56]

Other allergens

Among the typical indoor allergens, house dust is a mixture of various organic substances such as food allergens, representing a possible source of exposure and facilitating allergic sensitization via the skin[4] and the airways.

Summary

Allergen exposure may have different roles for different phenotypes of atopic disease such as atopic eczema and asthma. Both diseases have allergen-dependent and -independent mechanisms of inflammation. In a comparative analysis of the Ga[2]len initiative, including the most important birth cohort studies with information on indoor mite allergen exposure early in life, the Swedish BAMSE showed the lowest mite allergen exposure and the lowest asthma prevalence rates during the first 8 years of life.[38] If a person is allergic, allergen exposure increases airway inflammation and may be associated with a risk of hospital admission, especially if sensitization had occurred early in life.[57] Allergen sensitization and high-level exposure to sensitizing allergen and respiratory virus infection synergistically increases the risk of acute exacerbation among adults and children.[58,59] Furthermore, the bacterial content in house dust during the first year of life, especially reduced exposure to specific Firmicutes and Bacteriodetes, was associated with atopy and atopic wheeze in the URECA population.[13]

Avoidance of ingested allergens, that is, of cow's milk, in early infancy clearly can prevent early atopic dermatitis (German Infant's Nutritional Investigation[60]) but results of primary prevention trials avoiding indoor allergens, especially house dust mite allergens, indicate that in most of the studies the effect on the clinical outcomes is weak.[15,44,61] In the Isle of Wight Study, which used a multifaceted approach,[44] there seemed to be some long-lasting effect on asthma, whereas the Manchester Asthma and Allergy Study saw a slight increase in sensitization during early childhood. One problem is that exposure to indoor allergens cannot be sufficiently reduced. Low exposure is achieved[62] by allergen avoidance, but exposure outside the household will still occur.

In the case of pets, there is some evidence that early-life exposure to pets is related to children's risk of developing allergic sensitization and asthma. However, there seem to be differences in population-based studies explained by the community prevalence of pets.[19,26,63–67] Furthermore, heredity may modulate behavior (reverse causation[68]). No single primary prevention study exists that avoids only pet allergens. Some primary prevention studies such as the MAAS and the Canadian trial avoided pet allergen in addition to house dust mite allergens. However, there is a clear link between sensitization to indoor allergens and the development of allergic airway

disease in urban populations.[6,13,22] In contrast, contact with livestock in a traditional farming environment seems to protect against allergies and asthma,[69] mainly because of the immune-modulating effect of microorganisms and other components from cowsheds.[70,71] A single dog or cat cannot imitate this environment in a nonfarming community.

So far, recommendations to families for primary prevention of asthma should be given with great caution, because no single approach can prevent children from asthma to a large extent. However, allergen avoidance as a part of treating allergic patients remains an appropriate measure. The indoor environment remains an important source of immune modulating antigens, potentially priming sensitization or tolerance toward allergens.

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Microbial Influences on the Development of Atopy

13

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A lively debate is ongoing about the potential beneficial or detrimental role of microbes on human health. Although there is no doubt that some microbes—bacteria, viruses, fungi, and parasites—can harm humans and elicit deadly diseases, there is growing attention to an immense microbial world in and outside our bodies that may be beneficial for our health.

In the field of atopy, these deliberations started with the advent of the hygiene hypothesis. In 1989, David Strachan published a seminal article showing an inverse association between the number of siblings and the prevalence of hay fever in young adults.[1] This inverse association was more pronounced for older than younger siblings. In this report, Strachan proposed the hygiene hypothesis, stating that "infection in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally from a mother infected by contact with her older children" may prevent the development of allergic diseases. This first observation has been repeated numerous times; many studies show a protective effect particularly of older siblings on the development of atopy, hay fever, atopic dermatitis, and, to a lesser extent, asthma. Subsequent surveys have attempted to link the frequency of early childhood infections to the protective sibling effect, but have failed to explain it to a large extent.[2] Further attempts to explain the sibling effect by parity have not been unequivocal.[3,4] Thus, the mechanisms underlying these strong and consistent epidemiological observations await further elucidation.

However, contact with other children occurs not only within the family but also in day care settings. Several studies have shown that early-life exposure to day care is inversely associated with atopy. In East Germany, the development of asthma and allergies at age 5–14 years was reduced if a child from a small family entered a day care nursery between age 6 and 11 months, compared with later in life.[5] These findings were corroborated by the Tucson Children's Respiratory Study, which showed that children in day care in the first 6 months of life were at significantly reduced risk of atopy and wheezing at school age and adolescence.[6]

Environmental factors contributing to exposure encountered by children in day care have not been identified. The facilitated spread of viral and bacterial infections may have a role. This notion has been supported by findings from other epidemiological studies showing an inverse association between positive serologies to *Hepatitis* A, *Toxoplasma gondii*, and *Helicobacter pylori* with lower rates of atopic outcomes such as sensitization, hay fever, and allergic asthma.[7,8] Because those diseases are

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more easily spread under crowded and more unhygienic conditions, they represent exposure to infectious diseases but also general higher exposure to bacterial products that may confer a protective effect. Microbes are ubiquitous in our indoor and outdoor environments. The microbial load indoors has been shown to increase with the number of inhabitants and pet keeping, and also in day care settings.[9] Whether the protective effect of siblings and day care centers is attributable to increased indoor exposure has not been investigated, however.

Many studies have addressed the potential role of outdoor microbial exposures. Particularly intriguing and informative studies have been performed in Finnish and Russian Karelia. The former Iron Curtain divides this rural area into areas of westernized lifestyle in Finland and unchanged living conditions since World War II. Compared with the Finnish region which is about 150 km away, the prevalence of atopy and other allergic diseases is exceedingly low in children and adults in Russian Karelia.[10] These investigators have shown over the past years that environmental microbial exposures in dust and drinking water may significantly contribute to these striking differences[11,12] Furthermore, an increasing number of positive serologies to bacteria and virus was shown to protect from atopy.[13]

Microbial products are particularly abundant in farming environments where animals such as cattle, pigs, and poultry are kept. Children growing up on farms have a significantly lower prevalence of atopic sensitization, hay fever, and asthma compared with children from the same rural areas but not raised on farms. This epidemiological observation has repeatedly been reported from many areas of the world, and thus has become a robust and consistent epidemiological finding. In alpine farming studies, the farm type most strongly associated with protection from asthma, hay fever, and atopy was characterized by keeping dairy cows and breeding cattle combined with cultivation mostly of grain and corn.[14] These farmers also typically fed corn silage and grain shred to their animals. The individual exposures of farm children that mattered most were contact with livestock, plant material such as hay and straw, and consumption of unprocessed cow's milk.[14] These protective exposures were not confounded by other lifestyle factors such as family size, nutrition, or socioeconomic factors. In fact, the farm effect was independent of the sibling effect.[15] These observations suggest that different biological mechanisms underlie these two protective factors.

Asthma is not one disease, but rather a syndrome. There is atopic and nonatopic asthma and early transient wheeze, which is triggered by viral infections, but does not progress to childhood asthma. Protective farm exposures affected all of these phenotypes, including transient early wheezing, but were not attributable to improved airway size and lung mechanics.[16] These findings suggest that the farm effect on asthma is not mediated solely by reduced rates of atopy, but most likely also involves antiviral and anti-inflammatory mechanisms.

The timing of exposure matters. In many farm children exposure starts early in life when pregnant farm women work in animal sheds and elsewhere on farms.[14] Their offspring are subsequently further exposed when they are taken to their parents' workplaces. To disentangle prenatal from postnatal effects, a large birth cohort, the PASTURE/EFRAIM study, was conducted in five European countries enrolling over 1100 families, about half living on and running a farm. Women were enrolled during

pregnancy and maternal exposures were assessed by a comprehensive questionnaire. At birth, cord blood specimens were collected and children are currently being followed up to age 10 years.

Clear prenatal effects of maternal exposures during pregnancy were found. In the PASTURE study, a total of 1063 children were investigated. Doctor-diagnosed atopic dermatitis was reported by parents of children aged of 1–2 years and the gene expression of Toll-like receptors (TLRs) and *CD14* was assessed in cord blood leukocytes by quantitative polymerase chain reaction.[17] Maternal contact with farm animals and cats during pregnancy had a significantly protective effect on atopic dermatitis in the first 2 years of life. The most important determinant was the number of farm animal species with which the mother had contact during pregnancy. In fact, the risk of atopic dermatitis was reduced by more than half among children whose mother had had contact with three or more farm animal species during pregnancy. Interestingly, elevated expression of *TLR5* and *TLR9* in cord blood was associated with an increased risk of atopic dermatitis.

Another cohort of 84 pregnant mothers was recruited before delivery (66 nonfarming and 22 farming mothers) in rural areas in southern Germany by Bianca Schaub and team.[18] Cord blood was stimulated with the microbial stimulus peptidoglycan, the mitogen phytohemagglutinin (PHA), and house dust mite extracts. Regulatory T cells were furthermore assessed. Cord blood T-regulatory (Treg) cell counts, both unstimulated and PHA stimulated, were significantly increased with maternal farming exposure and associated with higher *FOXP3*. Furthermore, Treg cell function in cord blood was more efficient with maternal farming exposure. Among maternal exposures, increasing numbers of farm animal species and stable contact had the strongest effects. In parallel, Th2 cytokine (interleukin-5) levels were decreased and associated with decreased proliferation. These data suggest that farm exposure during pregnancy increases the number and function of cord blood regulatory T cells, which in turn are associated with lower Th2 cytokine creation and lymphocyte proliferation on innate exposure.

In the PASTURE/EFRAIM cohort, cord blood mononuclear cells were stimulated with *phorbol myristate acetate* and ionomycin, and supernatants were assessed for the production of interferon-gamma (IFN-γ), tumor necrosis factor-α (TNF-α), IL-5, IL-10, and IL-12. Significantly higher levels of IFN-γ and TNF-α were found in newborns of farming mothers compared with newborns of nonfarming mothers.[19] In contrast, IL-5, IL-10, and IL-12 levels did not differ between groups. Important determinants for IFN-γ and TNF-α levels were maternal contact with different farm animal species, the barns, and the consumption of unskimmed milk, resulting in increased levels of IFN-γ and TNF-α in the cord blood. Furthermore, allergen-specific immunoglobulin E (IgE) antibodies were detected in cord blood of 23.9% of all newborns.[20] Contamination with maternal serum was excluded by several means of analysis, including the absence of IgA antibodies. Different sensitization patterns in cord blood of farm and nonfarm children were observed. In multivariable analyses consumption of boiled but not unboiled farm milk during pregnancy was positively associated with specific IgE to cow's milk, independently of maternal IgE. However, there was a significant and strong inverse relation between maternal exposure to animal sheds and cord blood IgE levels against seasonal allergens, mostly grass pollen, which was enhanced by maternal contact with hay.

Thus, maternal exposures during pregnancy influenced not only cord blood cytokine patterns, but also atopic sensitization. Interestingly, there was a link between cytokine levels and atopy in cord blood: higher cord blood IFN-γ production was related to lower allergen-specific IgE antibodies of fetal origin.[21] Thus, the beginning of atopy may have roots in fetal life, where it may be determined by immune processes related to Th1 lymphocytes or other IFN-γ–producing cells.

Atopic sensitization to common inhalant and food allergens was further assessed at age 1 year in this birth cohort.[20] Detailed information on children's health, nutrition, and farm-related exposures was collected by a pregnancy questionnaire, two questionnaires at age 2 and 12 months, and a diary covering the time in between. Sensitization was more common at age 12 months than at birth for almost all specificities. On an individual level, persistent sensitization to the same allergens was rare (1%), whereas transient (only at birth; 11%) and incident (only at 12 months; 34%) sensitization was seen in substantial proportions of children. Again, maternal exposure to animal sheds and their contents (more specifically, silage for this analysis) was inversely related to grass pollen sensitization at age 1 year.

Animal sheds are a constant and strong source of microbial exposures in these environments. Therefore, the question arises as to which are the protective factors within these stables. We measured levels of endotoxin, a marker of gram-negative bacteria, in the bedding of children in the ALEX survey, a cross-sectional study performed in 6- to 13-year-old schoolchildren living in rural areas of Germany, Austria, and Switzerland.[22] Endotoxin levels in dust samples from the children's mattresses were inversely related to the occurrence of hay fever, atopic asthma, and atopic sensitization. In turn, nonatopic wheeze was not significantly associated with endotoxin levels. Furthermore, mattress dust samples were assessed for muramic acid concentration, which is a marker of mostly gram-positive bacterial exposure.[23] Like endotoxin, concentrations of muramic acid were significantly higher in dust from farm children's mattresses than in dust from nonfarm children's mattresses. Higher levels were associated with a significantly lower prevalence of wheezing regardless of farming status and endotoxin exposure. In turn, no association was found with atopic sensitization. Thus, muramic acid provides an independent microbial marker that is inversely associated with wheezing rather than with atopic sensitization. In the PARSIFAL study, levels of fungal extracellular polysaccharides were measured in mattress dust samples of 440 children. Independently of endotoxin, the extracellular polysaccharide levels were inversely related to asthma but not atopy.[24]

All of these findings suggest that environmental microbial exposures can protect from asthma and atopy. However, it is not clear which microbes may account for these beneficial effects. To assess environmental microbial exposures more specifically, house dust was collected via electrostatic dust collectors in the GABRIEL survey, a cross-sectional study of school-age farming and nonfarming children. Conventional culture methods were used to assess viable counts of fungi. In the PARSIFAL study, samples of mattress dust were screened for bacterial deoxyribonucleic acid with the use of single-strand confirmation polymorphism analysis to detect environmental bacteria that cannot be measured by means of culture techniques. In both studies, the diversity of microbial exposures, whether bacterial or fungal, was inversely related to the risk of developing asthma[25] (Figure 1).

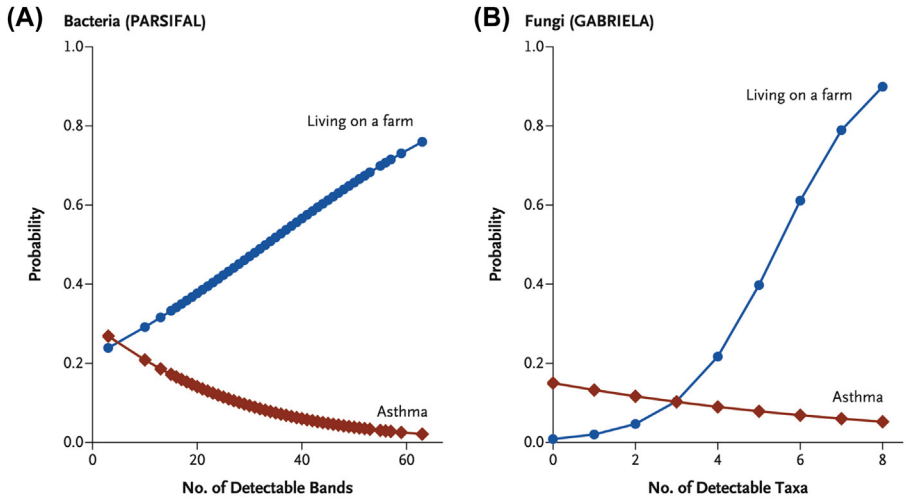


Figure 1 Relationship between microbial exposure and the probability of asthma. (A) Assessment of bacterial environmental exposure by SSCP. (B) Assessment of fungal environmental exposure by conventional culture methods.

© New England Journal of Medicine, Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrländer C, et al. Exposure to environmental microorganisms and childhood asthma. *N Engl J Med* 2011; **364**:701–9.

This diversity in exposures can be interpreted in a number of ways. Diversity clearly argues against the idea of the needle in the haystack, that is, a single microbe that would account for the protective effects seen in the farm studies. Rather, diversity suggests that more exposures are needed. However, diversity can be interpreted as the more, the better, or as an underlying effective cocktail of microbial exposures that may confer protection. In both the GABRIEL and the PARSIFAL studies, the presence of a certain mixture of bacterial and fungal exposures such as *Eurotium* and *Penicillium* for the fungi and *Listeria monocytogenes*, *Bacillus* species, *Corynebacterium* species, and others accounted for the asthma-protective effect. Indeed, these exposures explained a substantial fraction of the inverse relation between asthma and growing up on a farm. Taken together, all of these findings suggest that certain microbial exposures in the environment can protect children from developing asthma and atopy until school age. These protective effects are certainly in part mediated by changes in immune responses such as those previously described for cord blood studies. However, they might also affect the microbiome of the airways or the gut.

This novel dimension of microbial influences, namely the vast field of the human microbiome, is currently under intense scrutiny. The human body harbors 10-fold more microbial than human cells and orders of magnitude more microbial genes than the human genome.[26] So far, most attention has been given to the gut microbiome. In the intestines, commensal bacteria are involved in the conversion of indigestible food components, production of essential vitamins and cofactors, regulation of epithelial development, and instruction of immune maturation.[27–33]

There are several studies strongly suggesting that gut microbial colonization may have a systemic immune regulatory effect. Children developing asthma or allergies may have a lower gut microbial diversity in the first year of life compared with healthy children.[34–36] Not only diversity but also the composition of the gut microbiome may matter. Infants developing allergic sensitization or atopic dermatitis later in life had less *Enterococci* and *Bifidobacteria* but more *Clostridiae* in their early gut microbiome.[37–39] It is not well understood how the gut microbiome may affect inflammatory diseases in other organs such as skin (atopic dermatitis) and airways (asthma). A number of experimental studies in mice point to communication between the gut and the lung via a number of different immune cells.[40–43] For example, the oral application of *Helicobacter pylori* resulted in significantly reduced airway hyperresponsiveness, tissue inflammation, and goblet cell metaplasia, which in turn were associated with increased pulmonary infiltration of Treg cells and impaired dendritic cell maturation.[41] In germ-free mice, asthma development is enhanced, which has been shown to be mediated by invariant natural killer T cells.[28] Bacterial metabolic products such as short chain fatty acids[36,44,45] or zwitterionic polysaccharides such as polysaccharide A from *Bacteroides fragilis*,[46,47] which are produced by bacteria colonizing the gut, may have a further important role in suppressing inflammation. However, dead bacteria such as *Enterococcus faecalis* have been shown in animal models to suppress Th17 cell development in the lung and therefore to attenuate allergic airway responses.[42]

The hygiene hypothesis has had a long journey since its first appearance in 1989. Initial observations in epidemiologic surveys suggested that family size, day care, and oro-fecal as well as other infections may lower the risk of developing atopy. The next powerful set of studies investigated the strong protective effect of being raised on a farm in numerous countries around the world. Microbial exposures assessed by proxy, such as endotoxin, muramic acid, and extracellular polysaccharides, have been found to be inversely related to atopy and allergic illnesses not only in farm studies, but also in urban areas. Among those microbial exposures, a cocktail, or a mixture of different bacteria and fungi, may be needed to confer protection. These microbial exposures may act through the structures of their cell wall such as lipopolysaccharide, lipopeptides and others that bind to microbe-associated molecular patterns and activate innate immune responses. Thus, dead microbial debris may also be relevant. Alive bacteria may colonize mucosal surfaces of skin, airways, and gut and activate immune responses directly or by producing metabolites such as short chain fatty acids, as animal studies have elegantly shown. All of these mechanisms are conceivable. In fact, there seems to be no magic bullet, but many components of such diverse environmental microbial exposures may act in concert to stimulate innate immune responses and reduce adaptive immune responses that contribute to the development of atopy and asthma.

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Role of Vaccines

14

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Introduction

Active immunizations against diseases that cause substantial morbidity or potential mortality represent one of the greatest advances in medicine over the past century. Allergic patients should receive the same publicly recommended vaccines unless there is an allergy-specific risk that outweighs the risk of the infectious disease to be prevented.

Despite the dramatic benefits provided by immunization to vaccine-preventable infectious diseases, concerns regarding the potential for adverse reactions to immunizations have persisted since their introduction more than 200 years ago. Among potential adverse events following immunization (AEFI), concerns regarding vaccine allergy are commonly expressed by parents, patients, and immunizing physicians.

Allergic vaccine reactions to vaccines certainly occur, but severe allergic events are rare. For many routine vaccines the incidence is <1/100,000 doses.[1] Patients with an antecedent allergic reaction to a vaccine or to a component contained in the vaccine are at heightened risk. Unfortunately, most allergic reactions occur in patients without known risk factors and cannot be predicted by currently available tools. Unfortunately, lack of knowledge about whether and how to immunize patients with concerns regarding possible allergic vaccine reactions can contribute to incomplete immunization coverage.

Aside worries regarding possible allergic reactions to the vaccine itself, considerable fear exists that routine childhood immunization may promote development of allergic sensitization and allergic disease. In one survey, apprehension that vaccines may increase the risk of developing allergy was identified as a major impediment to timely routine immunization.[2]

In this chapter we summarize current evidence with regard to allergic vaccine reactions and the impact of vaccines on the development of allergy. We also summarize expert recommendations with regard to immunization of children predisposed to allergy.

Allergens that may be contained in vaccines

Vaccines usually represent a cocktail of potentially allergenic components. Aside from microbial components, which act as genuine immunizing antigens, vaccines may contain adjuvants, preservatives, stabilizers, and contaminants from the manufacturing process.

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Microbial antigens (e.g., toxins, toxoids, attenuated live microbes) typically induce an immunoglobulin G (IgG) response that is taken as a proxy for immunogenicity and protection from the vaccine target. Microbial antigens can induce a concomitant IgE response. Immunoglobulin E (IgE) responses to vaccine antigens are not rare. After primary immunization, IgE against diphtheria and tetanus toxoids were detected in about 50% of an atopy-enhanced infant cohort,[3] and after booster doses, vaccine antigen-specific IgE was found in more than 90% of vaccinees.[4] Atopic vaccinees express more frequent and pronounced vaccine-specific IgE responses.[3,5] However, because severe allergic AEFI to microbial vaccine antigens are rare, IgE responses to microbial vaccine components do not appear to predict a severe allergic AEFI.

Antibiotics (e.g., neomycin, streptomycin, gentamycin, polymyxin B) are sometimes used in vaccine manufacturing to prevent bacterial contamination. Residual antibiotic may be contained in vaccines but the concentrations are small and allergic reactions to these components are rare or nonexistent. There is one report of an anaphylactoid reaction in a boy with contact allergy to neomycin after administration of a *measles, mumps, and rubella* (MMR) vaccine, but because specific allergy testing for neomycin was not established, the causative allergen remains uncertain.[6]

Gelatin of porcine origin is used as a stabilizer in some vaccines. Gelatin can induce immediate-type and delayed-type hypersensitivity reactions. Evidence for gelatin as a true cause of anaphylactic reactions to vaccines was initially reported for gelatin-containing d*iphtheria, tetanus, and pertussis (*DTaP), Japanese encephalitis, varicella, and MMR vaccines,[7–10] in which gelatin-specific IgE was confirmed in the serum of patients with reactions. Subsequently, gelatin has been removed from many vaccines but it is still present in many, including varicella. Because gelatin ingested with food is more often of bovine origin, a patient history of food gelatin tolerance does not rule out the possibility of vaccine allergy resulting from porcine gelatin.

Aluminum salts are added to some vaccines as adjuvants to improve their immunogenicity. Routine vaccines usually contain <1 mg of aluminum salt. IgE formation to aluminum has not been observed and there are no published reports of anaphylaxis caused by aluminum. Aluminum can sometimes induce a type IV immune reaction with formation of an itching nodule at the injection site that may persist for months or longer.[11,12] Nodules may be larger with subsequent booster shots. However, among patients who developed a nodule after vaccination with an aluminum-containing vaccine, only a minority developed a nodule also after the booster shot.[13]

Thimerosal is also sometimes added as a preservative to vaccines to prevent bacterial and fungal contamination. In an epidemiological study in North America, it has been reported to be the fifth most common contact allergen,[14] and in Denmark, contact allergy to thimerosal has been demonstrated in 24% of children.[15] Supported by data from an experimental model, immunization in early childhood has been implied as a cause of sensitization.[16] Whereas thimerosal is mainly known to cause contact allergy, it has also been reported to cause systemic allergic reactions in nonimmunization settings.[17] With regard to immunization, one case has been reported with development of a generalized pruritic rash attributed to thimerosal in an influenza vaccine,[18] but no cases of severe anaphylaxis have been documented. Thimerosal has been estimated to induce transient local reactions in about 9% of individuals with thimerosal contact allergy, but it seems safe in these patients with regard to more severe allergic

AEFI.[19,20] Thimerosal has been removed from most vaccines because of unjustified concerns linking thimerosal in vaccines to neurological disorders.

Formaldehyde is used in the production of vaccines because of its capacity to inactivate bacterial toxins and viral replication.[21] In routine vaccines, formaldehyde can be found in the microgram range. One case with exacerbation of hand eczema after immunization with a formalin-containing hepatitis B vaccine in a formaldehyde-allergic patient was described,[22] but there are no reports with regard to anaphylaxis.

2-Phenoxy-ethanol is added as a preservative against gram-negative bacteria to vaccines. There is one report describing generalized eczema twice in an infant with atopic dermatitis within 24 h after diphtheria, tetanus, and pertussis (DTP) immunization; patch testing identified 2-phenoxy-ethanol as the most likely causative allergen and subsequent DTP immunization without 2-phenoxy-ethanol caused no eczema exacerbation.[23]

Chicken and hen's egg–derived media are commonly used to grow viruses for vaccine production. In the manufacturing process, vaccine viruses are purified as far as possible from residual ovalbumin. However, depending on the culture medium used, different amounts of residual ovalbumin have been detected in vaccines. Low amounts are found in MMR, *tick-borne encephalitis,* and rabies vaccines (vaccine produced in chicken fibroblast cell culture, ovalbumin <50 ng/dose, and <1 μg/dose in most current vaccines), higher amounts in influenza vaccines (vaccine produced in embryonated hen's egg, ovalbumin <150 ng/dose), and highest amounts in yellow fever vaccines (vaccine produced in chicken embryo, ovalbumin <3 mg/dose). Residual ovalbumin in vaccines is a potential hazard for highly egg-allergic patients and has been implicated as a culprit allergen after anaphylactic reactions to vaccines.

Residual yeast (*Saccharomyces cerevisiae*) from the vaccine production process has been found in hepatitis B and human papillomavirus (HPV) vaccines. Analysis of reports of patients with preexisting yeast allergy in the United States (US) Vaccine Adverse Event Reporting System identified 11 patients with probable or possible anaphylactic AEFI related to yeast sensitivity.[24]

Residual cow's milk from the vaccine production process may be contained in some vaccines, but because of the high prevalence of cow's milk allergy in childhood and a relatively small number of possible allergic AEFI, even most severely cow's milk allergic children seem to tolerate minute amounts of cow's milk in the vaccine. However, some cases were reported in children with severe cow's milk allergy with anaphylactic reactions after booster immunization against diphtheria, tetanus, and pertussis (DTaP or Tdap), in which casein was detected in sister lots of the vaccines administered.[25] Oral polio vaccine (OPV), which is still widely used in polio endemic areas, was suspected as a cause of anaphylactic AEFI in four children with IgE-mediated cow's milk allergy, who also reacted positive to a skin prick test with the vaccine; vaccine-coated enzyme-linked immunosorbent assay plates incubated with α-lactalbumin inhibited binding of patient serum.[26]

Latex is used in stoppers of multiuse vaccine vials, potentially leading to contamination of the vaccine. Analysis of >160,000 AEFI reports in the US Vaccine Adverse Event Reporting System revealed 28 cases of possible immediate-type hypersensitivity reactions in vaccine recipients with a history of allergy to latex; only in one case was latex considered to be the most likely culprit allergen.[27]

The content of known allergens in currently used vaccines is summarized in Table 1.

Table 1 Allergens in vaccines (calculated per 0.5 mL dose, if not stated otherwise)

Allergen	Vaccines	Amount
Amphotericin B	• Rabies	<2 ng
Casein, bovine	• DTaP, DTaP + IPV, DTaP + HepB + IPV	<20 ng/mL (DTaP)
	• Meningococcal	Ingredient of medium
	• Typhoid	Ingredient of medium
Chlortetracycline	• Rabies	<20 ng
Egg (ovalbumin)	• Influenza (IIV)	≤1 mcg
	• Influenza (LAIV)	<0.24 mcg
	• MMR, MMR + varicella	residual
	• Rabies	<3 ng/mL
	• Yellow fever	residual
Gelatin (bovine)	• Rabies	<12 mg polygeline
Gelatin (porcine)	• Influenza (IIV)	0.05%
	• Influenza (LAIV)	2 mg/0.2 mL
	• Japanese encephalitis	0.5 mg/1 mL
	• MMR, MMR + varicella	≤14.5 mg
	• Varicella	<16 mg
	• Yellow fever	As stabilizer
Gentamicin sulfate	• Influenza (IIV, LAIV)	<15 mcg
Latex	• Various	Vial stopper, syringe tip caps, rubber plunger
Neomycin	• Hepatitis A, AB	≤40 ng/mL neomycin sulfate
	• Influenza (IIV)	≤2.5 mcg
	• MMR, MMR + varicella	≤25 mcg
	• Polio	<5 ng
	• Rabies	<1 mcg
	• Smallpox	Trace amounts
	• Varicella	Trace amounts
Polymyxin B	• DTaP-IPV, DTaP-HepB-IPV, DTaP-IPV-HiB	≤0.01 ng
	• Influenza (IIV)	≤3.75 mcg
	• Polio	25 ng
	• Smallpox	Trace amounts
Serum, fetal bovine	• MMR, MMR + varicella	<1 part per million (ppm) 0.5 mcg
	• Polio	<1 ppm
	• Rotavirus	Trace amounts
	• Varicella	Trace amounts
Sodium taurodeoxycholate	• Influenza	<10 ppm
Streptomycin	• Polio	<200 ng

Table 1 Allergens in vaccines (calculated per 0.5 mL dose, if not stated otherwise)—cont'd

Allergen	Vaccines	Amount
Thimerosal	• DT	≤0.3 mcg
	• HiB	≤0.3 mcg
	• Influenza	≤24.5 mcg
	• Japanese encephalitis	0.007%
	• Meningococcal	25 mcg
	• Tetanus toxoid adsorbed	≤0.3 mcg
Yeast	• Hep AB, Hep B	≤5%
	• HPV	<7 mcg
	• Meningococcal	Ingredient of medium
	• Pneumococcal	Ingredient of medium
	• Typhoid	Ingredient of medium

Derived from Institute of Vaccine Safety, Johns Hopkins Bloomberg School of Public Health (http://www.vaccinesafety.edu/components-Allergens.htm, accessed 19.01.15).

Allergic immune responses to vaccines

Vaccines induce the protective IgG response but they can also induce a specific IgE response. After primary immunization against diphtheria and tetanus, about 50% of infants have detectable IgE against the diphtheria and tetanus toxoids.[3] After booster immunization later in life, detectable IgE antibodies against vaccine antigens have been observed in more than 90% of vaccinees.[4] The IgE response to vaccine antigens seems to be more pronounced if the vaccine is atopic.[3,5] Although IgE levels against vaccine antigens can be high (e.g., up to radioallergosorbent test [RAST] class IV with pertussis booster immunization), no relevant clinical allergic reaction has been shown[5]; patients even with high IgE responses tolerate the vaccine well. To the best of our knowledge, no published reports have established a link between vaccine antigen-specific IgE and immediate-type allergic reaction to the vaccine.

Immunization and development of allergy

Immunizations have been suspected by the public and by physicians to promote the development of allergy, and concerns in this regard contribute to delays or incomplete immunization. Because the onset of atopic disease, especially infantile atopic dermatitis, is often temporally associated with immunization, parents frequently question a possible association between immunization and the emergence of eczema or food allergy (Figure 1). Further, because early childhood infections are inversely associated with allergic disease, it has been hypothesized that deprivation of T helper 1 (Th1)-type

immune response inducing childhood infections owing to early immunization may skew the immune system toward development of Th2-dependent allergic disease (Figure 2).

Epidemiologic studies have addressed the possible effects of immunization, and overall have shown no effect on allergic disease[28–30] or even an inverse effect of cumulative vaccine antigen dose numbers and allergic sensitization, allergic disease, and disease severity of infant eczema.[31,32] In concordance, regional immunization rates were inversely associated with allergic disease.[33] These studies provide no justification

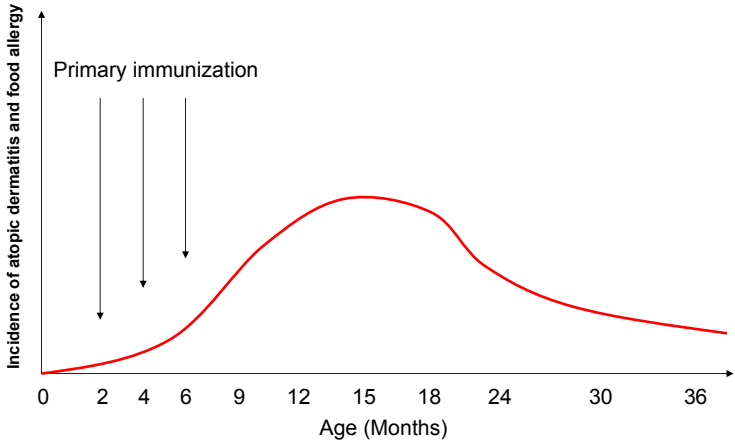


Figure 1 Occurrence of atopic dermatitis and age at primary immunization.

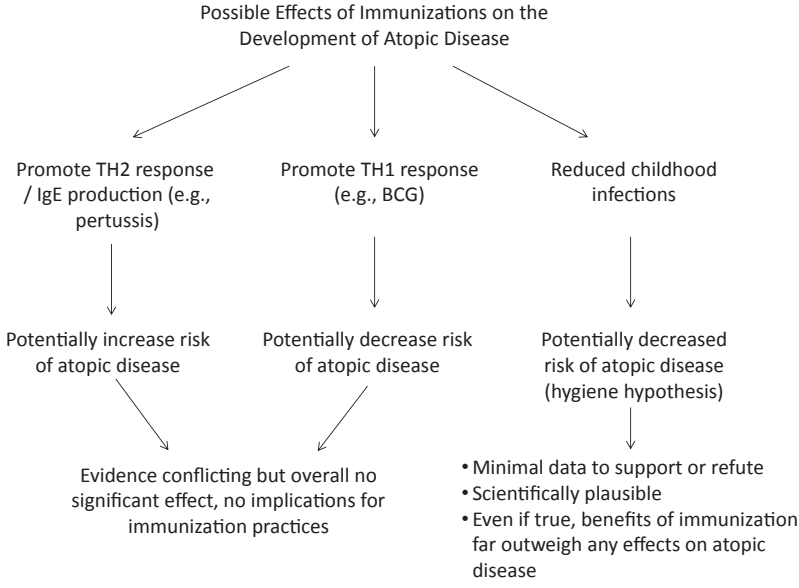


Figure 2 Possible effects of immunizations on the development of atopic disease.

to delay or withhold routine immunizations in children at average or heightened risk for development of allergic disease.

Specific vaccines have also been investigated in this regard. Pertussis immunization has been suspected to be proallergic because pertussis toxin can enhance IgE-formation. Data from a randomized intervention trial show no increased risk of allergic disease up to age 7 years.[34] Lower rates of MMR immunization have been associated inconsistently with reduced rates of allergic sensitization and allergic respiratory in some studies of children with an anthroposophic background.[35,36] Diphtheria-tetanus immunization was associated with asthma in one study[37] but not in others. Further studies found no effect of MMR,[31,35,38,39] *Haemophilus influenzae* type B,[40] polio,[41,42] varicella, or DTP immunization[29] on allergic sensitization or allergic disease.

Mycobacterial lipoproteins are inducers of a strong Th1-type immune response. In experimental models, they suppress allergic airway disease.[43] Children with evidence of mycobacterial infection display reduced proallergic Th2-type cytokines, allergic sensitization, and clinical allergy.[44] A systematic review and meta-analysis suggested that Bacillus Calmette–Guérin BCG vaccination is unlikely to be effective in preventing allergic sensitization or eczema but it might possibly offer some transient benefits against the development of asthma.[45]

Considerations for some specific vaccines

Diphtheria/tetanus/pertussis

Anaphylactic reactions to DTP vaccines are rare (<1/100,000 doses).[46,47] Pertussis immunization has been suspected to promote allergy in susceptible infants,[48] and in experimental models pertussis toxin is used to enhance IgE responses. In children, particularly acellular pertussis vaccines induce a Th2-type cytokine pattern and a vaccine antigen-specific IgE response, even enhanced in atopic infants.[49,50] Elevated serum levels of pertussis-IgE may also be associated with local reactions.[4,51] Even high specific IgE-titers against diphtheria, tetanus, and pertussis antigens are relatively frequently observed and thus are not useful in predicting anaphylactic reactions.[3,5,52] Overall, DTP immunization is unlikely to promote allergic disease in childhood; prospective data from randomized-controlled trials show no excess risk of pertussis-immunized children up to adolescence.[34,53]

Casein has been implicated as a potential cause of anaphylaxis in a few severely cow's milk–allergic children who reacted to DTP immunization.[25] Trace amounts of residual casein from the vaccine production process have been detected in the vaccine, but because it is not entirely clear whether casein is the culprit allergen in these few cases, and because the vast majority of severe cow's milk–allergic patients tolerate the vaccine, regular DTP immunization of cow's milk–allergic patients seems to be justified.[1,54]

Rotavirus

Immunization against rotavirus has now been performed in hundreds of millions of cases without exceptional risk of allergic reactions. The oral applicator of one brand

(Rotarix) includes latex rubber. In highly latex-allergic children, an alternative brand should be used.

Measles, Mumps, and Rubella

Viruses for most MMR vaccines are grown on chick embryo fibroblast cell culture. Historically, residual egg protein has been a concern in patients allergic to hen's egg. MMR vaccines for which viruses are produced in egg-free culture have been developed but are not licensed for children and not uniformly available in all countries. Concerns have also been raised regarding possible inferior immunogenicity of these vaccines.

Most important, the amount of ovalbumin in current MMR vaccines is extremely low (<1 ng/dose) and data are clear that MMR immunization is well-tolerated in egg-allergic patients.[55–58] Consequently, all guidelines have removed earlier contraindications for egg-allergic patients who can be immunized with a regular MMR vaccine in a regular setting.

Although neomycin has been suggested to cause adverse reactions to MMR, it is likely that gelatin is the specific vaccine component that is the most common cause of immediate-type reactions to this vaccine.[7,59]

Influenza

Influenza is a known trigger of asthma exacerbation[60] (Jain et al., 2009). Yearly immunization against seasonal influenza is recommended for asthmatic children and in most countries for all children aged >6 months. Two types of vaccines against influenza are licensed for children: injectable inactivated influenza vaccines (IIV) and nasally applicable live attenuated influenza vaccine (LAIV).

Injectable IIV is safe in asthmatic children.[61] Application of LAIV to the airways of infants aged <2 years is associated with increased wheezing compared with IIV, but not in older children.[62–64] Nasally applicable LAIV thus is not recommended for infants aged <2 years. In children aged 2–8 years, the immunogenicity of LAIV is superior to that of IIV.[65]

The safety of LAIV in children with asthma still has not been resolved. In children aged 6–71 months with mild to moderate asthma or prior wheezing, data from two large immunization trials showed no difference in wheezing rates between IIV- and LAIV-immunized children.[66] Currently in the US, LAIV is not approved for use in children with asthma, whereas in Europe, LAIV is contraindicated for children with severe asthma or active wheezing.

LAIV and IIV are produced in embryonated hen's eggs and contain amounts of residual ovalbumin that may be considerably higher (up to about 1 µg/dose) than in MMR vaccines. However, there is accumulating evidence that injectable IIV are safe in patients with even severe egg allergy.[67–73] A US joint task force representing national allergological societies updated its recommendation for immunization of egg-allergic patients with no special protocol, although this approach is not yet universally accepted.[74] The safety of LAIV in egg-allergic patients has yet not been

established; therefore, LAIV is not recommended in this risk group. Recommendations may change in this regard depending on the results of ongoing trials in egg-allergic patients.

Diagnostic aspects

The occurrence of severe allergic reactions to vaccines is rare but relevant. Severity ranges from mild local reaction to life-threatening anaphylactic shock. Anaphylaxis is a rapidly evolving hypersensitivity reaction to allergens that may cause death. Symptoms usually start within an hour after immunization, most often in the first 30 min after immunization with a delay of only a few minutes. Anaphylaxis is to be differentiated from vasovagal reaction on immunization.

If a patient has reacted allergic to a vaccine, identification of the culprit allergen is important because it may help identify an alternate vaccine formulation without the offending allergen for the next vaccine dose, and because the allergen (e.g., a stabilizer) may be contained in other vaccines as well. The availability and accuracy of these diagnostic tests are unfortunately limited.

Before any immunization, the child's guardian should be asked about possible allergic reaction to a previous immunization. History taking should include questions about allergic reactions to allergenic substances in the vaccine (e.g., hen's egg, gelatin, antibiotics). Important questions before immunization are: "Is your child allergic to hen's eggs, gelatin, milk, antibiotics, or yeast?" "Has your child had any reactions to an earlier immunization? If yes, how and how rapid after the immunization?" Allergic reactions should be discriminated from nonallergic local reactions, not vaccine-related exacerbations of a preexisting allergic disease, and coincident allergic reactions to allergens not contained in the vaccine. In case of a past allergic vaccine reaction, the vaccine product, manufacturer, and batch should be determined if possible.

Skin prick tests with allergen are less sensitive but possibly more specific; there is a risk of false-negative tests. Intradermal tests are highly sensitive but less specific. There is a relatively high risk of false-positive tests.[75] For the prediction of allergic vaccine reactions, no thresholds are defined. A pragmatic approach of sequential skin testing with a vaccine and vaccine constituents may include:

- skin prick test with 1:10 dilution (only if index of suspicion is high)
- if negative, skin prick test undiluted
- if negative, intradermal test, 1:100 dilution
- if negative, intradermal test, 1:10 dilution
- if negative, intradermal test, undiluted

The chance for a false-positive test is high with 1:10 dilution and undiluted allergen, particularly for influenza, MMR, and varicella vaccines.[75] All test results should be interpreted with caution.

Few IgE tests are commercially available for vaccine antigens or components, including hen's eggs, gelatin, and cow's milk. For research purposes, it is possible to couple vaccine antigens with a carrier substance in an immunological assay.[5,52,76] Like skin tests,

IgE tests may support the identification of culprit allergens, but their results have to be interpreted with caution. In particular, IgE-formation to vaccine antigens is part of the regular immune response and most likely is not evidence of an allergenic vaccine reaction. Depending on the vaccine antigen, vaccine antigen-specific serum IgE can be detected in more than 30% of atopic vaccines and sometimes with a titer as high as RAST class 4 in patients who tolerated the vaccine without anaphylaxis.[5] There are no established IgE cut-off values for the prediction of allergic vaccine reactions, and they are not to be expected.

For patients who reacted allergic to a vaccine, revaccination is an option in selected cases in which immunization is strongly indicated. Earlier allergic reaction to a vaccine defines high risk for a subsequent allergic vaccine reaction, although in one study all 38 patients with anaphylaxis at previous immunization tolerated the next dose with no problem.[77] Because of the high risk, written consent should be obtained and revaccination should be performed under direct observation in a clinical setting, allowing rapid intensive care if needed. As a pragmatic approach, fractionated dosing (administration of 10% of the full dose; if tolerated, followed after 30 min by the remaining 90%) and graded desensitization (sequential administration of a 0.5-mL dose at 15-min intervals as tolerated: 0.05 mL, 1:10 dilution; 0.05 mL, full strength; 0.1 mL full strength; 0.15 mL full strength; and 0.2 mL full strength) have been suggested for highly sensitive individuals.[78]

Identification of patients at heightened risk

Most anaphylactic vaccine reactions happen in a low-risk stratum, that is, in patients who have not reacted previously to a vaccine. Currently available tools do not allow good prediction of patients who will react to a vaccine. An anaphylactic vaccine reaction can occur in any patient.

Atopy or a positive family of allergy are not a contraindication for immunization. Patients with allergic disease such as atopic dermatitis, bronchial asthma, or hay fever should be regarded as an average risk population for most vaccines. They deserve timely immunization according to public immunization schedules because of the risk of being not immunized, and thus being exposed to vaccine-preventable diseases outweighs the risk of being immunized and being exposed to the risk of vaccine side effects.

Patients with an antecedent allergic reaction to a vaccine or a current reaction to one of its components should be considered at high risk for a subsequent allergic reaction. History taking before immunization should include questions after previous allergic reactions to a vaccine or constituents of the vaccine to be administered.

Immunization of patients at heightened risk

Because most allergic vaccine reactions are unpredictable, immunization should generally be performed in a setting that allows rapid and effective first intervention in the rare case of an anaphylactic vaccine reaction. Most anaphylactic reactions occur

within 30 min after immunization. Because of the relatively low incidence of anaphylactic reactions, most guidelines do not demand a mandatory observation time for standard-risk patients. If an allergic type I reaction occurs, observation until the resolution of symptoms is recommended.

Patients with an antecedent allergic reaction to a vaccine or to one of its components are at heightened risk for a subsequent allergic reaction. Generally, if a patient has reacted to a vaccine and if the culprit allergen has been identified, the allergen should not be administered again. If protection by immunization is needed (high risk without immunization with low protective immunization titer), an alternative product without the allergenic component should be sought.

It has been discussed whether a delay of routine immunization prevents the development of allergic disease. In a cross-sectional survey in the US among 117 infants with asthma onset within the first 15 months of life, those with a delay in DTP, MMR, and OPV immunization had significantly fewer visits to a subspecialist for asthma than those who were up-to-date.[79] This could be the result of febrile infections (Th2-type antagonizing) or less concerned parents. In a retrospective study from Canada, among 11,531 children who received at least four doses of DPT, the risk of asthma was reduced to half in children whose first DPT dose was delayed by more than 2 months, and the likelihood of asthma in children with delays in all three doses was 0.39 (95% confidence interval, 0.18–0.86).[80] In two United Kingdom (UK) preschool cohorts, a protective effect of early immunization against asthma was found.[81] In a further UK study with 29,238 children up to age 11 years, no association was found between the timing of DTP, polio, and MMR immunization and consultation frequency because of asthma and atopic dermatitis.[82] Considering that the bulk of the data do not support a possible link between immunization and allergy, there is no justification to delay routine immunization in an effort to prevent the development of allergic disease.

Conclusion

Immunizations effectively protect from infectious diseases that could otherwise lead to severe disablement and death. For many vaccine-preventable diseases, the risk of severe sequelae is 1000-fold higher than the risk of a severe side effect from the vaccine. Severe allergic reactions to vaccines are rare and are not predictable in most cases. Efforts should aim to keep risks of both vaccine-preventable disease and immunization as low as possible. Careful immunization policy allows immunization of almost all allergic children and will contribute to the better health of this population.

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Breast—Always Best?

15

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Introduction

Is breast always best? What a question! Humanity would not have survived without breastfeeding (Figure 1). Breastfeeding is natural, and human milk is the optimal source of nutrition for the term infants during the first 6 months of life. It contains all of the nutrients and important protective factors the infant needs to survive and thrive outside the womb. Certainly, even under the best conditions, defaults, misfortunes and accidents might have happened. This had to be tolerated in a natural environment. But we have changed and optimized our world, and we are not willing to accept any inconvenience, even if failures or diseases were produced by our own modern lifestyle.

The benefits of breastfeeding for infants include optimal growth, development, and nutrition, defense against infections, the enhancement of maternal–infant bonding, and possibly avoidance of allergic diseases. During the past decades, breastfeeding has been associated with decreased risk and severity of infants' infectious such as diarrhea and respiratory infections, especially in developing countries.[1,2] The protective activity of breastfeeding against infections is explained by antimicrobial factors present in human milk[3,4] and a lessened risk of contamination with pathogenic microorganisms, particularly when hygiene is poor. Although knowledge is still accumulating, breast feeding may also impart specific immune advantages to the neonate through enhancement or induction of the still-developing neonatal immune system.

From the immunologic perspective, the first few months of life represent a window of opportunity for neonatal oral tolerance induction and prevention of allergy. During this critical time period, both genetic and environmental factors, including maternal immune status, the age of introduction of a foreign food protein, and immunoregulatory factors in breast milk may have important roles in tolerance development. The maternal rather than paternal atopic status has a greater effect on the atopic outcome of the progeny, which raises the question of whether maternal factors such as maternal immune status, immunologic constituents of breast milk, and dietary and environmental exposure in utero and through breast milk have a role in tolerance induction. In mice, breastfeeding has been shown to have a crucial role in tolerance induction using adoptive nursing models.[5–7] On the contrary, in epidemiologic studies, there is conflicting evidence on the protective impact of breastfeeding against allergic diseases, which may at least partly result from differences in breast milk composition. This chapter will review the historical aspects and epidemiologic studies assessing the protective role of breast milk, and the impact of maternal interventions during lactation

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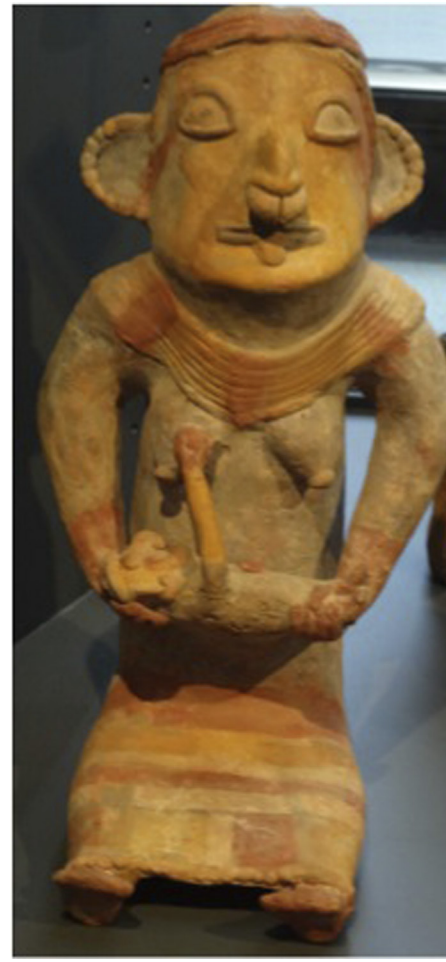


Figure 1 Without breastfeeding, humanity would not have survived. A pre-columbian statue of a breast-feeding mother in the museum of pre-columbian art in Santiago de Chile, Santiago, Chile (Inka mother).

on development of atopic diseases in infants and young children. The immunologic composition of breast milk, which could affect development of the immune system and allergies, will also be discussed.

Historical aspects

In 1572, the Italian physician Girolamo Mercurialis considered that an oozing pruritic condition of the head occurring in suckling infants may be linked to mother's milk.[8]

However, in 1936, two pediatricians in Chicago reported that of the 20,061 infants they had seen in 5 years, "infantile eczema" occurred seven times as often in infants fed cow's milk formula and twice as often in partially breast-fed compared

with fully breast-fed infants.[9] All infants received small amounts of orange juice from week 4, cod liver oil from 6 weeks, and solid food after 4 months.[10] Each infant was observed for 9 months. An infant was considered to have infantile eczema if there were any lesions on the face and body (not including cradle cap) at the time of examination. The eczema definition was probably not precise. For example, age- and season-specific rates of gastrointestinal and respiratory infections of these infants revealed distribution shapes similar to those for eczema, which suggests that some infectious manifestations might have been included in the eczema diagnosis.[11]

The evidence that breastfeeding reduces infectious morbidity, and in developing countries even mortality, has not changed to date, but the discussion on the protective role of breastfeeding in atopic diseases is ongoing.[12–14]

Challenges with studies

The clinical outcome of atopic disease is not a uniform phenomenon. Although clinical conditions have been known since the ancient times, the symptoms and definitions of the clinical manifestations have to be readapted from time to time, at least as knowledge increases regarding the etiopathology.[15] The clinical course and the morphology of each atopic manifestation, such as eczema, allergic rhinoconjunctivitis, and asthma, are variable and age-dependent. Especially in early life, it is difficult to assign symptoms for an atopic condition. Outcome definitions vary between studies and may be based on sensitization or self-reported allergy as opposed to clinical allergy exemplified by food allergy, for which the reference standard is the oral food challenge. Standardized definitions might be helpful, but it is also necessary to standardize the examiners; of course, this is impossible when relying on parental reports or doctors' diagnoses, or when looking at results from earlier decades.

Likewise, the definition of breastfeeding varies among studies. According to the World Health Organization, exclusive breastfeeding requires breast milk feeding (including feeding expressed milk) with no intake of any other liquids or solids, except for medicine, drops, syrup, and oral rehydration solutions.[16] Predominantly breast-fed describes an infant who receives water, or water-based drinks or juices, but no baby formula. Partial breastfeeding refers to the combined use of breast milk and formula. However, there are different definitions for breastfeeding among studies. Furthermore, the mode of timing of introducing solid foods is a significant confounding factor that may affect results.

To avoid the impact of reverse causation, for highly evident results, as in double-blind, randomized, prospective studies, the exposure or predictor "breast feeding" would have to be randomly assigned. However, neither the observer, nor the mother nor the child can be blindly assigned to breast or bottle feeding. Anyhow, random assignment to breastfeeding or formula feeding would be unethical. Therefore, the short-term and long-term effects of breastfeeding compared with bottle feeding a formula are derived from observational studies implying that other factors may confound

the association. In addition, the accuracy of the determinant breastfeeding depends almost exclusively on mothers' reports.

Evidence for breastfeeding in allergy prevention

In the 50 years after publication of the article by Grulée and Sanford, numerous studies have investigated the effect of breastfeeding on atopic diseases. The results were conflicting; some authors claimed a protective effect, some were undecided, and a few even suspected that breastfeeding might promote the development of atopic diseases.[17] In a review article on these studies, Kramer worked out 12 methodological standards for rating the original research articles, eight of which were concerned with biological standards (four exposure and four outcomes), whereas four standards dealt with statistical methods.[17] According to these standards, the nine studies claiming a protective effect of breastfeeding on *atopic eczema* performed less well than the 12 studies not affirming the protective role of breastfeeding. Of the 12 studies on *asthma*, seven found a protective effect and six denied it, whereas the seven studies on *allergic rhinitis* mostly did not claim that breastfeeding was beneficial. To avoid another 50 years of unresolved controversy, an improvement in design and analysis was demanded.[17]

Gdalevic and coworkers conducted a systematic review with a meta-analysis of prospective studies published in the years 1966–2000 that evaluated the association between breastfeeding and atopic diseases. They selected articles complying with the Kramer standards, except that they could not take into consideration the dose–response relationship for breast-feeding exposure (intensity and duration) and the statistical power of the results. Instead, they selected only studies with at least 3 months of exclusive breastfeeding. According to these criteria, they found 18 prospective studies from developed countries on childhood eczema and conducted a meta-analysis.[18] The results of the meta-analysis showed that at least 3 months of exclusive breastfeeding protected from *eczema* (odds ratio [OR], 0.68; 95% confidence interval [CI], 0.52–0.88). The effect estimate was even greater with a family history of atopy (OR, 0.58; 95% CI, 0.41–0.92). Similarly, a meta-analysis of 12 studies on the association between breastfeeding and *asthma* showed a protective effect on asthma, with an OR of 0.70 (95% CI 0.60–0.81), and an even stronger effect for children from atopic families.[19] The effect on *allergic rhinitis* from six strictly selected prospective studies was smaller (OR, 0.74; 95% CI, 0.54–1.01).[20] The mean age of children who were observed was 4.5, 4.1, and 2.25 years in the analyses of eczema, asthma, and rhinitis, respectively, which means that most of the outcomes encompassed early disease outcomes.

A prospective study in Arizona used maternal asthma and atopic sensitization of the child as a risk factor for later asthma. It could be shown that breastfeeding was associated with protection against *recurrent wheeze* in early life, but it was associated with an increased risk of *asthma* beginning at age 6 years, especially in children exclusively breast-fed for at least 4 months, but only in atopic children with asthmatic mothers.[21] Although the results from a long-term prospective study from New Zealand[22] could somehow be explained by these findings, they produced general excitement and even

outrage: Using the strict standards proposed by Kramer's 1988 review, except for dichotomizing breastfeeding to under 4 weeks or at least 4 weeks, formerly breast-fed children reported more often current *asthma* at each assessment between ages 9 and 26 years. In contrast, in a large prospective study in Australia, the overall effect of exclusive breastfeeding for at least 4 months was protection against asthma in children at age 6 years.[23] However, looking closely at the results, one can clearly see that the 6-year asthma prevalence in each of the stratified risk groups is slightly higher for children breast-fed less than 4 months exclusively, but not significantly different from those with a longer breast-feeding duration.

In the German Multicenter Allergy Study (MAS), the prevalence of *atopic eczema* in the first 7 years increased significantly with each additional month of breastfeeding, but the risk was higher if parents had a history of atopic eczema and if the child had other signs and symptoms of an atopic condition.[24] Although in the data analysis possible confounders were controlled for, reverse causation could not be excluded. Mothers probably extended breastfeeding once the infant had developed eczema, because they believed that breastfeeding is protective.

In the Melbourne Atopy Cohort study, in which infants were observed at 4-week intervals, Cox model data analysis demonstrated that early signs of atopic disease or sensitization were associated with a reduction in the risk of ceasing exclusive breast feeding, which demonstrated that reverse causality is a problem in interpreting the association of breast feeding and early atopic disease.[25]

To avoid this disease-related modification of exposure, infants in a large prospective birth cohort study in Sweden who had symptoms of eczema or asthma while being breast-fed were removed from the dataset.[26] In the remaining children, who obviously until then tolerated breastfeeding, the authors claimed that exclusive breastfeeding for at least 4 months reduced the risk of *eczema* at age 4 years.

As is clear for asthma, atopic eczema in early infancy is probably not a unique entity. It is a heterogeneous clinical phenotype caused by genetic predispositions, environmental factors, barrier disruption, and a hyperreactive immune system.[27] To find out which risk factor caused eczema, a purely descriptive diagnostic approach was therefore developed for the MAS project by two of the authors (RLB and KEB) that relied on elementary recordings of the skin manifestations and was reproducible in all subsequent analyses. In a significant proportion of children, the eczema manifestations disappeared at around age 3 years. The prognosis for an eczema prolongation up to 7 years was determined by early onset, severity, and other atopic markers and manifestations.[28] As was be shown in a large, international, multicenter cohort study, high levels of immunoglobulin E sensitization to milk, eggs, and/or peanuts were greater in patients whose eczema developed in the first 3 months.[29]

To exclude confounding and reverse causation, in a prospective study in Denmark, a sophisticated risk analysis on the association between breastfeeding and atopic eczema was performed using a regression analysis of exposure before disease onset and incidence of atopic eczema per person-months.[30] Adjusted for demographic variables, parents' eczema, pets at home, and genetic variants of the epidermal barrier protein filaggrin, exclusive breast-feeding duration significantly increased the risk of *eczema* in a dose–response manner. In contrast, after adjusting for day care and

mother's smoking, the risk of *wheezing episodes* in the first 2 years was reduced by exclusive breastfeeding with no further effect from duration.

To overcome the limitations of exposure in observational studies, for example, the assignment to a breast-feeding group, a large, prospective, birth cohort, cluster-randomized study was started by Kramer in Belarus in 1996–1997 with over 17,000 mother–infant pairs in 31 maternity hospitals and polyclinics.[31] Sixteen sites were randomly assigned to receive an experimental intervention concerning breastfeeding exclusivity and length, and 15 sites served as controls. Infants in the intervention sites were significantly more likely than control infants to be breast-fed exclusively at 3 months (43.3% versus 13.2%) and more likely to be breast-fed to any degree at 12 months. During the first year of life, they had a significant reduction in the risk of one or more gastrointestinal infections and of *atopic eczema*, but not of respiratory tract infections. However, in the 6-year follow-up, there was no difference in the prevalence of eczema, asthma, or sensitization between groups.[32] Remarkably, according to the ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire used in the 6-year follow-up, the lifetime prevalence of eczema in the control group was only one-sixth the prevalence diagnosed in the first year. This demonstrates that there is a problem in the diagnosis and definition of atopic outcomes for different age groups.

In an invited commentary to the "best systematic review and meta-analysis of the association between breastfeeding and childhood asthma" provided by Dogaru et al.,[33] Kramer stated "that there is an enormous heterogeneity among the studies." which "likely stems from the fact that asthma is a term to denote a highly variable phenotype."[14] In the German birth cohort MAS, even when 36 early exposure factors and four asthma definitions were used to predict asthma risk up to age 20 years, there was no difference between children who had been breast-fed at least 6 months compared with a lower breast-feeding duration.[34]

The conflicting data have been reconciled in a couple of reviews and meta-analyses. The American Academy of Pediatrics Committee on Nutrition clinical report concluded that there is evidence that breast feeding for at least 4 months, compared with feeding formula made with intact cow's milk protein, prevents (or delays) the occurrence of atopic dermatitis, wheezing, and *cow's milk allergy* in early childhood.[35] One multidisciplinary review of scientific literature (1966–2001) of 132 studies with 56 deemed "conclusive" determined that breastfeeding is protective of atopic diseases, and that the impact appears to be stronger in children with atopic heredity.[36] Another review found that exclusive breastfeeding for at least 4 months was associated with a lower cumulative incidence of cow's milk allergy until age 18 months.[37] In their systemic review and meta-analysis, Kramer and Kakuma[2] concluded that breast feeding does not appear to prevent challenge-confirmed food allergy,[38] although this was based on only one single qualified study. More recently, the Tasmanian Asthma Study reported that exclusively breast-fed children also had a reduced risk of asthma and food allergy at age 7 years.[39] Alltogether, although data on the protective effect of exclusive breastfeeding on atopic dermatitis are accumulating[40], few studies have examined the effects on development of food allergies, other than cow's milk allergy.

The Boston birth cohort study of multiethnic mother–infant pairs demonstrated that in children observed to about 2.5 years, the adjusted risk of *food sensitization*

against one of eight common food allergens was 1.5 times higher (95% CI, 1.1–2.1) in breast-fed children compared with never-breast-fed children, and slightly higher with exclusive breastfeeding for at least 4 months.[41] This association was modified by a variant in the interleukin-12 (IL-12) receptor β1 gene (*p* for interaction 0.0007). Breast feeding increased the risk of food sensitization in children carrying the GG genotype but decreased the risk in children carrying the GT/TT genotype. Similar associations were found for gene markers in the Toll-like receptor-9 (TLR9) and thymic stromal lymphopoietin genes.

Likewise, a cross-sectional study in Korean schoolchildren found a decreased adjusted risk of *aeroallergen sensitization* in children who carried a CT/CC genotype.[42]These gene–environment interactions may explain why in many studies breast feeding was protective against atopic markers or diseases in children of nonatopic families, and in others, only in children of atopic families.

Possible mechanisms by which breastfeeding may protect against allergies

The mechanisms by breastfeeding might affect (prevent or predispose) development of allergies may result from its impact on delaying introduction of sources of foreign proteins (infant formulas or solid foods) to the infant or from immunologic factors in human milk that can provide help to the maturing immune system of the neonate and therefore prevent allergy development.

Use of infant formulas versus breastfeeding

The predominant alternative for breastfeeding is infant formula, which is typically based on cow's milk. Such feeding can be a specific risk factor for development of cow's milk allergy (CMA). In fact, early exposure to cow's milk protein was shown to be a risk factor for developing CMA. A total of 6209 unselected healthy, full-term infants, 5385 of whom (87%) required supplementary milk while in the hospital, were randomly assigned to receive cow's milk formula, pasteurized human milk, or whey hydrolysate formula.[43] The comparison group was composed of infants who were exclusively breast-fed. The infants were observed for 18–34 months for symptoms suggestive of CMA. As a result, the cumulative incidence of CMA in the infants fed cow's milk formula was 2.4%, compared with 1.7% in the pasteurized human milk group (OR, 0.70) and 1.5% in the whey hydrolysate group (OR, 0.61). In the comparison group (exclusively breast-fed), CMA developed in 2.1% of the infants, which suggests that exclusive breastfeeding does not completely eliminate this risk of CMA.

The German Infant Nutritional Study observed 945 high-risk newborn infants in a randomized trial investigating the effects of breastfeeding supplemented with a partially hydrolyzed whey formula (pHF-W), extensively hydrolyzed whey formula (eHF-W), or extensively hydrolyzed casein formula (eHF-C) in the first 4 months of life.[44,45] Mothers were encouraged to breast feed exclusively for 4 months; however,

about two-thirds in each formula group introduced a study formula during the first month. Clinician diagnosis of any allergic disease (atopic dermatitis, food allergy, allergic urticaria, asthma, and allergic rhinitis) was significantly lower in the eHF-C and pHF-W groups at age 3 and 6 years (eHF-C: OR, 0.76, 95% CI, 0.63–0.93; and OR, 0.80, 95% CI, 0.69–0.93, respectively; pHF-W: OR, 0.78, 95% CI, 0.64–0.95; and OR, 0.82, 95% CI, 0.70–0.96, respectively). However, rates of urticaria, food allergy, and asthma were not affected at age 3 or 6 years for any of the formulas.[45,46] On the contrary, another study showed that early exposure in infancy (less than 14 days of age) to cow's milk protein via formula as a supplement to breastfeeding might protect against IgE-mediated CMA.[47] This large prospective study of 13,019 infants in Israel investigated by telephone interview the association of age of introduction to cow's milk formula and development of CMA. All mothers were encouraged to breast feed. It was noticed that introduction between 4 and 6 months of age was associated with the highest risk for CMA. In the study, feeding formula on a daily basis was qualified as introduction of formula. Sporadic exposures such as in a nursery were not counted toward formula introduction, which may be a confounding factor. Symptoms after the start of formula were questioned, but symptoms before initiating formula feeding were not, which may have influenced feeding decisions, raising issues of potential reverse causality. Also, the amount needed to prevent allergy was not determined from the study. Furthermore, the incidence of CMA in this Israeli cohort was surprisingly low compared with other studies from Westernized countries, which suggests differences in patient populations, potentially based on different genetic backgrounds.

Similarly, the introduction of solid foods may be related to the duration of (exclusive) breastfeeding. This is covered in other chapters of this book.

Human milk immunomodulatory components

Human milk originates in lactating mammary tissue. The basic structural unit is the alveolus, which consists of lactating cells that secrete milk into an adjoining lumen. The lumen connects to a duct system that drains the collected milk to outlets at the skin's surface. Individual arteriovenous capillary systems provide each alveolus with the individual nutrients needed to produce milk. Milk lipid, lactose, and most milk proteins, which currently add up to over 900 proteins,[48,49] are produced in the lactating cells.[50] Human milk contains immune cells, immunoglobulins, cytokines, chemokines, growth factors, lactoferrin, oligosaccharides, enzymes, peroxidases, lysozyme, secretory component, hormones, and foreign food antigens, bacteria, and viruses. Several of these bioactive factors have been assessed in relation to development of allergies in the infant. Several of these immunologically active factors in human milk are missing in processed cow's milk and infant formulas and the whey-to-casein ratio is markedly higher in human milk.

In early life, intragastric protein digestion is underdeveloped and high concentrations of antiproteases, alpha 1-antichymotrypsin, and alpha 1-antitrypsin, which impede proteolysis, are present in human milk.[51] Previous work on secretory IgA in breast milk has shown that it is resistant to the proteolysis in the gut and may be recovered in active form in the feces of infants.[52] As for IgA, it may exert its function

locally, because specific IgA antibodies are not absorbed in significant amounts from human milk.[53] The fate of breast milk–derived cytokines in the neonatal and infant gut is under debate. In the current study, most of the cytokines were detected in quantities known to exert their function in in vitro assays. With an exclusively breast-fed infant ingesting about 500 mL of human milk a day in early life, the level of cytokines equals micrograms per day. Therefore, it is possible that breast milk–derived cytokines can persist in the recipient in biologically active quantities and possibly exert their function acting on the gut epithelium.

Secretory IgA

The predominant immunoglobulin in breast milk is IgA, most of which is in the secretory IgA (SIgA), with smaller amounts of IgG and IgM.[54] Human milk IgA is produced by B cells in the mammary gland that have migrated from the mother's intestine via the enteromammary link.[55] Therefore, antigenic stimulation encountered by the maternal gut directs the antibody specificity of breast milk.[56,57] Secretory IgA reinforces the epithelial barrier and thus inhibits inappropriate immune activation by microorganisms and antigens in the lumen of the intestinal and respiratory tracts.[58] Maternal SIgA is provided by breast milk during the early postnatal period to support dimeric IgA produced by plasma cells, which is generated only after birth.[58]

Studies have been conflicting in the association between total and specific IgA levels in breast milk and development of allergic disease in older children.[59–61] Because food allergy represents a failure to develop mucosal tolerance to foods and typically develops during the first year of life, immune factors in human milk may have an important and direct impact on protection against food allergy compared with development of other atopic diseases in which oral exposure has a lesser role and development of a disease is more remote in relation to the breast-feeding period. It has been consistently reported that lower levels of total and CM-specific IgA are present in colostrum and breast milk of mothers with offspring developing CMA.[62,63] The etiology of low breast milk IgA is unknown but is unrelated to maternal atopy.[60,63] Because it is known that IgA responses to microbes are constantly modified to reflect the microbiota present in the gut lumen, it is possible that continued oral exposure to a particular antigen is required to maintain specific IgA responses.[64] Low levels of cow's milk–specific IgA levels have been associated with maternal avoidance diets restricting cow's milk protein,[65] although with some conflicting findings.[66,67] Likewise, the exact function of human milk IgA is unknown, although in a model of gut epithelial uptake, human milk with high levels of specific IgA was shown prevent uptake of cow's milk antigen better than low levels of IgA, which suggests a role in immune exclusion.[68,69] Alternatively, SIgA has been shown to target dendritic and T cells in Peyer's patches after transport by intestinal M cells, which could favor focused antigen uptake as immune complexes targeting antigen for presentation, and which could favor tolerance development.[70] It is also possible that changes in maternal diet and milk IgA levels reflect the mother's gut microbiota or that breast milk IgA is capable of influencing the infant microbiome.[71]

Cytokines, chemokines, and growth factors

Human milk is a rich source of immunostimulatory and immunoregulatory cytokines as well as chemokines and growth factors.[72] Immune markers in human milk may originate from human milk cells,[73–75] mammary gland cells,[76] or serum.[77] Immunomodulatory cytokines in breast milk, including transforming growth factor-β (TGF-β), have been shown to influence the development and maturation of the mucosal immune system of the neonates in mice[78] and possibly also in humans.[79,80] Transforming growth factor-β is a regulatory cytokine that in human milk has been associated with prevention of atopic dermatitis at an early age,[79] although subsequent studies have been conflicting.[59,81,82] In food allergy, the concentration of TGF-β1 in colostrum from mothers of infants with IgE-mediated CMA was lower than that from mothers of infants with non–IgE-mediated CMA; however, the levels in healthy controls were in between.[83] Reports on other human milk cytokines, chemokines, and growth factors in CMA are largely lacking.

Few studies have identified levels of cytokines, chemokines, and growth factors in breast milk associated with atopic diseases. Earlier studies using an enzyme-linked immunosorbent assay found that levels of IL-4 are lower and IL-8 and CCL5 (RANTES) are higher in milk from atopic compared with nonatopic mothers[84,85]; others[86] found that cytokine levels were largely not related to maternal atopy. It was shown that other maternal characteristics, such as gestational smoking and race, and season, affected levels of IL-6, IL-8, CXCL8, and CXCL10.[87] Groër et al.[88] showed that exercise was associated with elevated milk levels of proinflammatory cytokines, including IL-1β and IL-17. Probiotic supplementation has been associated with increased IL-10 levels in mature breast milk.[61] Levels of cytokines in general decrease from colostrum to mature milk, although such a decrease may be insignificant because the amount of human milk ingested by an infant increases with age. Interestingly, some growth factors and chemokines increased as a function of duration of lactation, resulting in increasing amounts of these factors ingested with age.

More recently, multiplex assays have allowed simultaneous measurement of multiple cytokines, chemokines, and growth factors present in breast milk at low concentrations.[77,86–90] Some of these studies have related levels to atopic outcomes in the offspring. Soto-Ramirez et al.[77] showed that IL-5 and IL-13 levels in milk, although extremely low, are risk factors for asthma at age 12 months, and high levels of IgA and TGF-β1 were protective. Ochiai et al.[86] showed that high levels of eotaxin (CCL5) in milk were the strongest risk factor for developing atopic dermatitis. Because food allergy represents a failure to develop mucosal tolerance to foods, immune factors in human milk may have a more important and a direct impact on protection against food allergy compared with development of other atopic diseases in which oral exposure has a lesser role. In fact, another study showed that a panel of proinflammatory and regulatory cytokines including IL-1β, IL-6, IL-10, and TGF-β1 in human milk were associated with protection against CMA.[91] It is unclear whether these bioactive factors are directly related to protection or whether they are biomarkers of another protective mechanism.

Leukocytes

Human milk contains 10,000 to 13×10^6 cells/mL, the number of which decays rapidly at 4 days postpartum with transition from colostrum to mature milk.[92] It can therefore be estimated that a neonate ingests billions of breast milk cells daily in the first few days of life. Breast milk contains maternal cells, from leukocytes to epithelial cells of various developmental stages that include stem cells, progenitor cells, lactocytes, and myoepithelial cells, and cell counts as well as leukocyte proportions vary greatly from one mother to another (Figure 2). The predominant cellular component, up 60–90% of milk leukocytes, is the macrophage, followed by neutrophils (8–50%) and lymphocytes (5–10%).[92–97] T cells account for 74–83% of milk lymphocytes.[98,99] There is a small proportion of natural killer cells, 2% eosinophils, and occasional basophils.[94,100] Stem cells have also been detected in breast milk.[101] It is not until the past decade that technological advances allowed characterization of breast milk cell types at the protein and messenger ribonucleic acid (RNA) levels, beyond their morphological appearance.[92]

Macrophages display lipid-filled vacuoles, milk fat globules, and casein micelles[93,96,97,102] and represent a fully mature tissue macrophage population.[103] They show high phagocytic activity and respiratory burst.[93,104,105] Polymorphonuclear cells are highly activated, as evidenced by their high expression of CD11b and decreased expression of L-selectin,[106] although with less locomotive, adherence, microbicidal, and respiratory burst capabilities than those of their counterparts in blood.[107] T cells primarily present memory T cell phenotype and the great majority of T cells express antigens involved in intercellular adhesion (LFA-1 and ICAM-1) and T cell activation (CDw29 and human leukocyte antigen-DR).[99,108,109] Milk T cells also exhibit good

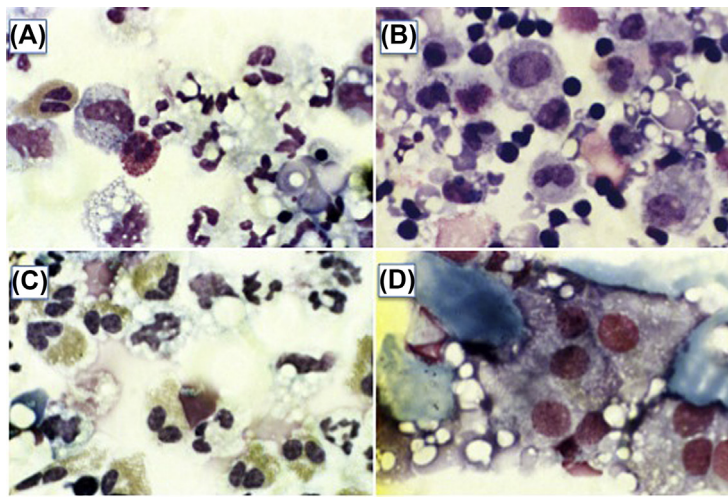


Figure 2 Examples of breast milk cells from different donors. (A) Breast milk contains many types of leukocytes including macrophages, neutrophils, lymphocytes/plasma cells, and even eosinophils and basophils. (B) Lymphocyte predominance. (C) Neutrophil predominance. (D) Secretory epithelial cell cluster.

responsiveness to a variety of bacterial and viral antigens[93,110] and produce significant amounts of interferon-gamma.[108] The proportion and activation of CD8+ cells are higher than in peripheral blood.[94,99] B cells produce IgA[111,112] and the vast majority home from *gut-associated lymphoid tissue* to the mammary gland and transform into plasma cells that remain sessile in the mammary gland.[55,112,113] The migration to the mammary gland appears to be directed by homing receptors.[114]

The function of human milk leukocytes is largely unknown. Although animal studies have shown transfer of milk leukocytes through the intestinal epithelium into the blood circulation in the young and engraftment in different immunologic organs,[98,115–118] this has not been shown in humans. One study showed that the predominant leukocyte in mothers of healthy infants was the macrophage, whereas in mothers of CMA infants, the proportion of macrophages was significantly smaller and large proportions of eosinophils and neutrophils were found more often.[95] Human leukocyte antigen-DR expression was also shown to be higher in milk from mothers with healthy infants as opposed to those with CMA infants.[119]

Soluble CD14

CD14 is the soluble component of TLR4, which has a role in innate immunity. It binds to lipopolysaccharides (LPS) from gram-negative bacteria and intestinal enterocytes. Its absence reduces the TLR4 response to LPS. Colostrum is rich in soluble CD14, with levels decreasing over time, whereas neonates lack CD14. Soluble CD14 levels have been found to be lower in colostrum and breast milk of mothers with children who develop atopy or eczema sensitization.[120] More recent studies either confirm or refute these results.[82] Omega-3 polyunsaturated fatty acid (PUFA) levels were positively associated with IgA and sCD14 levels, which suggests a relationship between fatty acid status and mucosal immune function.[121]

Fatty acids

Studies have shown that milk from atopic mothers has aberrations in its fatty acid content. Atopic mothers' milk showed lower levels of n-3 long-chain PUFA at 1 month of lactation than nonatopic mothers.[122] Human studies have been conflicting with respect to fatty acid composition in human milk and development of allergy. A number of studies examined fatty acid profile in breast milk as a risk factor for subsequent atopic disease. These studies have shown mixed results, but generally found that n-3 PUFAs in breast milk possibly protect against atopic diseases.[122–127] The conflicting findings may be a result from the complex interactions between different fatty acid types and the divergent functions on immune system based on the dose.[128]

Human milk microbiome

Bacterial colonization of the newborn intestine may contribute to the development of neonatal immune functions or susceptibility to immune-mediated disorders in early (and later) life.[129,130] Animal studies have provided direct evidence of the role

gut microbial colonization has in immune developmental processes and the orchestration of appropriate or aberrant immune responses.[131] Human observational studies have also reported imbalances in gut microbiota composition that precede the development of specific immune-mediated diseases later in life.[132] Although colonization seems to be host-specific, breastfeeding is able to influence initial neonatal gut colonization and maturation of the immune responses.[133] In recent years, several culture-dependent and -independent studies have revealed that colostrum and breast milk contain a diversity of bacterial communities colonizing the infant's gut. The amount of bacteria ingested by an infant with 800 mL milk is estimated at 1×10^5 to 1×10^7 daily.[134] The initial culture-dependent studies demonstrated predominance of staphylococci, lactic acid bacteria, streptococci, and propionibacteria, and closely related gram-positive bacteria, as reviewed by Fernandez.[133] Culture-independent molecular techniques, especially those using 16S rRNA genes have confirmed a diversity of bacteria mentioned above, but also the presence of several others including gram-negative bacteria.[135–139] Among hundreds of operational taxonomical units, only nine were found in each woman, but bacterial communities appear to be relatively stable over time within individuals.[136] The microbiota of the breast-fed infant is related to that found in the breast milk.[140] There appears to be bacterial transference from the mother's milk to the infant's gut at least for lactobacilli, staphylococci, enterococci, and bifidobacteria.[141] Comparison of skin and breast milk bacteria confirmed differences in genotypes and the presence of bacteria and suggests an endogenous route for breast milk colonization.[135,142]

Oligosaccharides

Human milk oligosaccharides are complex glycans containing a lactose moiety at the reduced end. They are the third largest solid component in human milk and are minimally hydrolyzed by human enzymes and reach the large intestine, constituting the main substrate for the infants' gut microbiota, particularly for bifidobacteria.[143–145] They have a prominent role in driving the diversity of infant's gut microbiota composition.[146] Some have an inhibitory effect on growth in intestinal cells[147] and some bind specifically to dendritic cells through the lectin receptor dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin.[148] Human milk oligosaccharides have been shown to attenuate mucosal responses to surface inflammatory stimuli during early development, while enhancing signals that support maturation of the intestinal mucosal immune system.[149]

Exosomes

Exosomes are small nanosize membrane vesicles that are released after endosome fusion with the cell membrane in many mammalian cells, including dendritic cells, mast cells, B and T cells, and epithelial cells. The function of exosomes on the immune system is far from understood, and those found in breast milk induce Foxp3 expression on *peripheral blood mononuclear cells* in vitro[150] and contain RNA.[151,152] Although their phenotype may vary with maternal sensitization and lifestyle,[153] their

role in induction of the infant immune system and the development of allergies is largely unknown.

Impact of maternal interventions during breastfeeding on prevention of allergy

Maternal interventions during pregnancy and lactation may affect the composition of breast milk and thus development of immune system in the recipient. The following summarizes studies assessing maternal interventions during lactation and their impact on development of allergy in breast-fed children.

Food avoidance diets

The United States (US) Dietary Prevention Program[154,155] was a randomized, prospective study including 288 subjects with one parent with atopy and sensitization assessing the impact of not only maternal, but simultaneous infant dietary interventions including maternal avoidance and delayed introduction of milk, eggs, and peanuts. There was a decrease in food allergy in the prophylaxis group; however, milk allergy was responsible for the difference seen and by age 7 years differences disappeared. The Isle of Wight study, with an additional aeroallergen avoidance program during infancy, discovered an overall reduction in asthma prevalence from age 1 to 18 years (OR, 0.51; 95% CI, 0.32–0.81), but no difference was found in sensitization at age 18 years between the intervention and control groups.[156]

Kramer and Kakuma in their systematic review from 2012 summarized the quasirandomized and randomized controlled trials (RCTs) looking at the impact of maternal dietary interventions on atopic diseases.[157] They identified four studies that fulfilled their criteria. Intervention period included third trimester in all four and lactation in most. One study looked at peanut, tree nut, and fish avoidance, whereas others assessed milk and egg avoidance. The authors concluded that maternal avoidance diets during pregnancy were not effective in preventing allergic diseases (eczema, asthma, and allergic sensitization) in children. None reported adequate concealment of study allocation and because mother, infant, and environmental intervention varied, it is difficult to draw robust conclusions.

Emerging data on peanuts create even more controversy. Two case-control studies,[158,159] although small, did not detect a significant association of frequent maternal peanut ingestion during pregnancy or lactation on peanut sensitization or peanut allergy in infants, but showed a significant independent relation of peanut allergy with the use of skin preparations containing peanut oil. Observational data from the US support the concept that exposure to peanuts in pregnancy and lactation may be sensitizing. This includes a human cohort of 503 high-risk infants in whom maternal peanut ingestion during pregnancy, but not breastfeeding, predisposed infants to peanut sensitization.[160] The most recent studies include a prospective cohort study of 10,907 in which data on maternal diet during or shortly

before or after pregnancy were collected, as well as information on offspring-reported physician-diagnosed food allergy.[161] The incidence of peanut and tree nut allergy was significantly lower among children of nonallergic mothers who consumed more nuts in their peripregnancy diet. By contrast, a nonsignificant positive association was observed between maternal peanut consumption and risk of peanut or tree nut allergy in the offspring of 146 nut-allergic mothers. As a limitation, all of the studies possessed potential recall bias, and no controlled human clinical trials are available. Furthermore, infant diet, which is typically linked to maternal diet, may be a confounding factor.

Maternal probiotics, PUFA, vitamins, and antibiotics

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. The probiotic/prebiotic supplementation approach aims to shape the gut microbiome toward healthy commensals. A meta-analysis of 14 RCTs showed that maternal and infant use of probiotics modestly decreased the incidence of atopic dermatitis and IgE-associated atopic dermatitis at 12 and 24 months.[162] The favorable effect was similar whether mother or infant, or both, were receiving therapy. However, the therapy was typically administered to mothers starting in pregnancy and the impact attained via breast milk alone is unclear. Evidence is lacking on the effect on eczema severity and duration of effect, and there are limited data to compare probiotic types except for *Lactobacillus rhamnosus*.

Prebiotics are oligosaccharides that contribute about 10% of the solid components in human milk. They promote favorable colonization and via fermentation afford direct anti-inflammatory properties. There are no studies assessing prebiotic supplementation during lactation.

Omega-3 (n-3) PUFAs are found in fish oils and are considered to have anti-inflammatory properties. They have been found in decreasing amounts in Western diets, whereas omega-6 PUFAs, which favor Th2 deviation, are found in increasing amounts. Docosahexaenoic acid and eicosapentaenoic acids are two biologically active omega-3 PUFAs. The impact of perinatal maternal n-3 PUFAs on allergic diseases was summarized in a systematic review of four studies.[163] In these studies, n-3 supplementation during pregnancy reduced the 12-month prevalence of egg sensitization and childhood asthma, but supplementation during lactation did not prevent the development of atopic diseases.

Maternal vitamins and Mediterranean diets have been assessed in a meta-analysis of 62 reports, most of which are not RCT.[164] High maternal dietary vitamin D and E intakes and Mediterranean diet were protective against development of wheezing illnesses. As a whole, most studies with fruit- and vegetable-rich diets associated higher consumption during pregnancy with reduction of asthma, although studies in lactation are lacking.

Maternal use of antibiotics has been associated with increase in asthma, atopic eczema, allergic rhinitis, and cow's milk allergy, but the data are not consistent between studies. Neither eczema nor atopic sensitization was significantly associated with antibiotic exposure through breastfeeding.[165]

Conclusions

In summary, although there is a great controversy regarding the overall impact of breast feeding in preventing allergies and atopic diseases, there is evidence that 3–4 months of exclusive breastfeeding may be enough for such a protective effect. The exact mechanisms providing protection through breastfeeding remain to be elucidated. Studies so far have largely not accounted for gene–environment interactions, and the fact that breast milk varies between individuals and geographic locations, and its protective properties may have changed over time. Breast milk contains networks of immune factors that provide protection against respiratory and intestinal infections and promote intestinal homeostasis and possibly development of oral tolerance (Figure 3). This support may include a concerted action of antibodies, proinflammatory and regulatory cytokines, microbiome, and human milk oligosaccharides, which regulate the gut microbiota and host gene expression. The evidence for allergen avoidance during breastfeeding is in equipoise and the evidence is inadequate to advise avoidance diets in allergy prevention. Probiotic use in late pregnancy throughout lactation may decrease the incidence of atopic dermatitis modestly, although the long-term effect is unclear. Therefore, more

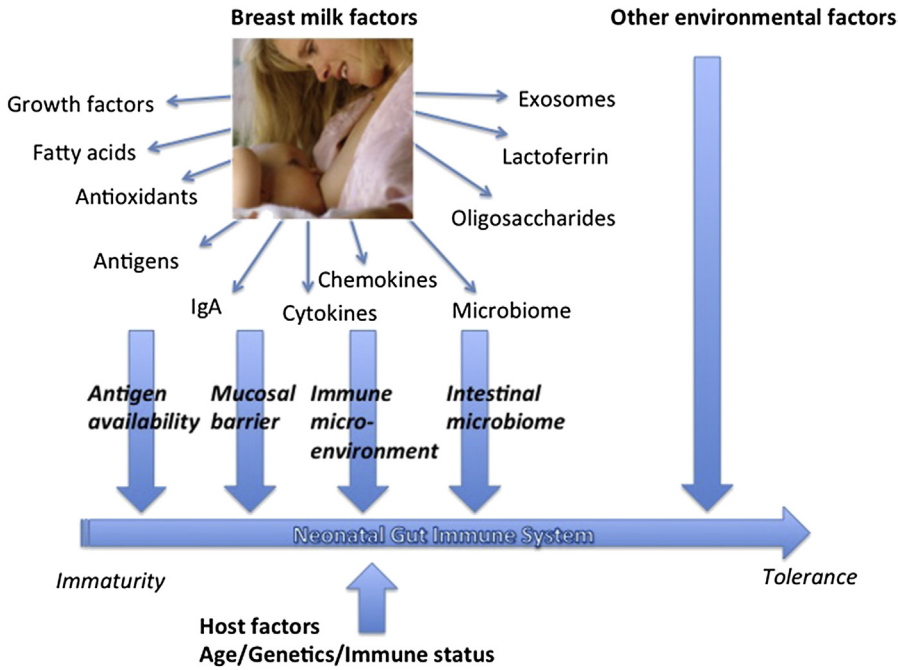


Figure 3 Both environmental and genetic factors influence the development of the neonatal gut immune system. The support provided by breast milk includes a concerted action of antibodies, proinflammatory and regulatory cytokines, microbiome, and human milk oligosaccharides, among other factors, which regulate the gut microbiota and host gene expression. Source of photograph: http://www.bfr.bund.de/de/nationale_stillkommission-2404.html.

studies on maternal/environmental factors, their influence on breast milk composition, and gene–environment interactions are needed for prevention strategies in the future.

Abbreviations

CMA Cow's milk allergy
eHF Extensively hydrolyzed formula
HMO Human milk oligosaccharides
LPS Lipopolysaccharide
OR Odds ratio
pHF Partially hydrolyzed formula
PUFA Polyunsaturated fatty acid
RCT Randomized controlled trial
SIgA Secretory IgA
TLR Toll-like receptor

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Dietary Interventions in Infancy

16

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Introduction

Allergy is a serious health concern that might have life-threatening consequences. In recent years there has been a tendency for the number of allergic disorders to increase. One group of allergic disorders particularly prevalent in infancy and early childhood is food allergy. Estimates show that approximately 8% of children compared with 3–4% of adults have food allergies.[1,2] Together with atopic eczema, food allergy is one of the earliest manifestations of the atopic march, which is defined as the progression from a food-allergic state in early life to inhalant-triggered hypersensitivity and/or asthma later in life.[3] An important characteristic of the allergic sensitization state is the presence of allergen-specific immunoglobulin E (IgE) levels at various stages of life, including the prenatal period.[4] About 80% of food allergies are triggered by proteins such as milk, peanuts, eggs, soybeans, tree nuts, and wheat.[5] The most common food allergy, but also the earliest, occurring within the first year of life is cow's milk allergy (CMA). Although early-life food allergies are spontaneously outgrown with time, those tend to become more persistent. Cow's milk allergy is outgrown in about 85–90% of children by age 3,[6] and egg allergy in about 80% by age 5.[7] However, studies show that percentages of spontaneous remission are declining to 79% for CMA and 68% for egg allergy by age 16.[8,9] Other food allergies, such as peanut and tree nut allergy, are more persistent (only 20% might spontaneously resolve) and are associated with most life-threatening and fatal anaphylaxis.[10,11]

Currently, there is no treatment for food allergy. Both preventive and therapeutic approaches in the clinic focus on avoiding the culprit food. In the meantime, research has been considerably influenced by the hypothesis that food allergies result from the inability to build oral tolerance or from defects in already established oral tolerance to the ingested food proteins. Therefore, scientific interest has been intensively shifting from the allergen avoidance to the tolerance induction approach. The first days of life, or maybe even the time before birth, are considered an important window of opportunity for applying preventive strategies to reduce the risk of food allergic sensitization. Preventive approaches in this period might be able to interfere with the

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immune system development and maturation in a beneficial way to promote the proper establishment of naturally occurring tolerance.

The underlying mechanisms and the etiology are still largely unclear; food allergy is believed to rely on a complex interaction between genetic and environmental factors. Predisposition, or high-risk status, is considered when at least one first-degree relative is diagnosed with allergy. However, many children present with atopic diseases early in life even with no family history of allergy.[3] This highlights the importance of environmental exposure in early life, especially its considerable implication in food allergy development. One of the most significant exposures for immune maturation in early life is the diet. The increasing prevalence of atopic diseases is suggested to relate to decline in breast feeding, changes in modern Western diet (e.g., increased intake of n-6 over n-3 polyunsaturated fatty acids [PUFA]), and reduction in microbial exposure (hygiene hypothesis).[12,13] Furthermore, some dietary components are suggested to possess beneficial immunomodulatory effects. All of these point to the major role of environmental and dietary exposures in the pathogenesis of allergy. It is therefore of great interest to consider dietary interventions with specific dietary components or supplements with recognized beneficial effects on the immune system for prevention strategies in early life. Many dietary components such as breast milk, probiotics, prebiotics, synbiotics, n-3 long-chain PUFA (LCPUFA), and antioxidants are currently being investigated for their potential effect on altering food allergy outcome. In addition, modifying food proteins to reduce their allergenicity might contribute to food allergy prevention and oral tolerance induction. Most important, some strategies might be applicable not only postnatally, but also prenatally because of the implications that certain nutrients in the maternal diet can influence predisposition to allergies via epigenetic changes.[14,15]

The gut, the diet, and oral tolerance in early life

The gastrointestinal (GI) tract is the largest immune organ in the human body. In early life, it is the central site for natural immune tolerance evolution. Rapidly after birth, depending on the method of delivery, the neonatal GI tract is colonized by many types of microorganisms. This process takes about a week, but the number and composition of the bacteria are relatively unstable during the first year of life.[16] A complex interplay between those commensal microorganisms, the mucosal barrier, oral exposure to dietary factors, and host-related factors is responsible for maturation of the gut-associated lymphoid tissue (GALT) and mucosal immune network.[12,14] The importance of the gut microbiota for the immune response is emphasized in studies with germ-free animals that present with abnormal development of Peyer's patches and mesenteric lymph nodes (MLN), together with an impaired resistance to infections.[17] Beyond microbiota, mice fed a balanced protein-free diet were reported to present with poorly developed GALT, reduced levels of IgG, IgA, and lymphocytes, and a predominant T_h2-type immune response.[18] Such studies point to the importance of exposure to both microbes and dietary antigens for proper development and maturation of the immune system in the newborn.

Inflammatory responses to harmless antigens are undesirable and are prevented by the evolution of default homeostatic mechanisms leading to local and systemic specific unresponsiveness, also referred to as oral tolerance.[19] This phenomenon specifically occurs in the gut and develops after exposure to a dietary antigen via the oral route. This highlights the fact that the normal development of oral tolerance is strongly antigen dependent and might require early exposure to food proteins.[20] The mechanisms underlying oral tolerance are not yet understood, but some studies indicate that they involve induction of anergy or deletion of antigen-specific T cells and differentiation of T regulatory (T_{reg}) cells.[21] The induction of T_{reg} cells is facilitated by professional antigen-presenting cells (APC) with regulatory properties, such as dendritic cells (DC), and suppressive soluble factors, such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β) cytokines.[22] The role of T_{reg} cells in inducing and maintaining oral tolerance is considered crucial. Furthermore, Peyer's patches and MLN are important sites for tolerance induction because they accommodate the interactions between DC and naïve T cells.[23] The MLN appear to be the major site for mucosal allergen presentation and tolerance induction because lack of MLN results in no tolerance development.[24] Other factors such as the availability of the antigen, the immune microenvironment, the type and activation status of the APC, the intestinal flora, and the age and genetics of the host are essential for tolerance development.[25,26]

Although neonates have an immature gut and immunomodulatory network, their immune system is considered prone to tolerance induction. Regardless of the T_h2-skewed effector response, it is suggested that any kind of immune response could be induced in the neonate if the appropriate conditions are provided.[27] Therefore, modifiable factors such as gut colonization and diet in early life can be targeted for optimizing immune system maturation and susceptibility to tolerance induction.

Allergens in the diet: from avoidance toward tolerance induction

The classical approach for preventing and reducing food allergic reactions is to avoid the offending food. Restriction diets and strict allergen avoidance have been applied not only to adults but also to infants, neonates, and even fetuses by implementing allergic protein avoidance during early life, lactation, and pregnancy.[27] However, the dogma of allergen avoidance is currently challenged by increasing interest in early life, even prenatal, allergen exposure for its crucial role in tolerance induction.

Allergens in the maternal diet

Next to early postnatal exposures, prenatal exposure to food allergens may have a crucial role in allergy development, and therefore preventive measures during pregnancy might prove effective. In utero exposure to food allergens is suggested to take place via transplacental transport[28] and/or the amniotic fluid.[29] Reports describe that the fetal immune system might be ready for sensitization as early as 20 weeks of gestation,

based on the presence of IgM-positive B cells.[30] Around this time, allergen-specific responses to mitogenic and allergenic stimuli have been demonstrated in cord blood.[31] Furthermore, allergen-specific IgE has been detected at birth and allergic disease has been reported as early as 8 days after birth, both of which imply that in utero acquired antigens could initiate fetal lymphocyte proliferation and sensitization.[32] Such evidence suggests that mothers should avoid allergenic proteins in their diet during pregnancy. However, maternal restriction diets during pregnancy have not resulted in protection from food allergy in high-risk offspring. On the contrary, adverse effects on the maternal and fetal nutrition together with higher, although not significant risk of preterm delivery and lower birth weight were documented.[33] Maternal exposure to food allergens during pregnancy, on the other hand, can benefit allergy prevention. Studies in guinea pigs have shown that oral antigen exposure not only during pregnancy, but also before conception is important for developing tolerance and can prevent postnatal IgE sensitization.[32] Mechanistically, this effect could be attributed to the transfer of maternal allergen-specific IgG, which (1) blocks IgE, (2) suppresses B cells by facilitating the cross-link between neonatal FcγRIIb and B cell receptor, or (3) helps antigenic determinants destruction by phagocytosis.[32] Results from studies in rats confirm that maternal IgG that is passively transferred to the offspring ensures long-lasting suppression against IgE sensitization.[34]

After birth, there is also a risk that allergens from the maternal diet can be transferred during breast feeding. Intact dietary antigens such as bovine β-lactoglobulin (BLG), hen's egg ovalbumin (OVA), gliadin from wheat, and Ara h1 from peanuts have been detected in human breast milk.[35] This implies that mothers should avoid allergens during lactation as well. However, maternal restriction diets during lactation have failed to prevent food allergy development.[33] Instead, evidence from animal studies suggests that maternal exposure to a dietary antigen during lactation facilitates antigen-specific tolerance induction.[36] Studies in nonatopic mice revealed that combined exposure to allergen and TGF-β in breast milk was necessary for the development of regulatory T cells and allergen-specific tolerance in the offspring.[16] In contrast, allergen exposure of lactating allergic mice induces tolerance in the offspring via antigen–IgG complexes formed in breast milk, rather than TGF-β.[37]

The intake of allergenic proteins by pregnant or lactating mothers can influence the immune system of the newborn. However, the exact mechanism of oral tolerance induction in the child via maternal exposure to allergens or allergen alternatives alone or combined with dietary components needs to be clarified.

Allergens in the infant diet

Allergen avoidance in children at risk is an intensive topic of debate. The gold standard for primary allergy prevention in high-risk infants is avoidance of allergens. The rationale behind this approach is that delaying the introduction of complementary feeding and avoiding allergenic foods would reduce the risk of systemic allergic responses in the leaky gut of infants whose mucosal immune system is still immature.[38] In case of peanuts, this approach was applied for up to age 3 years.[39] However, later findings in the field triggered reevaluation of the practices, and in 2008 it was

concluded that there is no sufficient evidence to support specific food avoidance and complementary feeding delay.[40] In their article, Palmer et al. reviewed older and newer (after 2008) birth cohort studies and suggested that early allergen avoidance is associated with increased risk of sensitization.[38] This has been further confirmed in animal models in which allergen restriction in early life was linked to increased IgE sensitization.[41] Studies in humans and animals suggest the existence of alternative routes of sensitization in early life, such as via the skin, which could contribute to the disruption of normal oral tolerance development.[18,42] By avoiding mucosal allergen exposure in early life, the normal acquisition of tolerance upon oral exposure is circumvented and as a consequence the risk of allergen sensitization via the skin is increased.[18]

In past years, scientific interest has shifted toward immune tolerance induction in early life as a prospective preventive strategy. Evidence from animal studies shows that the process of oral tolerance is strongly antigen dependent and that exposure to the certain antigen during a specific time period might be essential for the process.[43] Studies that support early-life allergen exposure as an important factor for tolerance induction in toddlers have observed that children from families in which a peanut snack is consumed early in life are reported to have a low incidence of peanut allergy.[44] A key study emphasizing the importance of early introduction to allergenic foods in oral tolerance reported a lower peanut allergy rate in Israeli infants fed roasted peanut butter products in their first year of life, compared with Jewish infants on a restricted diet in the United Kingdom (0.17% vs 1.85%, respectively).[45] Animal studies further suggest that early-life exposure to a large amount of allergen is more prone to promoting tolerance by means of suppressing IgE responses.[46] To further investigate the potential effect of early allergen exposure on tolerance induction and prevention of allergic sensitization, a number of randomized controlled trials were started.[47,48]

In general, the strategy of active tolerance induction for food allergy prevention shows promising efficacy, but more insight is needed into the proper presentation of the allergen (such as form, dose, and timing) that will benefit the natural mechanisms of tolerance.

Immunomodulation via dietary interventions

The paradigm for allergen avoidance is being challenged; currently, restriction diets for both mother and child are not recommended as long as they are not already diagnosed with food allergy. Early dietary interventions for allergy prevention therefore focus on alternative protocols or dietary supplements with immunomodulatory properties (Figure 1). The underlying reasoning is that such dietary components might be able to modulate food allergy development by actively improving immune system maturation and supporting natural oral tolerance induction. An important determinant of the efficacy of dietary interventions aiming to prevent allergy in early life is the timing of the supplementation. Most clinical studies focus on dietary interventions after birth, but suggestions point to prenatal supplementation as a potential contributor to oral tolerance induction.

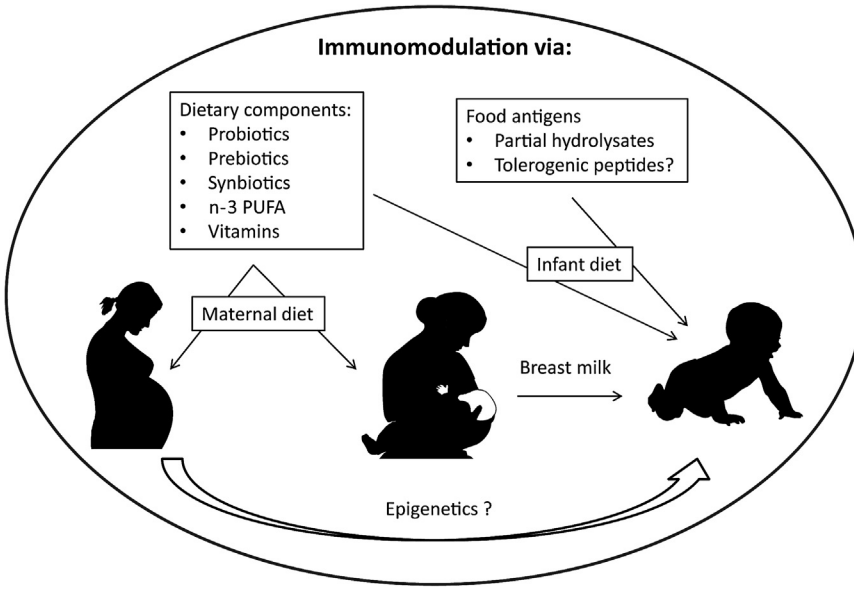


Figure 1 Early dietary interventions with immunomodulatory effect.

Breast milk

In early life, the most important dietary source that offers ideal nutritional, immunologic, and physiologic provision for healthy growth and development is breast milk. Because the developing immune system of the newborn requires not only protective factors against infections but also a suitable microenvironment for oral tolerance development, breast milk provides nutrients, growth factors, immunomodulatory molecules (e.g., immunoglobulins, lactoferrin, oligosaccharides, LCPUFA, cytokines, hormones, antioxidants), and antigens from the maternal diet and maternal immune cells.[12] Therefore, additional information on maternal diet and its influence on breast milk composition might create new horizons for the use of maternal imprinting or epigenetic modifications for allergy prevention strategies.

Hydrolysates

Because it is not always possible to feed an infant exclusively with breast milk, infant formulas based on cow's milk proteins are given in the first months of life. Cow's milk protein formulas are considered to provide infants with 106 times higher oral antigenic load compared with breast milk.[49] Modifications of the standard milk formulas are necessary to reduce the antigenic load, especially in infants at high risk of developing allergy. The most common modifications of cow's milk proteins are enzymatic hydrolysis, heat treatment, and/or ultrafiltration. The goal is to reduce the molecular weight, peptide size, and allergenicity of cow's milk proteins.[50] Lower-molecular-weight proteins are normally expected to induce immunological tolerance rather than sensitization upon uptake by GALT cells.[49] Depending on the cow's milk

protein source, hydrolyzed formulas can be casein-based or whey-based. In addition, the degree of hydrolysis and the length of the remaining peptides classify hydrolysates as partial and extensive. In some cases, even amino acid–based formulas are used. Although no clear definitions exist, partially hydrolyzed formulas are considered to contain a high percentage of fractions with a molecular weight between 3 and 10 kDa, whereas extensive hydrolysates mainly contain peptides of less than 3 kDa.[49] Partially hydrolyzed formulas are often used for prevention in high-risk babies, whereas extensive and amino acid formulas are recommended for infants with a diagnosed food allergy or high risk of anaphylaxis, respectively.[51] This reflects the fact that partial hydrolysates contain larger fragments and may still induce allergic symptoms. Both partially and extensively hydrolyzed infant formulas have been investigated for food allergy prevention in the past decade, and both have shown a potential to reduce the risk of developing atopic dermatitis (AD) and CMA in high-risk infants compared with standard infant formula.[13,50,52–55] Most of the effects observed, regardless of their magnitude, have been reported to persist for up to 24 months.[49] Long-term preventive effects of partial whey hydrolysates have been monitored after 5 years; they reduced the cumulative number of atopic manifestations significantly compared with standard formula.[56] A large clinical trial on the preventive effects of partial whey, extensive whey, and extensive casein hydrolysates demonstrated that the cumulative allergy-preventive effects on AD of both partial whey hydrolysate and extensive casein hydrolysate persist after 6 years[55] and 10 years.[57]

Not much is known about the mechanisms by which hydrolysates might reduce the risk of allergy. In most clinical studies, hydrolyzed infant formulas have been used to avoid the sensitizing epitopes, so there is not much information about whether the clinical effects could result from active oral tolerance induction. Suggestions are that the larger fragments in partial hydrolysates account not only for higher allergenicity compared with extensive hydrolysates, but also for the higher immunogenicity that might lead to allergy prevention via tolerance induction.[58] Such hypotheses have been strongly supported by data from animal studies in which partial rather than extensive hydrolysates were demonstrated to induce tolerance to the allergen, implying the presence of tolerogenic milk protein fragments or peptides in the former but not in the latter one.[59–61] In a more recent study, Van Esch et al. investigated the tolerizing capacity of hydrolyzed whey formulas in an animal model of orally induced CMA. They not only demonstrated oral tolerance induction after feeding naïve mice partial hydrolysate before sensitization, but they also observed an increased frequency of $Foxp3^+$ T_{reg} cells in the MLN. In addition, they reported that the protective effect against allergy development is transferrable to a recipient animal by means of an MLN transfer.[62]

An important aspect is that most studies testing the capacity of hydrolysates to prevent allergic sensitization have been conducted in infants at high risk for allergic disease. However, atopic diseases in early life have also been observed in children without a family history of allergy. Therefore, the allergy-preventive effect of partial hydrolysates-based infant formulas might need to be investigated further in a wider group of children who are in need of breast feeding supplementation or substitution.[49]

Tolerogenic peptides

Preexposure to intact allergen via the oral route results in oral tolerance to the protein. Previously, we described that partial hydrolysates, although having higher residual allergenicity, have better tolerance-inducing capacities than extensive hydrolysates. This suggests possible oral tolerance induction by peptide fractions present in the partial hydrolysates. Knipping et al. demonstrated a specific time at which protein hydrolysis resulted in peptides possessing reduced IgE cross-linking capacity and preserved T cell–activating properties.[63] An attractive approach for oral tolerance induction is the selection of specific peptides with tolerogenic capacities to supplement protein mixtures with low allergenicity.[64] Tolerogenic peptides are relatively small (around 20 amino acids) protein fragments, comprising T cell-binding epitopes. In a preventive animal model, it was demonstrated that prior intradermal administration of a T cell–dominant α_{s1}-casein peptide results in immunologic tolerance in both the T cell and the antibody response to the native protein.[65] Focusing on specific peptides from hydrolysates might enhance oral tolerance induction via regulatory T cells and also direct the immune system away from T_h2 phenotype and allergic sensitization. A good example of this hypothesis is the study of Bogh et al. which showed that co-immunization of intact BLG with BLG-derived peptide fragments can reduce the sensitizing capacity of the intact protein.[66] This emphasizes that the locations of allergenic and tolerogenic sites on proteins might be independent from each other, resulting in the possibility of separating tolerogenicity from allergenicity.[67] In a later study, it was shown that appropriate selection of peptides with tolerogenic properties after tryptic hydrolysis of BLG and their enrichment in a formula reduced the allergic response to the naïve protein in a mouse model of CMA.[67] It was indicated that this specific fraction was 50 times less allergenic compared with the total BLG hydrolysate. Both intestinal and serum antigen-specific IgE were suppressed, whereas delayed-type hypersensitivity and proliferative responses were prevented. Another study on tolerogenic peptides from BLG reported that early exposure to specific T cell epitope-containing peptides is able to prevent the allergic skin response in a murine model of orally induced CMA.[68] The tolerogenic potential of one of the peptides was remarkable because it suppressed the frequency of T_h2 cells while it enhanced $Foxp3^+$ T_{reg} cells in the MLN and tended to reduce the allergen-specific antibody response.

Regardless of the great potential of this approach to induce immunologic tolerance, there are still many questions concerning the underlying mechanisms and the various MHC class II molecules expressed on APC of different individuals. This emphasizes the need for further research that might lead to a more personalized tolerogenic approach to prevention and therapy for food allergies. Furthermore, combining this strategy with a tolerizing dietary component might result in even more powerful protection against allergy development.

Probiotics

During pregnancy, the immune system of the infant is skewed toward a T_h2-dominant profile to reduce the risk of uncontrolled inflammation that could lead to premature

birth.[69] The balance between T_h1 and T_h2 cells needs to be restored after birth to avoid the development of atopic disorders. Commensal microbes present in the infant gut (such as bifidobacteria and lactobacilli) stimulate the maturation of T_h1 and T_{reg}-mediated immune responses, thereby supporting the development of immune homeostasis.[70] A strong association has been found between the development of atopic disorders in early childhood, changes in the diet, and microbial colonization pattern.[14] Studies on delayed gut colonization describe failure in oral tolerance development,[71] which suggests that allergen exposure alone is not sufficient and healthy gut colonization is necessary for tolerance to both self-antigens and allergens.[43] On the other hand, disruption of the microflora by antibiotic treatment enhanced the allergic sensitization to peanut protein in a murine food allergy model.[72] In line with this, significant differences were found when intestinal microbiota composition of healthy infants and infants with atopic disorders were compared.[73]

Beneficial microbes ensure mucosal integrity and are involved in maturation of the immune system of the infant by inducing oral tolerance.[74] The presence of beneficial microbes prevents colonization by exogenous pathogens and simultaneously stimulates the production of secretory IgA (sIgA) antibodies by the mucosal immune system.[75] Secretory IgA binds to microorganisms in the gut and prevents adherence to the mucosal surface, contributing to intestinal homeostasis. In allergic diseases, it is suggested that beneficial microbes induce proinflammatory and regulatory cytokines that favor the development of the T_h1 and regulatory T cell phenotypes, skewing the immune response away from the T_h2-mediated allergic phenotype. Levels of inflammatory markers were elevated in eczema-free infants receiving probiotics, which indicates that a low-grade inflammatory status may reduce the risk of developing eczema.[76]

Providing a favorable microflora via probiotic supplementation in the diet might be a promising strategy to support tolerance induction and suppress the allergic response. Probiotics are defined as "live microorganisms which, when consumed in adequate amounts, confer a health benefit on the host."[77] Long-term outcomes of clinical trials using probiotic interventions in early childhood are inconsistent in terms of effective allergy prevention. A randomized placebo-controlled study performed by Jensen et al. used *Lactobacillus acidophilus* as a supplement during the first 6 months of life in a high-risk group of children.[78] After 5 years, all participants were invited to the clinic for a diagnosis of eczema, IgE-mediated food allergies, allergic rhinitis, asthma, and sensitization to common food and aeroallergens. No significant differences were observed between the placebo group and the probiotic group regarding allergy prevalence, indicating that postnatal *L. acidophilus* supplementation does not provide long-term protection against allergic manifestations.[78] Two other clinical studies investigated the long-term effects of probiotic supplementation during the last 4 weeks of pregnancy and the first 6 months of life of the neonate.[79,80] Kalliomaki et al. showed a reduction in the development of eczema during the first 7 years of life after both prenatal and postnatal *Lactobacillus rhamnosus GG* supplementation.[79] Kuitunen et al. used a probiotic mixture (*L. rhamnosus GG, L. rhamnosus LC705, Bifidobacterium lactis Bb12*, and *Propionibacterium*) and found a decrease in IgE-mediated allergic disorders only in the cesarean-delivered infants and not in the total cohort after 5 years of follow-up.[80] Starting probiotic supplementation before birth might enhance

the efficacy of a probiotic intervention in early childhood.[32,81] Maternal microbial transfer to the offspring might result in imprinting of the microbiota of the fetus during a critical window in immune development.[82] A review published by Kuitunen regarding clinical trials aimed to prevent eczema with probiotics and stated that most studies indeed show a protective effect until age 2.[83] However, the effect is highly dependent on the strain used, the timing of supplementation, duration and dose, mode of delivery, and allergy risk status of the infant.

Prebiotics

Human milk is composed of several immunomodulatory components that ensure optimal maturation of the infant immune system. It also provides beneficial microbes that will colonize the infant gut.[84,85] Furthermore, the growth of beneficial microbes such as lactobacilli and bifidobacteria is supported by human milk oligosaccharides (HMOS) because they function as a substrate in fermentation by the microbiota.[86] In addition, a growing body of evidence suggests that HMOS can interact directly with cells of the immune system.[86] The prebiotic effect of HMOS is suggested to be of key importance in preventing the onset of atopic manifestations such as eczema, food allergies, allergic asthma, and allergic rhinitis in early childhood.[87,88] Human milk contains 10–12 g/L oligosaccharides, which makes them the major component in the diet of breast-fed infants after lipids and lactose.[89] Endogenous enzymes active in the digestive tract are unable to cleave the specific linkages present in HMOS, which explains the finding that they reach the intestine intact.[90]

To enhance the immunomodulatory capacity of infant formula, dietary nondigestible oligosaccharides with specific functional capacities similar to HMOS were identified. Nondigestible oligosaccharides include, among others, galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS). Galacto-oligosaccharides are β-galactosidase–linked galactose monomers derived from lactose, whereas FOS are obtained from enzymatic hydrolysis of chicory-derived inulin. The degree of polymerization is an important factor that determines functional activity; therefore, a distinction has been made between short-chain and long-chain oligosaccharides.[86] A specific combination of short-chain GOS and long-chain FOS (scGOS/lcFOS ratio 9:1) has been studied extensively in both preclinical and clinical setup. A large prospective, randomized, double-blind, placebo-controlled study with formula-fed infants at risk of developing atopic manifestations investigated the effect of prebiotic supplementation on the development of allergic diseases in early childhood. Prevalence of both atopic manifestations and infections was reduced in the prebiotic group during the first 6 months of life compared with the placebo group. The protective effect was still detectable 5 years after the prebiotic intervention, indicating that immune programming in early life has a long-term protective effect.[87,91] The addition of scGOS/lcFOS to infant formula enhanced colonization with bifidobacteria and lactobacilli, resulting in an intestinal flora more similar to the one in breast-fed infants.[92] Supplementation with scGOS/lcFOS is associated with an increase in sIgA production, which indicates that prebiotics can modulate the mucosal immune system.[93] Furthermore, an intervention with scGOS/lcFOS in formula-fed infants resulted in a metabolic profile analogous to

breast milk-fed infants.[94] Fermentation of nondigestible oligosaccharides by bifidobacteria leads to the production of specific short-chain fatty acids (SCFA) and lactate and lowers the pH of the GI tract.[75] Short-chain fatty acids are involved in maintaining the epithelial barrier function and contribute to oral tolerance induction. Via direct interaction with cells of the immune system, SCFA are involved in regulating the inflammatory response.[95]

Studies in mice using a dietary intervention with nondigestible oligosaccharides showed an enhanced T_h1 and regulatory T cell response to the expense of T_h2 cells in both an allergic asthma model and CMA model. The acute allergic response was suppressed and a reduction in the number of inflammatory cells was observed.[96,97] Transferring splenocytes from oligosaccharide-treated cow's milk allergic mice to naïve recipient mice could prevent an allergic response. Ex vivo depletion of $CD25^+$ T_{reg} cells before the splenic transfer inhibited the protective effect, indicating that nondigestible oligosaccharides exert systemic effects via the induction of T_{reg} cells.[98,99] In vitro studies were conducted to investigate the systemic uptake of nondigestible oligosaccharides. In the gut, a monolayer of intestinal epithelial cells (IEC) forms a barrier between the luminal content and the mucosal tissue and selectively allows passage of food antigens into the lamina propria. The HMOS and dietary oligosaccharides were able to cross the intestinal epithelium via both receptor-mediated transcytosis and paracellular transfer through a Caco-2 monolayer.[100,101] Eiwegger et al. showed in an in vitro study that HMOS shifted the T_h2-dominated profile typically seen in neonates toward a more balanced T_h1/T_h2 profile as they suppressed T_h2-type cytokine production by $CD4^+$ T cells in cord blood.[102] Co-culture experiments using human IEC in combination with human peripheral blood mononuclear cells (PBMC) were used to elucidate the immunomodulatory mechanisms of scGOS/lcFOS. Incubation with oligosaccharides and a synthetic Toll-like receptor-9 ligand (based on bacterial CpG DNA) increased the IEC expression and secretion of galectin-9.[103] Galectin-9 is a soluble-type lectin with sugar-binding capacities. In addition, galectin-9 is able to bind cells of the adaptive immune system, thus enabling IEC to influence the effector response.[104] Increased galectin-9 secretion was associated with skewing toward a T_h1 and regulatory T cell profile and an increase in both interferon-gamma (IFN-γ) and IL-10 production. It could also bind to IgE and prevent antigen-IgE complex formation in a rat basophilic leukocyte assay, thus preventing degranulation in vitro.[105] Dietary intervention with scGOS/lcFOS increased serum galectin-9 levels in a murine model of CMA, which was shown to contribute to the suppression of allergic symptoms.[106] The immunomodulatory characteristics of nondigestible oligosaccharides, such as their interaction with cells of the GALT via galectin-9, are suggested to contribute to tolerance induction and intestinal homeostasis in a microbiota-independent manner (Figure 2).

Synbiotics

The beneficial effect of supplementation with either probiotics or prebiotics has led to the hypothesis that an additional or synergistic effect could be induced when both components are combined. A study performed by Schouten et al. showed that a dietary

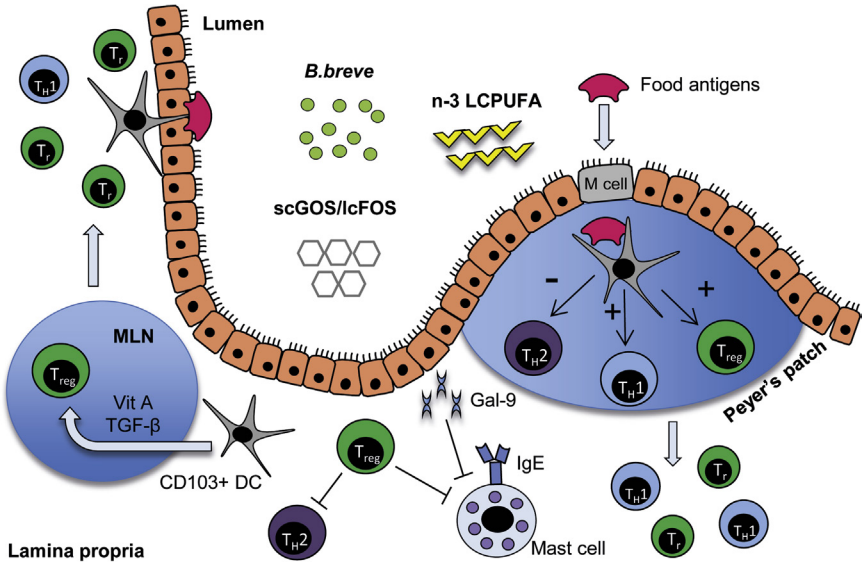


Figure 2 Effects of dietary components on the mucosal immune system. Upon sampling antigens, antigen-presenting DC present the proteins to naïve T cells and stimulate their differentiation into T_h1, T_h2, or T_{reg} cells. A special subtype of $CD103^+$ DC in the lamina propria migrates to the MLN, where it converts vitamin A into retinoic acid, and in the presence of TGF-β, induces T_{reg} cells differentiation. T_{reg} cells can suppress T_h2 effector cells and mast cell degranulation and contribute to oral tolerance to the food antigens. Dietary components such as *B. breve* (probiotics), scGOS/lcFOS (prebiotics), and/or n-3 LCPUFA might directly interact with adaptive immune cells, induce T_{reg} cells, or enhance suppression of the allergic effector response via Gal-9. Gal-9, galectin-9; MLN, mesenteric lymph nodes; DC, dendritic cells; T_{reg}, Tr, regulatory T cells.

intervention with scGOS/lcFOS and *Bifidobacterium breve* before sensitization could more efficiently reduce the allergic response in cow's milk allergic mice compared with an intervention with either scGOS/lcFOS or *B. breve* alone.[96] Furthermore, the bifidogenic effect of the synbiotic concept was confirmed in a clinical study in which infants aged less than 7 months with AD were fed extensively hydrolyzed formula supplemented with a synbiotic mixture (scGOS/lcFOS and *B. breve*) for 12 weeks.[107] The prevalence of AD was not influenced by the dietary intervention, but the severity of symptoms in infants with IgE-mediated AD was reduced.[107] De Kivit et al. measured serum galectin-9 levels at baseline and after the 12-week intervention period and found a significant increase in the synbiotic group compared with the placebo group.[106] The 1-year follow-up of this study showed a reduction in asthma-like symptoms and asthma medication use in the synbiotic group.[108] However, no significant difference was observed between the placebo and the synbiotic group in plasma markers of allergy, cytokine profiles after ex vivo restimulation of PBMC, and percentage of T_{reg} cells present in the circulation.[109] Another randomized, double-blind, placebo-controlled clinical trial conducted by Kukkonen et al. provided high-risk neonates with a combination of four probiotic strains and GOS during the first 6 months of life. A reduction

in the prevalence of eczema, but not in other allergic diseases, was observed after 2 years in the intervention group.[110]

Underlying mechanisms of allergy modulation by probiotics, prebiotics, and synbiotics are not completely understood; however, both animal and clinical studies show promising results in terms of allergy prevention and suppression. Variation in protocols, probiotic strains, and type of nondigestible oligosaccharides complicates interpretation of the results. Timing of dietary interventions appears to be crucial for development of the immune system and intestinal flora of newborns. Even prenatal administration of probiotics and prebiotics might prove decisive for the development of a healthy gut flora and oral tolerance.

Polyunsaturated fatty acids

The change in composition of the Western diet involves a reduction of dietary fiber and antioxidant intake and an increase in the total intake of fat, all of which are suggested to contribute to the development of allergies.[111] In addition, the consumption of vegetable oils rich in n-6 PUFA has increased, leading to a rise in the n-6/n-3 PUFA dietary ratio. n-6 PUFA consists mainly of linoleic acid (LA), a precursor for the long-chain PUFA arachidonic acid (AA). Arachidonic acid can be converted into proinflammatory eicosanoids (e.g., prostaglandins) by cyclo-oxygenases and lipoxygenases.[112] Prostaglandins decreased the production of T_h1-type cytokines (IFN-γ and IL-2) and simultaneously stimulated the production of T_h2-type cytokines (IL-4 and IL-5) in an in vitro human whole-blood assay.[113] In addition, prostaglandin E_2 promotes IgE isotype switching in B-cells, indicating that an increased intake of n-6 PUFA may contribute to an allergic phenotype.[114] n-3 PUFA consists mainly of α-linolenic acid (ALA), a precursor for the LCPUFA-eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA are able to replace AA in membrane phospholipids and change the structure and functionality of different cell types. T cell proliferation and the interaction between T cells and APC can be altered via the incorporation of n-3 LCPUFA into the cell membrane of lymphocytes.[115] Furthermore, the conversion of EPA into its metabolite Resolvin-E1 results in a reduced production of IL-12 and blocked transfer of inflammatory cells across the intestinal epithelium.[116] Interaction with G-protein–coupled receptor 120 present in the cell membrane is a possible mechanism of action by which EPA and DHA exert their anti-inflammatory effects.[117] In a murine CMA model, partial exchange of n-6 PUFA rich soybean oil by n-3 LCPUFA-rich tuna oil in the diet largely prevented allergic sensitization in mice and enhanced $Foxp3^+$ T_{reg} cells in the spleen.[118] Transferring splenocytes or isolated $CD25^+$ T_{reg} cells from these mice to naïve recipients protected the latter from developing CMA.[119]

During pregnancy, epigenetic factors such as nutrition and microbial exposure influence the immune status of the mother and have an indirect effect on fetal immune development.[120] Maternal dietary changes, resulting in an increased n-6/n-3 PUFA ratio, are proposed to contribute to a disturbed maturation of the neonatal immune system by inducing T_h2-type cytokines. Dietary supplementation with fish oil rich in EPA and DHA might be involved in restoring immune balance. Maternal n-3 LCPUFA

supplementation during pregnancy decreased cord blood levels of the T_h2 cytokines IL-4 and IL-13.[121,122] In another intervention trial with prenatal n-3 LCPUFA supplementation from 20 weeks of gestation until birth, higher proportions of n-3 LCPUFA were found in neonatal erythrocyte membranes combined with reduced levels of IL-5, IL-13, IL-10, and IFN-γ.[123] However, at 1 year of age no difference in the frequency of AD was found between the n-3 LCPUFA and the placebo group, although symptoms appeared to be less severe.[123] In another randomized, placebo-controlled intervention study, n-3 LCPUFA supplementation during both pregnancy and lactation significantly decreased the prevalence of food allergies and eczema at 1 year of age.[124] The preventive effect of fish oil against IgE-mediated disorders was sustained even after 2 years of follow-up.[125] Furthermore, allergy prevention by postnatal n-3 LCPUFA supplementation in newborns until age 6 months resulted in higher DHA and EPA levels in plasma and reduced AA levels in erythrocyte membranes.[126] However, clinical outcomes of allergy were not significantly different between the placebo and the fish oil group, which indicated that postnatal supplementation with n-3 LCPUFA may not be sufficient to reduce the risk of developing allergies in early childhood. The same finding was observed in other postnatal fish oil supplementation studies: supplementation to the infant or to lactating mothers did not result in a reduced prevalence of allergies later in life.[120] The allergy-preventive effect of n-3 LCPUFA supplementation might depend on the presence of maternal factors involved in the development of the immune system in early life.

Antioxidants

Observational studies have linked the intake of dietary antioxidants such as vitamin A and its metabolites, vitamin C, D, E, zinc, selenium, and folate, to the prevalence of asthma and allergic disorders in early childhood.[127] Allergic disorders are known to cause oxidative stress, and it is difficult to define whether a reduced antioxidant status is a cause or a consequence of the allergic diseases in infants.[128] A systematic review published in 2011 by Patelarou et al. found an association between inadequate antioxidant status in utero and during early childhood and the development of allergic disorders.[129] Elevated antioxidant concentrations in maternal, child, or cord blood serum were related to a decreased prevalence of asthma, wheezing, or eczema during early life. A Danish birth cohort included 44,594 mother–infant pairs and examined the relationship between maternal intake of vitamins A, E, and K during pregnancy and the development of asthma and allergic rhinitis in the offspring during the first 7 years of life. A weak beneficial effect of vitamin A and E intake during pregnancy on the prevalence of allergic rhinitis at a young age was found. In contrast, high vitamin K intake was linked to an increased prevalence of asthma.[130]

To date, evidence from well-conducted randomized controlled trials is lacking and the exact mechanism of action of antioxidants in the development of a balanced immune system in neonates has not been clarified. Some studies point to $CD103^+$ DC present in the lamina propria that convert vitamin A into retinoic acid, which is essential for the development of T_{reg} cells in the MLN and the promotion of an sIgA switch of B cells in the presence of IL-10 and TGF-β.[131,132] The influence of vitamin A and

its derivatives, the retinoids, on postnatal lymphocyte proliferation and differentiation was determined in an in vivo OVA-induced postnatal allergy model in mice. Dams were fed a regular diet, a vitamin A elimination diet, or vitamin A-supplemented diet during lactation, and similar diets were fed directly to the pups after weaning. Allergic sensitization was induced via a single intraperitoneal OVA injection in 28-day-old pups. Vitamin A supplementation was associated with a decrease in the amount of $CD4^+$ and $CD8^+$ splenic lymphocytes and significantly enhanced IL-4 and OVA-specific IgE concentrations after allergic sensitization. The authors concluded that despite its suggested role in T_{reg} induction, vitamin A supplementation of the maternal diet during lactation and of the pups' diet after weaning increased the severity of allergic sensitization in the offspring.[133]

Animal studies found a positive effect of vitamin E on IgE-mediated atopic disorders, and a similar association was found in a human cohort in which the relation between vitamin E intake, serum IgE concentrations, and allergic skin sensitization was investigated.[134] Vitamin E suppressed the production of IL-4 in peripheral blood T cells by blocking transcription factor NF-κB and thus inhibiting activation of the promoter sites.[135] An inverse association between maternal vitamin D status during pregnancy and the development of asthma and wheezing in early childhood was found in several birth cohort studies with more than 750 mother–infant pairs.[136] To test the hypothesis of whether maternal supplementation with vitamin D from 27 weeks of gestation could reduce the prevalence of wheezing at age 3 years in the offspring, a randomized controlled trial was conducted. No significant differences were found between the vitamin D and control groups for infant wheezing, atopy, or eczema at age 3.[137] On the other hand, high maternal and cord blood vitamin D status was positively associated with a risk for the development of food allergies during the first 2 years of life in infants enrolled in the German LINA birth cohort.[138] These studies show conflicting results on allergic outcomes upon vitamin D supplementation, revealing both inhibitory and stimulatory properties, depending on the timing and/or dosing and duration of supplementation.[139–141]

Clear evidence from randomized controlled trials is needed to determine the role of antioxidants during postnatal immune development and to learn whether supplementation during pregnancy and lactation decreases or increases the risk of atopic disorders in early childhood. Until safe recommendations can be made, a healthy balanced diet is the best way to control the development of allergic diseases in infants.

Conclusion

The increasing prevalence of allergic disorders among infants might reflect changes in Western diet composition and reduced exposure to microorganisms in early childhood. Food allergy is one of the first allergic outcomes developing from a T_h2-prone state that can progress to respiratory allergies and asthma later in life. It is important to intervene as early as possible, even prenatally, to actively prevent sensitization to foods and stop the development of the atopic march. Early introduction to larger protein fragments or small peptides with tolerogenic capacities appears to be an attractive

approach for oral tolerance induction and allergy prevention. The exposure to food proteins with reduced allergenicity and tolerogenic properties might prevent allergic disease development by supporting maturation of the neonatal gut and/or by stimulating normal tolerance establishment. Clinical trials are needed to further clarify the preventive effect of this approach, and might resolve the dilemma of early-life exposure to allergenic proteins. Using dietary components with immunomodulatory properties as adjuvants is another approach to skew the T_h2-dominated immune response toward a more tolerogenic response in early childhood. Although evidence from clinical trials points to the beneficial effects of supplementation with dietary components on preventing allergic sensitization, this approach has not been implemented as an allergy-preventive strategy yet. Supplementing the diet with probiotics, prebiotics, n-3 PUFA, or specific antioxidants is suggested to support oral tolerance induction and prevent allergy development in early childhood. Contradictory findings on some dietary components, such as vitamins, emphasize the importance of clarifying the exact window of opportunity in early life, before specific recommendations on this approach can be made. Recent evidence suggests that dietary intervention should start during gestation because maternal factors may influence the development of both the microflora and the immune system of the offspring. Epigenetic changes induced by maternal (dietary) factors might be needed for lifelong protection against allergic disorders in the infant. More clinical trials are needed to confirm the beneficial effects of dietary interventions and to promote their use as a strategy for (food) allergy prevention.

Abbreviations

AA	Arachidonic acid
AD	Atopic dermatitis
ALA	α-Linolenic acid
APC	Antigen-presenting cells
BLG	β-Lactoglobulin
CMA	Cow's milk allergy
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FOS	Fructo-oligosaccharides
GALT	Gut-associated lymphoid tissue
GI	Gastrointestinal tract
GOS	Galacto-oligosaccharides
GPR120	G-protein coupled receptor 120
HMOS	Human milk oligosaccharides
IEC	Intestinal epithelial cells
LA	Linoleic acid
LCPUFA	Long-chain polyunsaturated fatty acids
MLN	mesenteric lymph nodes
OVA	Ovalbumin
PBMC	Peripheral blood mononuclear cells
PGE_2	Prostaglandin E_2

PUFA Polyunsaturated fatty acids
SCFA Short-chain fatty acids
TGF-β Transforming growth factor-β

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Allergen Exposure and Avoidance as Part of Early Intervention and Prevention

17

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Exposure to an inhalant allergen is a major factor in both sensitization and the subsequent development of nasal, lung, or skin symptoms. However, there are dramatic differences in exposure by season of the year and by individual houses and also because of changes in the climate of different geographic areas. In some countries such as the United Kingdom and New Zealand, the climate is consistent in different areas of the country. By contrast, in Sweden and the United States (US), there is a wide range of climate that can dramatically alter the allergens to which children are exposed. Most studies on the effects of early exposure have been based on the view that sensitization and symptoms are directly related to exposure, and that the relevant allergens have been identified.[1] Allergens are antigens, usually protein in nature, with the ability to sensitize the host for subsequent allergic reactions. When genetically susceptible subjects are exposed to airborne allergens, they can become sensitized. These subjects may remain asymptomatic, and sensitization may be detected only by skin testing or by specific immunoglobulin E (IgE) measurements with appropriate allergens. However, on subsequent exposure, possibly in the presence of an adjuvant factor, many would develop one or more of the allergic disorders (Figure 1).

Three major types of studies have been designed to understand the role of exposure in the development and severity of allergic diseases in childhood:

1. Studies in which a cohort or cohorts of children are followed over the period of sensitization. These include studies within a geographic area where the effects of individual homes can be assessed, and those including populations raised in dramatically different environments.
2. Cohort studies usually of at-risk infants or children in which the homes or some other aspects of their environment are modified to decrease the exposure to one or more perennial allergens. This type of study is particularly relevant to investigating factors in the environment that are directly related to the allergic march. It is important to remember that many other factors can influence the response to allergens, including tobacco smoke, animals, bacteria, and products of bacteria such as endotoxin.

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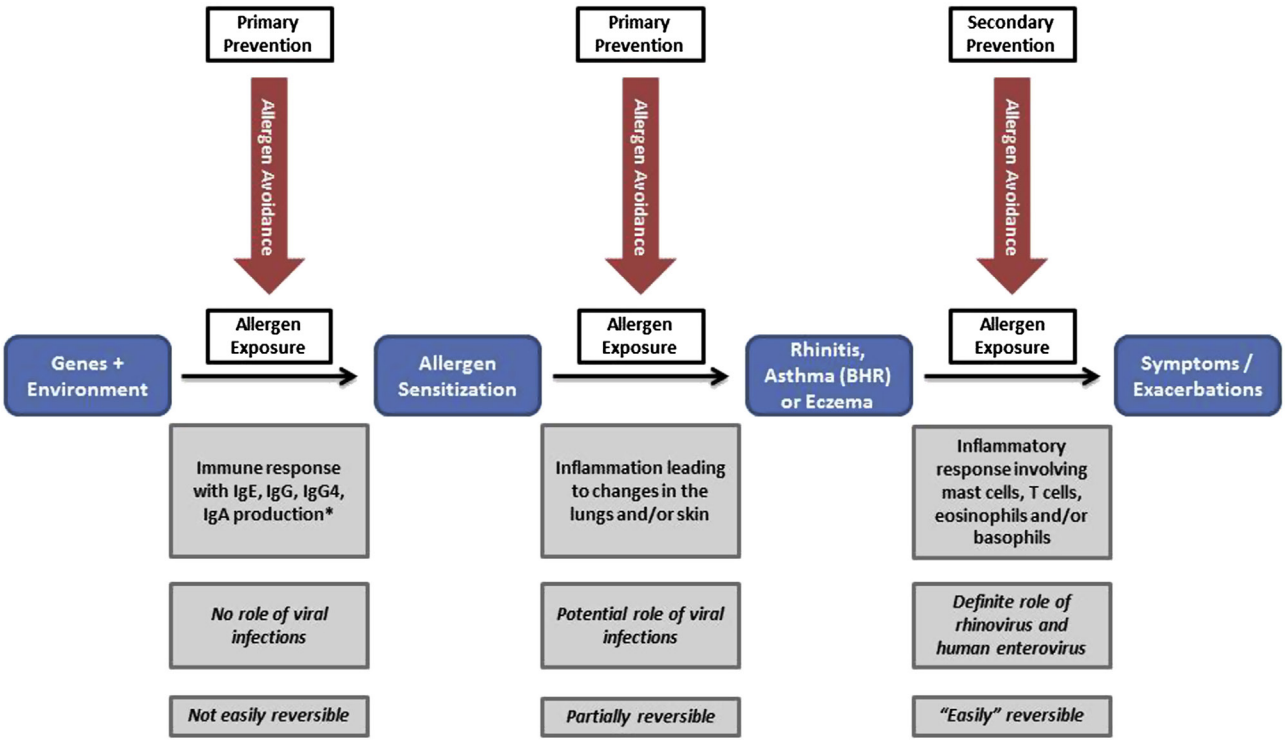


Figure 1 Allergy prevention using allergen avoidance.

3. Controlled trials of the effects of decreasing exposure to a specific allergen or a group of allergens (such as mites and cockroaches) in children who are already symptomatic and sensitized.[2] These studies are of two kinds:
   a. Those in which the children are moved to a hospital or sanatorium where conditions can be maintained with very low levels of allergen exposure.
   b. Those that make changes to each patient's home to decrease exposure. Most of these studies have focused on dust mite allergens, but a few have (correctly) addressed more than one allergen.

Before considering the results of intervention studies, it is important to recognize that major assumptions are involved in carrying out the studies and in interpreting the results. Some of clearly recognized assumptions are that (see Table 1)

1. It is possible to measure the quantity of relevant allergens in the subject's environment, and that these measurements are sufficient to evaluate the relationship of the allergens to either sensitization or symptoms.
2. Exposures that are important occur in a defined space that can be controlled.
3. The effects of allergen exposure are primarily a function of the protein rather than the adjuvant effects of other exposures.
4. The relevant exposures in each environment have been identified. It is important to recognize that over the past 40 years, we have become progressively aware of the effects of dust mite, cockroach, and mouse allergens.

Techniques for changing exposure in a home: Any approach to avoidance in a house has to be tailored to what is known about the relevant allergen and what is practical. As dust mites were first shown to be a major cause of sensitization, they were sometimes referred to as "bed mites." This was because the highest numbers of mites were routinely found in bedding or mattresses. However, at that time, that is, in the 1960s, many houses were still kept cold, that is, ambient temperatures below 60 °F. Thus, the bedding may have been the only site with optimal temperatures and humidity. Over the next 15 years, progressive changes in ventilation, furnishing, and heat generation of homes, particularly in the United Kingdom, will lead to foci of high mite growth throughout the homes. Despite the obvious evidence that mites were growing in many parts of the home, some important studies in the 1990s focused only

Table 1 Assumptions related to allergen avoidance studies that may influence the interpretation of the results

1. That it is possible to measure the quantity of relevant allergens in the subject's environment.
2. That these measurements are a good evaluation of the exposure that is relevant to (i) sensitization or (ii) symptoms.
3. That exposures that are relevant occur in a defined space, that is, bedroom or bedroom plus living room, which can be controlled.
4. That effects of dust in the environment are primarily related to allergen-derived proteins rather than other adjuvants in the dust, such as endotoxin or bacteria.
5. That relevant allergens in each environment have been identified. It is important to remember that we have become progressively aware of the effects of dust mite, cockroach, and mouse allergens over the past 40 years.

on the bedding. Indeed, one study on dust mite "avoidance" simply provided active and placebo mattress covers.[3] It is important to understand why studies of that kind were bound to fail[4]:

- First, a mattress cover alone will not control other parts of the bedding, so that the studies either ignored the rest of the bedding[3] or recommended hot washing for bedding in both active and placebo groups.[4]
- Second, there is a large Hawthorne effect with studies of this kind, such that subjects enrolled in the study will tend to change their household management. This will tend to blur any effect of a single intervention.
- Third, in a modern overheated house, the highest levels of mite exposure may well be seen on carpets or sofas away from the bedroom.

Techniques for controlling exposure to other allergens have not been studied as extensively as those for dust mites. Techniques for reducing cockroach, cat, fungi, or mouse allergens each are different from measures recommended for dust mites. In each case, conclusions can be reached only if the recommended procedures decreased exposure by at least 75%, but preferably by nearer to 90%. For cockroaches and mice, measures include steps to prevent the entry of the organism, a strategy for killing them in the house, and obsessive cleaning. By contrast, for fungi, the primary strategy is to keep the house dry and remove any existing damp material. Cats and dogs represent a special case because the source of allergen is obvious, but the allergen can be found, sometimes at high levels, in homes without an animal. Furthermore, there is good evidence that children can become sensitized to cat or dog allergens on the basis of exposure in schools or homes without an animal.[5,6] In general, measures designed to control exposure with a cat or dog in the house (although possible) are too demanding to be part of normal practice let alone part of a controlled trial.[7]

Allergen avoidance

A number of allergen exposure measures have been tested and are recommended for both primary and secondary prevention of allergic disorders. However, there is significant uncertainty about where the exposure occurs, how to measure this exposure accurately, and the dissociation between the presence of allergens in the environment and the level of personal exposure.

Specific measures to reduce house dust mite (HDM) allergen rely on (1) killing the mites, such as washing at higher temperatures and using acaricides or liquid nitrogen on carpets and upholstery; (2) making the environment unsuitable for mite growth, such as replacing carpets with hard flooring and controlling humidity; and (3) protecting the individual from inhaling dust mite allergens by using vacuum cleaners with high-efficiency particulate air filters and allergen-impermeable covers for bedding (Figure 2). The allergen is usually measured in the house dust, and with these interventions, reductions in allergen levels have been confirmed.[8] However, whether personal exposure (allergens that an individual inhales) is also reduced with these measures has not been well documented. The most effective way of reducing pet

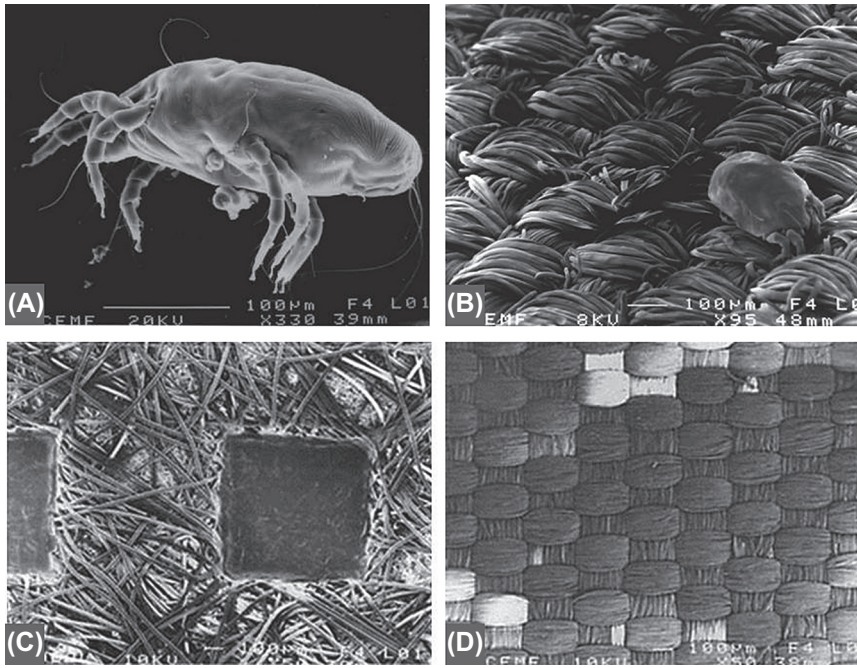


Figure 2 Dust mites and materials used for mite avoidance: (A) scanning electron micrograph of D. pteronyssinus; (B) mite on a fine woven fabric that does not allow access of mites or accumulations of debris; (C) non-woven material with spot welds; this kind of fabric prevents rapid passage of mites or allergen but allows accumulation of allergens, and may allow breeding of mites; and (D) fine woven material which can block very fine (i.e., 2 μm) particles from penetration.

allergen exposure is to remove a pet from the house. Other measures to reduce pet allergen with a pet remaining in the home include washing the pet, reducing direct contact, and using high-efficiency particulate air filter air cleaners, but these are likely to have a modest effect. Measures to reduce mold exposure include reducing humidity, removing old and damp furnishings, and even placing patients in low-allergen dwellings, which tend to have a beneficial effect on asthma symptoms.[9] Cockroach and rat infestations require specific measures to kill and remove these allergens.

Allergen exposure, sensitization, and disease

The concept of allergen avoidance in preventing the development of allergic diseases is simple as sensitization, and subsequent allergic manifestations cannot develop without some level of exposure to the allergen. Furthermore, exposure to common allergens such as HDM, pollens, mold spores, and allergens of animal origin is ubiquitous, although its extent may depend on the geographical area of residence and other

household and community factors. A number of studies in the past two decades of the twentieth century supported the view that a higher level of exposure to common indoor allergens increases the risk of allergic sensitization and asthma.[1,10] However, sensitization and clinical allergic diseases are different and the exposure outcome relationship may not be the same. Traditionally, sensitization is considered a first and essential step toward the development of allergic disease (Figure 1). There is a strong association between environmental allergen exposure and development of sensitization, and a dose–response relationship between the level of exposure to cockroaches, house dust mites and cats and the development of sensitization has been suggested.[11] However, despite extensive research, the direct role of exposure to indoor allergens in the causation of allergic disorders remains controversial. In the German Multicenter Asthma Study, exposure to HDM was associated with allergic sensitization but not asthma at age 6 years.[12] However, a relationship of exposure to asthma was apparent at age 12.[13] These uncertainties stem from several unresolved issues and gaps in knowledge, with implications for the role of allergen avoidance in primary prevention of allergic disease. For example, the relationship between allergen exposure and development of sensitization may not be linear. It has been suggested that a higher level of exposure to environmental allergens might be protective rather than sensitizing.[14] Alternatively, a bell-shaped relationship has been suggested so that both high and low exposures induce tolerance whereas middle levels could be sensitizing and predispose to asthma.[15] In other studies, higher exposure to environmental allergens led to a lower risk of allergic disease, whereas allergic sensitization increased or no effect was observed.[14] This disconnect between allergen sensitization and clinical allergic disease challenges the basis for using allergen avoidance as a means of preventing allergic disease. However, several other factors and exposures can have an influence and may act in synergy with allergen exposure, such as a diversity of microbes in the environment, exposure to cigarette smoke and other pollutants, and dietary deficiencies. This is the critical shortcoming of many previous epidemiological studies, which tried to associate allergen exposure with allergic outcomes without adjusting for a host of other important determinants, and came to different conclusions.

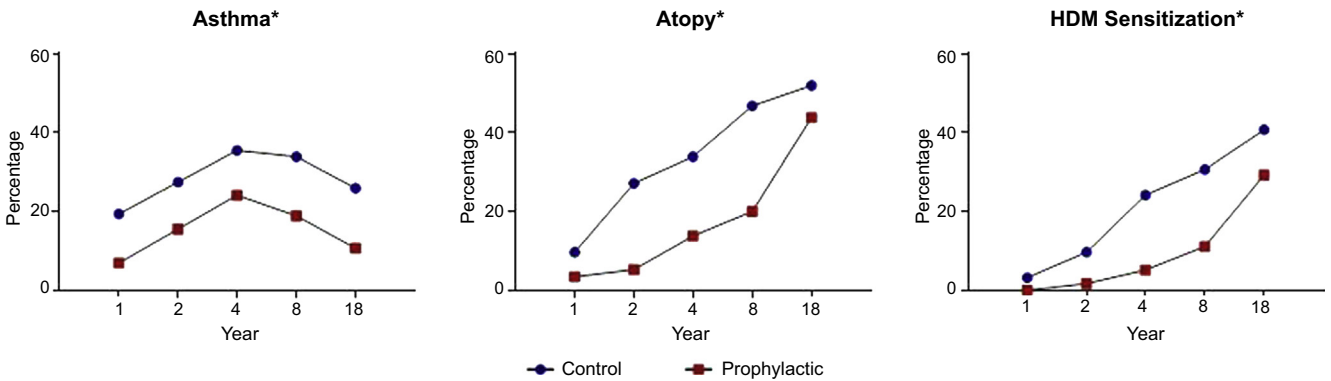
When considering allergen avoidance as a primary prevention tool, some other aspects are important, such as the nature of the allergen and the route of exposure. Epidemiological studies show that different allergens assume importance in different environments. Although, HDM is important in most temperate climates of Europe, the US, and Australia, cat allergen is predominant in some cold countries such as Sweden, *Alternaria alternata* in dry climates of the US and Australia, and cockroach, mouse, and rat allergens in many inner-city areas of the US. Although there is substantial evidence for exposure to HDM and cockroach allergens and development of sensitization and possibly asthma, this might not be true for an animal allergen. Indeed, exposure to cats and dogs has been suggested to increase the risk,[10,16] to have no effect,[17] or to protect from allergies.[18] Living in homes with dampness and visible mold has been associated with a number of adverse respiratory effects including asthma.[19] Furthermore, the issue of the optimal age at which allergen avoidance is likely to have the maximal effect has not been resolved. There is a general agreement that both prenatal and postnatal exposures are important, because allergic sensitization and eczema

often develop early in life. Based on the traditional concept (which may not always be true) that allergic sensitization precedes clinical disease, allergen avoidance has to be instituted before the child is sensitized, that is, from birth (or even during pregnancy) (Figure 2). However, studies show that interventions such as allergen immunotherapy can be effective in preventing allergen sensitization as well as asthma in later childhood. Environmental allergen avoidance is useful in secondary prevention and will be discussed later in this chapter. We will now focus on studies and evidence of where environmental allergen avoidance was used to prevent the development of allergic diseases (asthma, eczema, and rhinitis) and atopy (defined as sensitization to one or more common allergens) in early childhood.

House dust mite allergen avoidance in primary prevention

A key study published in 1990 showed that high exposure to the HDM allergen (>10 mg/g dust) during infancy increases the risk of HDM allergen sensitization and the development of asthma at age 11 years.[1] After the publication of this seminal article, a number of randomized controlled trials (RCTs) investigated the effect of reducing HDM exposure during the prenatal and postnatal periods and assessed the subsequent development of asthma in children. The first of these studies was the Isle of Wight primary prevention study, which randomized 120 pregnant mothers of infants at high risk of allergy into prophylactic and control groups.[20] The study used a multifaceted approach to reduce both common food allergens and HDM allergen exposure during infancy in the prophylactic group, whereas the control group was given standard advice prevailing at the time. A strict dietary regime was used to exclude all common food allergens from both infants and lactating mothers, whereas an extensive hydrolyzed formula was used as a supplement. This resulted in a 50% reduction in eczema in early childhood and a similar reduction in asthma over the entire childhood period.[20–22] Allergic sensitization was consistently reduced up to age 10 years (Figure 3). Furthermore, the intervention reduced the occurrence of persistent and atopic asthma up to age 18 years whereas there was no effect on nonatopic asthma.[22] There was no effect on allergic rhinitis. Thus, in highly selected children, it might be possible to reduce the occurrence of asthma and eczema with strict and comprehensive allergen avoidance measures.

A similar multifaceted approach was tested in the Canadian Asthma Primary Prevention Study. The researchers randomized 545 infants at high risk of asthma with the intervention consisting of avoidance of HDM, pets, and environmental tobacco smoke. Mothers were encouraged to breast feed and infants had delayed introduction of highly allergenic foods such as cow's milk, peanuts, and fish. They found a protective effect of this intervention with reduced asthma prevalence up to 7 years.[23,24] However, at 15 years the protective effect on asthma was seen only in females. Strangely, there was no effect on allergen sensitization at any age. Similarly, no difference was observed for eczema, rhinitis, or bronchial hyperreactivity (BHR). Hence, it is difficult to explain the biological pathway for the effect of allergen avoidance measures on asthma in this study. A Dutch study in primary care used environmental allergen avoidance measures as well as prolonged breast feeding and delayed solid food introduction in a primary



†Repeated measurements taken from birth to 18 years
‡p<0.001

Figure 3 Asthma and atopy from birth to 18 years in the Isle of Wight prevention study (repeated measurement analyses). *<0.001.

care setting. Although a reduction in allergen exposure to HDM, cats, and dogs was achieved, this did not translate into a protective effect on asthma or atopy.[25]

Overall, it seems that multifaceted allergen avoidance may have some protective effect on asthma, and to a lesser extent, on eczema and atopy. However, with combined intervention measures, it is difficult to know which specific intervention was effective. Two larger RCTs were carried out to assess the effect of HDM as a single intervention. The Manchester Asthma and Allergy Study recruited 291 pregnant mothers in an RCT and instituted extensive HDM avoidance measures as a sole intervention, whereas placebo intervention was used in the control group. The HDM levels were low at baseline and the comprehensive measures virtually eliminated this exposure in the intervention group.[26] However, the researchers did not find a significant preventive effect on asthma. Surprisingly, allergic sensitization was more common in the intervention arm.[27] In the Prevention and Incidence of Asthma and Mite Allergy study, 810 allergic mothers were enrolled during pregnancy and randomized to impermeable mattress and pillow covers or placebo covers. There was a significant reduction in HDM allergen levels in the intervention group.[28] However, no beneficial effect on the development of atopy, wheeze, hay fever, eczema, or asthma up to age 8 years was seen.[29] A Cochrane analysis concluded that multifaceted environmental interventions might be effective in reducing asthma, but current evidence does not support the use of a single intervention such as the HDM avoidance measure.[30]

The timing of allergen avoidance is an unresolved issue. Allergic sensitization to cockroach, dust mite and rat allergens may occur in utero as a direct consequence of the presence of these allergens in the maternal environment.[31] Although the significance of this sensitization to future development of allergic disease remains uncertain, most investigators share the view that allergen avoidance measures should be commenced either before birth or soon after. However, there is some suggestion of primary prevention later in childhood. For instance, HDM immunotherapy in asthmatic children may prevent the development of new sensitization. Furthermore, immunotherapy to grass pollen in children with allergic rhinitis may reduce the risk of development of asthma.[32,33] The Study on the Prevention of Allergy in Children in Europe took an innovative approach of using three childhood cohorts in different age groups in an attempt of preventing the development of asthma using HDM allergen avoidance. The cohorts were newborns as well as toddlers and schoolchildren who did not have asthma or atopy but were at high risk due to their family history. This was a large multicenter study in five European countries with dust mite–impermeable mattress covers, in addition to an educational package as the main intervention measure in an RCT.[34–36] In each cohort, sensitization to dust mite allergen was reduced to some extent, but reduction in asthma was modest and mostly nonsignificant.

Could HDM allergen avoidance, when combined with a dietary supplement, be more effective? Some Australian investigators set out to answer this in an RCT of infants at high risk of atopy. In a three-arm trial, HDM avoidance measures (use of impermeable mattress covers and an acaricide) were instituted during pregnancy and infancy in one arm. In the second arm, mothers and infants were supplemented with omega-3 fatty acids, and in the third arm both interventions were combined. Disappointingly, there was no effect on atopy or asthma of either the individual interventions or the combined one.[37]

In summary, RCTs to reduce HDM allergens have been generally disappointing. A combined approach of food and dust mite allergen avoidance seems promising in preventing childhood asthma and eczema, but the findings from the Isle of Wight prevention study have not been replicated in a larger trial, and in view of the complex nature of the interventions used, they may not be suitable for wider implementation.

Avoidance of other allergens

Pet Allergen Avoidance: There are no RCTs assessing the effect of pet allergen avoidance on primary prevention of allergic disorders as a single intervention. The Canadian Asthma Primary Prevention Study included pet allergen avoidance, among a number of other measures, to prevent the development of asthma in children at high risk. However, compliance with this advice was low and there was no effect on allergen sensitization to pets. Thus, this intervention is unlikely to have contributed to the preventive effect observed in this study. Observational studies show a complex picture. A systemic review found that cat allergen exposure is associated with the development of cat sensitization.[38] However, other studies suggest that exposure to high levels of cat allergen may induce immune tolerance.[39] Another study showed that nearly all children with filaggrin loss-of-function mutations who were exposed to cats developed eczema.[40] Many studies propose a protective effect of dog exposure in childhood on atopy and asthma, which may be related to an increase in exposure to bacterial products, because dogs tend to bring dirt into the home. However, not all studies support this notion.[41] A meta-analysis of birth-cohort studies could find no association, risk or protective, between pet exposure in early life and the development of childhood asthma.[17] Thus, to date, no specific recommendation has been made regarding the ownership of pets for high-risk children.

Cockroach and Mouse Allergens: Exposure to cockroach and mouse allergens is common in inner-city areas of the US, which also have a disproportionate burden of allergic diseases, especially asthma. No RCTs have been carried out to assess the effect of avoiding these allergens on developing asthma in at-risk populations. However, a number of observational studies suggest that exposure to cockroach and mouse allergens contributes to the development of allergic sensitization, wheezing, and asthma.[42,43] However, a study showed that although the cumulative exposure to cockroach, mouse, and cat allergens during the first 3 years of life is associated with allergic sensitization, exposure during the first year and particularly during the first few months was protective for wheezing. Other factors including bacterial diversity are important and are often linked to the presence of other allergens, especially pets.

Mold Allergen Avoidance: Between 10% and 50% of homes in Europe are reported to be damp, with a high risk of mold growth; and living in damp houses has been shown to increase the risk of incident asthma.[44] Reducing the level of dampness and moisture and removing mold in the home improve respiratory symptoms.[45] However, no primary prevention study has been carried out to investigate the effect of reducing exposure to mold allergens and the development of asthma.

Why environmental allergen avoidance in primary prevention is not effective

Most intervention studies have focused on HDM allergen avoidance. It is likely that reducing a single allergen such as HDM is not effective in primary prevention of asthma or atopy and a multifaceted approach is required. However, in some of the studies, which used mono-intervention aimed to reduce exposure to HDM allergens, such as the Prevention and Incidence of Asthma and Mite Allergy and Manchester Asthma and Allergy Study, the baseline mite allergen levels were so low that further reduction may not have had a significant effect.[26,28] The multifaceted approach tends to show some effect, but questions remain regarding its effectiveness and generalizability.[30] There may be other explanations for the lack of effectiveness of allergen avoidance measures. It might be that allergen reduction measures currently in use do not reduce personal exposure even though allergen levels in the dust collected from carpets and mattresses may be lower (Figure 4). It is also possible that allergen reduction measures remove protective factors from the dust, such as bacterial products that nullify any preventive effect. Furthermore, genetic variations in different populations may account for the variability in response. For example, variation in CD14 genotype may determine the response of the immune system to allergen exposure.[46] In addition, most of the focus for primary prevention using environmental allergen avoidance has been on asthma, which is a heterogeneous condition. Various phenotypes of asthma have been described with a potentially wide-ranging pathophysiological and

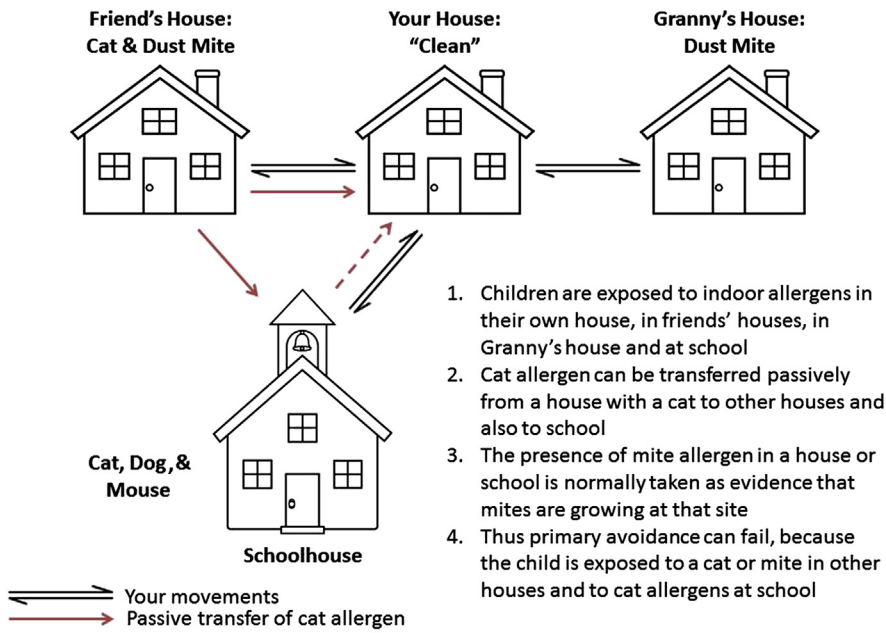


Figure 4 Possible routes of allergen movement and allergen exposure within a community.

etiological basis. The other major obstacle is the lack of predictive markers for asthma development. Most previous studies used a family history of allergy as a selection criterion for high risk, which is neither highly sensitive nor highly specific. For any primary prevention strategy to work, it would be essential to identify early biomarkers to select a population that is at high risk and thus stands to benefit most. The biomarkers are likely to vary according to the specific allergic disease, and indeed the specific phenotype of the disease. Any future trial in primary allergen avoidance will need to consider issues such as allergen dose, timing of exposure, type of allergen, concomitant exposures such as bacterial products and pollutants, as well as variations in individual susceptibility to these exposures. A primary prevention strategy tailored to the individual, with his or her specific genetic/epigenetic makeup and environmental exposures, is likely to be most effective. On the basis of current evidence from observational studies and RCTs, environmental allergen avoidance measures to reduce the risk of asthma or other allergic diseases in at-risk patients cannot be recommended.

Secondary avoidance

Allergists in practice advise avoiding secondary allergens for many of their allergic patients.[2] The basic principle is that you identify which allergens are contributing to the patient's symptoms and recommend that changes are made in the home to reduce exposure to those relevant allergens. Central to this approach is to obtain evidence that the individual is allergic, either by skin test or in by vitro assays of IgE antibodies. Thus, by definition, secondary avoidance is *not* relevant to preventing initial sensitization to allergens. However, secondary avoidance is relevant to understanding progressive development in allergic disease in at least two ways: First, much of the best evidence about how to change allergen exposure in a house has come from studying the effects of secondary avoidance. Second, there are real questions about whether ongoing inflammation contributes to the progression of allergic sensitization. This could relate to the development of sensitization to a new allergen or to epitope spreading within the response to a single protein. In addition, there is good evidence that eczema can allow increasing sensitization and that prolonged desensitization treatment can decrease sensitization to a new allergen.

There are two distinct forms of secondary avoidance:

1. Moving the symptomatic child or adult to an environment that has low or very low levels of allergens.
2. Making or recommending changes in the patient's home which are designed to decrease exposure to relevant allergens.

Studies involving moving patients to a sanatorium or hospital

The concept of changing environment actually started with treating tuberculosis (TB) patients in Davos, where it became clear that some patients did not have TB and that those patients, including those with asthma, were the ones who really benefited from

the mountain air. As early as 1929, Storm Van Leuven attempted to reproduce the beneficial effects of the sanatorium by building a climate chamber in Amsterdam.[47] It was already known that many patients with asthma got better simply by being taken out of their homes and admitted to a hospital. Indeed, when Rackemann defined "intrinsic asthma" in 1947, he listed the fact that these patients did not get better in the hospital as one of the defining characteristics of this condition.[48] The effects of moving children to high-altitude sanatoriums have been studied extensively in Davos, Misurina, and Briancon. The major study carried out in Davos during the 1960s included monthly tests of BHR over 1 year. Kerrebijn published the results of that study in 1970 and showed that many of the children not only became better symptomatically but that almost half of them "lost" their BHR.[49] At that time, this was an important and novel concept, because Roger Altounyan had just presented evidence that patients with severe hay fever who developed BHR during the pollen season generally returned to normal lung reactivity over the 4 months after the season.[50] The subsequent study carried out in a London hospital was designed to address the same question in mite-allergic subjects with moderately severe asthma. The results confirmed that some of the patients who were kept away from their homes for 5 months or more not only improved symptomatically, but also had major decreases in BHR to histamine.[51]

Interestingly, studies in which patients have been moved to a sanatorium or hospital have several features that are not always recognized[49,51,52]:

- First, the rooms in which the patients lived had no mites, no animal dander, and very little mold allergen. Thus, they generally avoided more than just the primary allergen that was being studied.
- Second, because the patients improved, they were able to reduce treatment; in particular, they required less bronchodilator.
- Third, patients were encouraged to progressively increase physical activity.

It is clear that each of these other changes could be significant in improving both symptoms and BHR.

Unfortunately, there are no studies to answer whether prolonged changes in environment could prevent the progression of allergic disease. On the other hand, taken together, studies in which patients are moved to a new environment argue strongly that decreases in lung function and increased BHR in asthma are reversible under conditions that include a major reduction in allergen exposure.

Secondary allergen avoidance by making changes in the patient's own home

Central to studies on avoidance in patients' homes has been the availability of assays to measure changes in the quantity of allergenic proteins in dust samples from the house.[53] However, even with sensitive assays it is not easy to estimate the quantities that are inhaled.[7,54] It is also difficult to reproduce in a patient's home the decreases that are routinely achieved in a hospital or sanatorium. To carry out a controlled trial in patients' homes, it is also necessary to overcome the problem of blinding. This is

daunting in a situation in which the patient inevitably knows that things are being changed in the home. We think that two important issues dominate the design and interpretation of studies in this field:

1. Should avoidance of an allergen such as dust mite address the whole house, or is it sufficient to focus on the bedding or simply the mattress?
2. Should enrollment be restricted to individuals who are specifically sensitive to allergens that are being studied? For example, in a study on mite avoidance, should enrollment be restricted to patients who are monosensitized to dust mites?

The importance of these questions became clear because of two Cochrane analyses of dust mite avoidance studies that made wrong decisions based on several questions.[55,56] Although they restricted the studies to those that evaluated dust mite avoidance, they did not address whether subjects enrolled in the studies were sensitized to multiple allergens. Worse, they included in the analysis studies in which the only intervention was a mattress cover or placebo, and another study in which covers were tested but both active and placebo groups were encouraged to hot wash the bedding regularly.[3,4] Only a few studies have achieved major reductions in mite allergens, were focused on suitably sensitized individuals, and have maintained a reduction for 6 months or more. Enert et al. made extensive efforts to identify monosensitized mite-allergic subjects and achieved major reductions in exposure to mite allergens. The result was an impressive reduction in symptoms and BHR in the active group.[57] The other study that addressed the question correctly was that of Morgan et al., who applied avoidance measures in the homes directed at each of the allergens to which the patient was allergic. Again, the researchers achieved a highly significant improvement in symptoms.[58] Neither of these studies was included in the final Cochrane analysis.[56] We previously analyzed a large number of studies and concluded that those that maintained a large decrease in mite allergen in the bedroom for at least 6 months consistently achieved a benefit.[2,59] However, it is clear that (1) simple measures such as covering the mattress are not effective in themselves, and (2) it is not easy to make major changes in a patient's home. Thus, unless the family is committed to changing the home, and it has the resources to make real changes (e.g., removing carpets), avoidance may not be achievable as part of the treatment or within a trial. Judging these studies on secondary avoidance from the point of view of primary avoidance, it seems clear that real changes in the homes are necessary to achieve a significant effect on exposure.

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Pharmacotherapies Early in Life to Prevent the Atopic March

18

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Introduction

There have been relatively few studies designed to demonstrate whether a pharmacotherapeutic approach could prevent allergic sensitization or interrupt the march from food allergy and eczema to inhalant allergy associated with rhinitis and asthma. Previously, the only trials have involved the use of therapies developed to treat established allergic diseases. However, as understanding of the mechanisms involved in primary sensitization and the subsequent evolution to allergic disease improves, ever more potential targets for prevention are being identified. We remain optimistic that we will identify effective strategies for allergy prevention and disease modification. However, other than in animal models, most studies have merely identified associations between particular factors and subsequent allergy and allergic disease. Association is not synonymous with causation, and as indicated in an editorial in *Pediatric Allergy and Immunology* by Ulrich Wahn, in some respects our concepts and expectations have failed to transpose into effective interventions.[1] Although the ontogeny of allergic disease in murine models is relatively well-characterized, the same is not true for humans. Furthermore, it is clear that influences on allergic sensitization are at least partially independent of those leading to allergic disease (Figure 1).

Taking the analogy of the prophylaxis and treatment of TB, we have reviewed the evidence in three categories:

1. *Primary prophylaxis*—directed at susceptible subjects before they have any evidence of allergic sensitization. Most research in this area has focused on infants born to allergic parents in relation to dietary variations, and manipulating the microbiome with probiotics or prebiotics. For atopic march, this would be prevention of atopic dermatitis.
2. *Secondary prophylaxis*—directed at those showing evidence of allergic sensitization but with no signs of allergic disease. Research currently focuses on dietary practices particularly during weaning. The other group of subjects that fall into this form of prophylaxis are those with allergic sensitization having the first manifestations of the allergic march. The intervention is designed to interrupt the progression from eczema and food allergy to rhinitis/asthma and inhalant allergy. This is one of the few areas in which pharmacotherapy and allergen immunotherapy have undergone clinical trials.
3. *Tertiary prophylaxis*—this is the fundamental approach to allergy clinical practice, which involves the institution of regular therapy to prevent exacerbation of disease and thereby improve the quality of life. It was hoped that by early intervention this might modify long-term outcomes, but other than for allergen immunotherapy there was no evidence for a positive effect.

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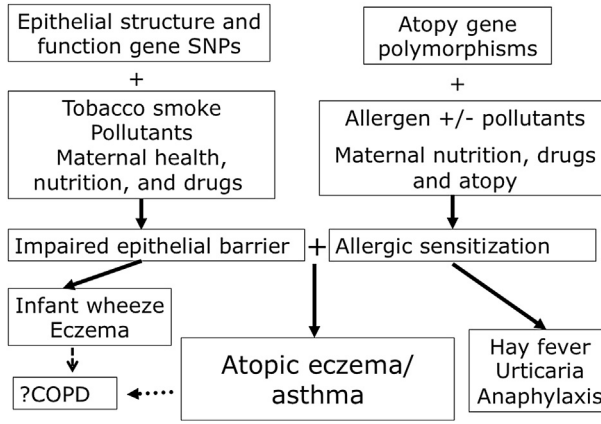


Figure 1 Risk factors for developing atopy and asthma. Potential risk factors and environmental factors leading to development of asthma. The genetic risk factors in conjunction with environmental exposures lead to impaired epithelial barrier dysfunction and allergen sensitization leading to atopy.

We have included an additional section on pharmacotherapy, which increases the risks of allergic diseases because remarkably there are more published articles on adverse rather than on favorable effects of drugs.

Pharmacological agents have been studied in other inflammatory diseases: multiple sclerosis, inflammatory bowel disease, and other autoimmune diseases. Multiple sclerosis is a chronic inflammatory disease of the central nervous system characterized by demyelination and axon loss. The antigen specificity of this autoimmune pathway is unknown. However, recent advances have called for the use of disease-modifying agents in national and international guidelines for relapsing and remitting multiple sclerosis (reviewed in Kamm et al.[2]). These drugs have reduced the number of exacerbations and prevented the development of new multiple sclerosis lesions on magnetic resonance imaging. The agents include interferon-beta-1a, glatiramer acetate (which interferes with the binding of the major histocompatibility complex), fingolimod (sphingosine-1-phosophate receptor antagonist), teriflunomide (which inhibits pyrimidine biosynthesis and disrupts the interaction of T cells with antigen-presenting cells), and dimethyl fumarate (neuroprotective). These drugs have significant side effects including an increased number of infections or cancer, depending on the agent. These agents would be tertiary or secondary prevention agents because they work after the disease is diagnosed.

The disease-modifying agents have also been studied in other autoinflammatory diseases such as juvenile idiopathic arthritis (previously known as juvenile rheumatoid arthritis); it has multiple forms with one just affecting the joints and another affecting multiple system disease involving joints, fever, and rash. Various biologicals and nonbiologicals have been studied. These drugs in general have a reduction in flare-ups and reduction in joint inflammation.[3] One of the most promising agents is the tumor necrosis factor-α (TNF-α) inhibitors. Early aggressive therapy with TNF-α inhibitors improved

symptoms; 68% had no disease after 52 weeks of therapy. This suggests that early therapy can prevent disease progression.[4] In general, other disease-modifying agents work on various key points of the pathway (antigen presentation or signaling pathways). These agents prevent disease progression, which makes them secondary prevention agents.

For treatment and prevention of the atopic march, it is important to look at what step can be blocked in a possible pathway. The process may start as early as pregnancy. Pregnancy is in some respects a T-helper 2 (Th2) lymphocyte-mediated phenomenon. Under normal circumstances, the maternofetal interface creates a Th2 and T regulatory environment by generating interleukin-4 (IL-4), -10, and -13 and transforming growth factor-β. These cytokines downregulate the maternal Th1 response against fetopaternal antigen, which is essential to sustain pregnancy and enhance fetal growth. Failure to achieve this balance in both murine and human models results in early miscarriage or intrauterine growth restriction.[5] The Th2-promoting cytokine milieu clearly affects the fetus and there is good evidence of a Th2-biased immune response in the neonate[6] that in some circumstances may be irreversibly committed as a result of a range of factors including maternal and fetal genes, maternal allergy, nutrition, allergen and drug exposure, and maternal gut microbiome.[7] The latter is critical to the inoculum received by the neonate during a normal vaginal delivery. The rapid development of diversity of the gut microbiome in the infant is likely to be a key factor switching the neonatal Th2-biased response to a more balanced pattern.[8]

The fetus is capable of mounting a T cell response to antigenic exposure from as early as 16 weeks' gestation. Although circulating cells are immature, in the fetal small bowel antigen-presenting cells and T cells demonstrate a full array of signaling molecules and maturity markers.[9] Furthermore, antigens and allergens can be detected in the amniotic fluid, providing a route of exposure via fetal swallowing.[10] In addition, maternal IgE present in the amniotic fluid and aggregated to IgE receptors on fetal antigen-presenting cells could facilitate antigen uptake and sensitization.[11] Thus, it is not surprising that peripheral cord blood mononuclear cells from some neonates proliferate in response to environmental allergens, and that the pattern of cytokine release is different in those destined to develop allergy.[12]

The identification of innate lymphoid cells with a type 2 (ILC2) phenotype has provided the missing link to explain how allergen exposure induces an adaptive Th2 response. It could also explain how the local airway immunopathology in nonallergic asthma is similar to that associated with allergy.[13] Many allergens have enzymatic activity that may well mimic those released by parasites, which open gut epithelial cells, facilitating attachment. The epithelial cell response is to release thymic stromal lymphopoietin (TSLP), IL-25, and -33. These cytokines activate ILC2 to release IL-4, -5, and -13, and amphiregulin. For parasite elimination, the IL-5 effect on eosinophils and the local effects of IL-13 are pivotal, but the IL-4 creates the environment for a Th2 adaptive response to the allergen. Amphiregulin induces tissue repair and remodeling through fibroblast activation (Figure 2).

The process continues from the fetal period through atopic dermatitis (AD) and allergic sensitization onto asthma—the atopic march (Figure 3). The hypothesis reflects a combination of genetic variants and defects in the skin barrier that initiates

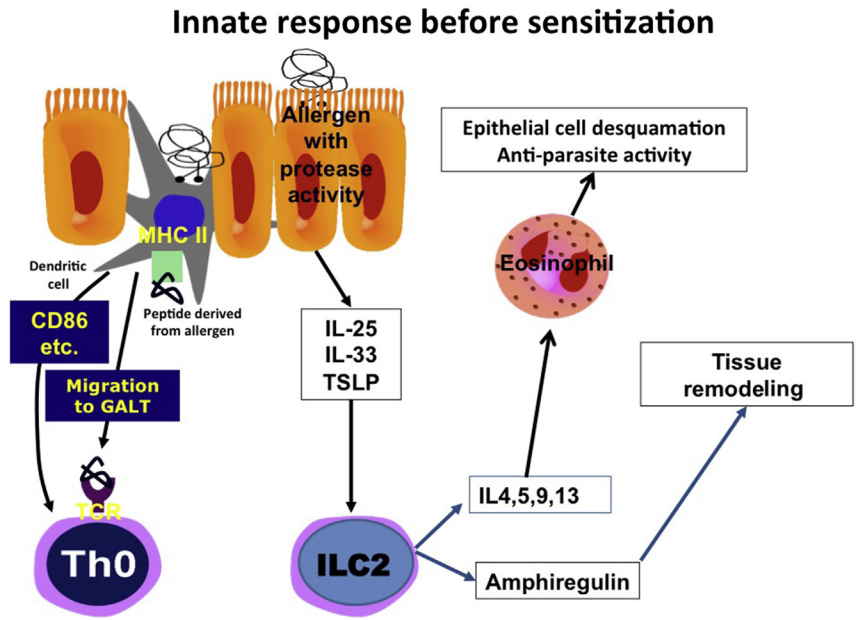


Figure 2 Innate Response in Allergy. Allergy Exposure can lead to epithelial damage and sensitization. Many allergens have native protease activity that leads to production of danger cytokines (IL-25, IL-33 and TSLP) leading to activation of innate lymphoid cells -2 (ILC-2) leading to production of Th2 cytokines (IL-4, 5, 9 and 13). In conjunction, allergen is presented to T cells with costimulation of cytokines leading to development of Th2 paper. These Th2 cells and cytokines lead to additional tissue damage and eosinophilic production.

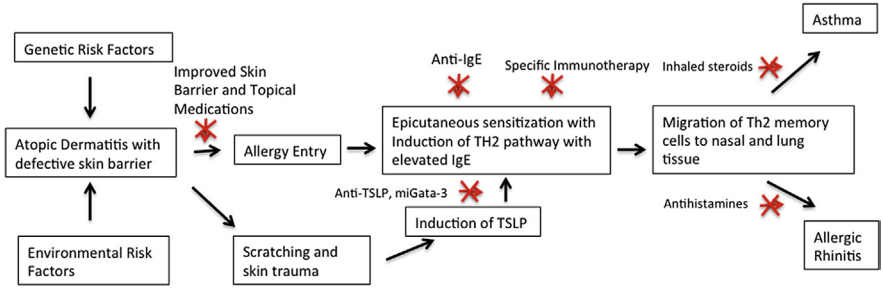


Figure 3 Potential pathways for blocking the atopic march. Potential mechanism for developing asthma from atopic dermatitis is shown from the genetic risks along environmental factors. The theory is altered barrier dysfunction leads to increased allergen sensitization and development of Th2 cytokines and asthma and atopy. Potential points of intervention to block atopic march are indicated with "X".

and potentiates the atopic march. It has been proposed that allergen penetration can lead to transcutaneous sensitization. Some key longitudinal studies have also highlighted the IgE in the process. Other studies have shown that skin barrier defects (in particular, filaggrin) are associated with AD and systemic responses such as

increased total IgE and airway hyperreactivity, possibly due to subsequent sensitization. Based on murine and human studies, keratinocyte-produced TSLP may also link AD and asthma. Because AD has elevated TSLP in the skin, an IL-7–like cytokine is released as the result of trauma, various microbes, and inflammation, which in turn initiates or exacerbates the allergic inflammation. Mouse models have shown that a significant elevation in serum TSLP triggers bronchial hyperresponsiveness to inhaled allergens in the absence of epicutaneous allergen sensitization. These findings suggest that the link between overexpression of TSLP in the AD skin and other allergic disorders could be independent of the skin barrier defect (reviewed in Spergel[14]).

Adverse effects of pharmacotherapies on allergy outcomes

As indicated in the Introduction section, epidemiological associations do not necessarily indicate causal relationships. Many studies have found associations between the use of a number of drugs in pregnancy and early life with subsequent allergic disease. Because maternal atopic disease confers the highest risk of early similar problems in offspring, it is likely that any drugs used by the mother to manage disease will associate with allergy in the infant. Thus, anti-asthma medications are obviously excluded, but the same may be true if maternal asthma increases risks of infection requiring antibiotics and antipyretics or increases reflux, leading to the use of acid-suppressive drugs. Thus, the results of studies discussed below should be interpreted with caution.

Paracetamol/Acetaminophen

Several observational studies have shown an association between increased use of paracetamol/acetaminophen in pregnancy[15–17] and increased risk of the child developing asthma (Figure 4). Whereas most studies support the association taking account of confounders, the concomitant use of other drugs such as antibiotics negates the significance in some studies.[18] Similar issues apply to the association found with use of paracetamol through early childhood.[19–22] Although the association with its use may be confounded, this seems unlikely in view of the consistent findings in studies from diverse communities and environments. The potential mechanism to explain the association is that a metabolite of this analgesic/antipyretic depletes antioxidant activity, thereby decreasing the elimination of proinflammatory oxygen radicals. There is an interaction between paracetamol use in pregnancy and glutathione *S*-methyl transferase gene polymorphisms, which enhances the risks of asthma, providing even greater credibility to the association.[16] It is unlikely that an intervention study will be conducted in pregnancy because there are contraindications to using alternative nonsteroidal anti-inflammatory drugs. None have so far been conducted in infancy.

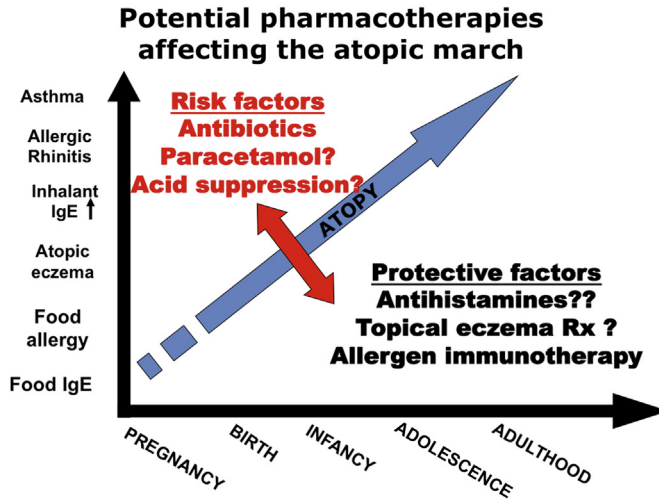


Figure 4 Risks for developing asthma and atopy. The development of atopic march is present in chronological order in y axis and age in the x axis. Risk factors (red (dark gray in print versions)) that have been identified based on observation studies that have been shown to increase the risk of development of atopy. Pharmacotherapy (black) that are hypothesized to inhibit the development of atopic march.

Antibiotics

Studies have shown associations between increased antibiotic use in pregnancy and infancy with higher risks of allergy/asthma.[18,23] Postnatal use may be confounded because those developing allergic disease may be more likely to receive antibiotics during acute infection episodes. However, a German study found that antibiotic treatment of infants increased the risk of atopic eczema whereas infections alone had no impact, even though they were of similar severity.[23] The antenatal effect is consistent and less subject to confounding. Mechanistically, alteration of the maternal microbiome by antibiotics will change the inoculum donated to the neonate, as also occurs in those delivered by cesarean section. In both cases, this is followed by a higher prevalence of allergic disease. Although avoiding antibiotics may not be possible, a trial of prebiotics or probiotics in infants whose mothers have received antibiotics would be worthwhile.

Acid-suppressive drugs

Inhibiting gastric acidity impairs peptic digestion, which in turn could result in intact allergenic proteins reaching dendritic cells in the small bowel with close access to lymphoid follicles and therefore increased risk of sensitization. Epidemiological studies have shown associations between both pregnancy and infant exposure to acid suppression and increased allergy.[23–25] Attempts to account for potential confounding have not changed the effect.[18,23]

Prevention strategies

Primary prophylaxis agents

Skin barrier lotions

Because the start of the atopic march is AD and the defective skin barrier is a key feature, four studies have examined whether improving skin barrier preventing inflammation and allergen entry would lower the incidence of AD and allergen sensitization. Simpson and colleagues examined the use of Cetaphil moisturizer as a preventive agent in 22 patients. They observed the infants for 547 days starting at 7–14 days of life. The infants were at risk for AD, defined as a sibling or one parent with atopy. They found that AD developed in 3% compared with 10–40% in a historical control. However, with a smaller number of patients and close follow-up in active therapy, the possibility of a placebo effect is high.[26]

Lowe and colleagues examined the use of ceramide-rich lotion to prevent AD. They examined the use of Epiceram® daily for 6 weeks in 10 infants (aged 0–4 weeks) with a family history of atopy (asthma, allergic rhinitis, or atopic dermatitis). They examined the safety and the rate of atopy at the end of 6 weeks and found no safety concerns. No significant differences for atopic features were noted; however, a period of 6 weeks is probably too short to identify any development of atopic status.[27]

Two studies also examined prevention of AD through barrier creams in larger studies. Simpson et al. conducted a randomized controlled trial of 124 infants in the United States and United Kingdom.[28] The active group applied an emollient therapy on a daily basis. The active group had a 50% risk reduction (relative risk [RR], 0.28–0.9) for developing AD compared with the control group at 6 months. The study patients were at risk for developing AD if they had a family history (first-degree relative) of atopic disease. In this study, there was concern for selection bias, because over 60% of eligible patients did not enroll.

Horimukai et al. examined the use of daily moisturizers in 59 Japanese infants compared with 59 control infants, all of whom were at risk of developing AD.[29] They used the standard risk factor of a family member with atopy. The active group had a risk reduction similar to the previous study, with an RR of 0.48 (95% confidence interval [CI], 0.27–0.86) at the primary end point of 32 weeks. In addition, they measured specific IgE to eggs. They found no difference in specific IgE to egg white, but the active group had a higher specific IgE to ovomucoid (19% with specific IgE > 0.35 kU_A/L compared with 6.8% in the nonintervention group; $P = .17$). These results suggest that they prevented AD but not allergen sensitization.

These four studies suggest that the use of early moisturizers in at-risk infants could prevent the development of AD with a reduction of 50% at 6 months in the diagnosis of AD. However, several concerns exist. The end points were short in time, because previous studies (probiotics and antihistamines) showed a limited effect at 6 months that disappeared by age 2–4 years. These studies examined only the first 6 months, raising the possibility of temporary effects. Also, the increase rate of specific IgE to egg protein in active therapy is of concern. It suggests that it does not prevent allergen desensitization; this concern needs to be studied in larger and longer studies.

The use of prebiotics and probiotics and vitamin D in treating the atopic march is reviewed in the chapter on Dietary Interventions in Infancy by Garssen and Kopp.

Secondary prophylaxis agents

Antihistamines

After the successful introduction of sodium cromoglycate (SCG) as tertiary prophylaxis for asthma, many pharmaceutical companies invested huge sums in developing an equivalent orally active compound. It was assumed that the effect of SCG was through stabilizing the mast cell. However, many subsequent compounds with this activity failed to have a beneficial effect on asthma. Ketotifen was a product with inhibitory properties on mast cell degranulation that was perceived to improve asthma and urticaria.[30] A subsequent systematic review of placebo-controlled trials suggested a significant benefit for established asthma, but with side effects including excessive weight gain and sedation.[31] Because the studies mostly included atopic subjects, the effects may primarily have resulted from antihistaminic properties, and most asthma guidelines do not include ketotifen as recommended treatment. Two trials investigated its properties as a secondary prophylactic agent. The first trial demonstrated significantly less subsequent asthma in infants with atopic eczema after 12 months of ketotifen compared with placebo[32]; the second showed similar effects over 3 years in infants with a family history of allergy and raised serum total IgE. Numbers were relatively small and the mechanism of effect was unclear. Furthermore, the appetite-promoting and sedative side effects were of concern. Other potent antihistamines, such as cetirizine, have been shown to inhibit allergen-induced upregulation of intercellular adhesion molecule-1 in nasal and conjunctival epithelium, and as a likely consequence reduce eosinophil recruitment.[33–36]

This provided rationale for two large placebo-controlled trials using cetirizine and levocetirizine, respectively. In a large multicenter study, the Early Treatment of Atopic Child conducted a randomized trial of cetirizine (0.25 mg/kg) versus placebo for 18 months in 817 infants (aged 12–24 months). The infants were treated for 18 months and followed for an additional 18 months. They examined the development of recurrent wheezing or nonviral-induced wheezing as a marker of asthma.[37] Based on the primary end point, there was no significant benefit (asthma by predefined criteria in 37.7% receiving placebo and 38% receiving cetirizine after 18 months) or the use of mild or moderate topical corticosteroids for AD. A post hoc analysis showed a significant reduction in asthma with active compared with placebo treatment over 18 months of treatment and at an 18 month posttreatment follow-up for those at highest risk of asthma, namely with eczema and sensitization to either house dust mite or grass pollen. Relative risks with cetirizine were for grass pollen (0.47; 95% CI, 0.26–0.86) and house mite (0.56; 95% CI, 0.35–0.89), with limited overlap of subjects between the allergic sensitizations.[38]

Based on the positive findings in the subgroup, an additional study focusing on this group was studied. A total of 2222 infants with eczema (aged 12–24 months) were screened and 514 with the appropriate allergic sensitization were randomized to either

levocetirizine 0.125 mg/kg twice a day or placebo for 18 months in a 1:1 fashion.[39] The infants had AD and specific IgE to dust mite or grass pollen and a family history of atopy, which put the infants at high risk of developing asthma. After 18 months' treatment, there were no significant differences between treatment groups, with asthma developing in 39.8% and 40.2%, respectively. These studies indicate that antihistamines cannot be used as a secondary agent to prevent the atopic march.

Topical calcineurin inhibitors

A secondary treatment effect is to treat AD aggressively to reduce inflammation and prevent secondary sensitization and progression of AD to asthma and allergic rhinitis. The Study of the Atopic March, funded by Novartis, examined whether the early use of pimecrolimus at the first signs of flare-up of AD would prevent the progression of AD to asthma. The multicenter study of young children aged 3 to 18 months with AD defined by American Academy of Dermatology Consensus Conference criteria,[40] with no less than 3 months and no signs of food allergy or asthma, enrolled 1091 patients, mean age 7.3 months, most of whom had mild or moderate AD. The study design was conducted in 36 clinics in the United States, which was divided into 2 phases. During the double-blind (DB) phase, a stepwise treatment plan was used in which emollients were given for dry skin (step 1) followed by the addition of study drug (step 2; patients randomized 1:1 to 1% pimecrolimus cream or vehicle cream in a DB manner) if pruritus or other AD signs were observed. From this point, if patients' symptoms failed to improve after 3 days of step 2 treatment, the treatment plan added topical corticosteroid (TCS) of increasing potency to study drug: midpotent TCS (fluticasone propionate 0.05% cream; step 3a) and more potent TCS (step 3b). These were to be followed by oral agents (step 4) if no improvement was seen. Caregivers were instructed in the first 3 months on how and when to use the midpotent TCS, and after 3 months they were responsible for making this decision. All patients who completed the DB phase and did not have a definitive diagnosis of asthma were enrolled in the open label (OL) phase and given 1% pimecrolimus cream with TCS rescue, as in the initial DB treatment plan.

The study was terminated before all patients completed the DB phase because many patients left the study after the Food and Drug Administration issued a black box warning on pimecrolimus and tacrolimus. However, sufficient data from DB and OL have shown that the group treated with pimecrolimus initially had no significant difference in food allergy, asthma, or AD rates. In fact, the trend was for increased atopy in the pimecrolimus-treated arm, which suggests that the early use of pimecrolimus does not prevent the atopic march; in fact, it might promote it. The early use of topical pimecrolimus cream was safe in the study, with no significant difference of adverse events between groups (Paller A, personal communication).

An alternative secondary strategy to reduce the inflammation and severity of AD is to use TCS or topical calcineurin inhibitors to reduce the flare-ups of AD. The general idea was to use them two to three times a week to prevent eczema flare-ups. Eight trials examined this method and a meta-analysis was published in 2011.[41]

All studies found that the active therapy had fewer flare-ups than placebo arms with an RR reduction of flare-ups with tacrolimus ranging from 0.61 (0.48–0.78) to 0.94 (0.76–1.16) and a reduction of flare-ups between 0.38 (0.30–0.49) and 0.58 (0.33–1.02) for fluticasone propionate ointment and cream, respectively. Interestingly, when the two studies using fluticasone propionate cream were combined in the meta-analysis (RR, 0.46; 95% CI 0.38–0.55), flare-ups were reduced in more than three studies of topical tacrolimus three times a week (RR, 0.78; 95% CI, 0.60–1.00). Regarding safety issues, there were more viral and ear–nose–throat infections in the topical steroid arms compared with the vehicle. One of the TCS trials reported 4.5% adrenal suppression in the active arm, whereas two other clinical trials saw no adrenal suppression in the active or placebo arms. None of the studies reported evidence of skin atropy. These studies indicate that topical corticosteroids and topical calcineurin inhibitors on a regular basis have tertiary preventative effects, reducing the number of flare-ups.

Specific allergen immunotherapy

Allergen immunotherapy has been in clinical practice for over a century after the pioneering work of Leonard Noon and his clinical colleague, John Freeman, at St. Mary's Hospital London, in 1911. They demonstrated that graded injections of grass pollen extracts reduced conjunctival sensitivity to grass pollen[42] and subsequently reduced the severity of hay fever.[43] The first DB, placebo-controlled trial confirmed the clinical benefit in 1954.[44] Only in the past three to four decades have immunological changes associated with benefit been elaborated. Favorable outcome is associated with abrogation of the late allergen-induced response,[45] reduced Th2 and increased Th1 activity, increased IgG4 antibodies, and regulatory T-cell responses,[46] and after 3 years of treatment a prolonged carryover effect.[47] This is the only treatment that has been shown to alter the natural history of established allergic disease. A few studies have investigated the potential for allergen immunotherapy to be used in primary and secondary prophylaxis.

One observational trial showed that women who continued rye grass immunotherapy during pregnancy had children who 2-12 years later were less likely to be rye grass allergic than were infants of rye grass–allergic mothers not receiving immunotherapy.[48] Until then, no controlled trial had been performed because of concern about the potential for an acute allergic reaction to the immunotherapy compromising the pregnancy. However, a clear mechanism could explain this effect. Allergen immunotherapy increases IgG4 allergen-specific antibody levels. Although it is unclear whether this change is the key event conferring benefit in established disease, high cat and birch IgG allergen-specific antibody in cord blood was associated with less subsequent allergy.[49] High egg exposure during pregnancy with high ovalbumin IgG in the cord blood was associated with less subsequent eczema.[50] There are IgG inhibitory receptors on antigen-presenting cells, which could provide a mechanistic explanation.

Jacobsen studied the effect of 3 years of specific immunotherapy for allergic rhinitis to prevent the development of asthma. A total of 205 children (aged 6–14 years) with grass and/or birch pollen allergy were randomized to 3 years of standard subcutaneous immunotherapy (SCIT) or placebo followed by 2 years of follow-up.[51] The study had 183 evaluable

patients at 5 years after starting SCIT and 147 were evaluable 10 years after the start of SCIT.[52] The odds ratio for developing asthma was 2.5 at 3 years after starting SCIT, 2.68 at 5 years (17 of 75 in the SCIT arm compared with 29 of 67 in the placebo arm developed asthma), and 2.5 at the 10-year point (16 of 64 in the SCIT compared with 24 of 53 control patients), with SCIT having more protective effect at all time points. They also examined conjunctival provocations and methacholine challenges. There was a statistical improvement for conjunctival provocations for the SCIT group compared with the placebo group at 3, 5, and 10 years.[51,52] There was no difference in the methacholine challenges between groups at 5 or 10 years. Overall, SCIT in this well-designed study shows a significant secondary preventative therapy for the development of asthma from allergic rhinitis. Several other studies have shown that sublingual and subcutaneous allergen immunotherapy in children with a single allergic sensitization reduced the subsequent development of new allergen sensitizations.[53,54] Trials are in progress to establish whether sublingual allergen immunotherapy will abrogate the allergic march from eczema to asthma. This is currently the most promising approach to allergy prophylaxis.

Tertiary prophylaxis agents

Inhaled corticosteroids

Large-scale studies have tried to prevent the progression of asthma, including the Childhood Asthma Management Program (CAMP) study. The CAMP study enrolled 1041 patients, focusing on long-term lung growth, as reflected in the choice of postbronchodilator forced expiratory volume in 1 second (FEV1) as the primary outcome. Patients were divided into three equal groups receiving nedocromil, placebo, and budesonide. They had rescue therapy with albuterol as quick relief and the use of beclomethasone if they had too many flare-ups or daily symptoms. While receiving budesonide, patients had improvement with more symptom-free days and fewer hospitalizations, urgent care visits, or prednisone courses. Prebronchodilator lung function (FEV1) and airway responsiveness were also improved in the budesonide group. However, the primary outcome measured by postbronchodilator FEV1 or postbronchodilator FEV1/foreced vital capacity was similar in all three groups, which indicated no disease-modifying effect. In terms of safety, the one issue was a 1.1-cm decrease in growth in the budesonide arm after 4 years of trial (reviewed in Strunk et al.[55]). This study had a positive tertiary protective effect but no secondary protective effect, because the natural history was not changed but symptoms were improved.

A proposed theory for why the CAMP study did not lead to improvement in lung function or a disease-modifying effect was that it was started too late. Therefore, two studies examined the use of inhaled steroids in wheezy infants before they were diagnosed with asthma. In these studies, they treated toddlers who were at risk of developing asthma, based on the Martinez risk factor for asthma (history of AD and family history of atopy). The first study by Guilbert et al. examined 285 toddlers randomized to fluticasone 88 μg twice a day or placebo for 2 years, and then both groups were monitored for 1 year (third year of the study). Patients receiving fluticasone had fewer exacerbations ($P < .001$) and more symptom-free days ($P = .006$) compared with the placebo arm. However, during the medication-free year (the third year), there was no

difference between groups in the number of exacerbations, symptom-free diseases, or lung function, and there was no disease-modifying effect. There was a similar adverse event because the active arm of fluticasone had a 1.1-cm decrease in growth ($P \leq .001$) in the first 2 years that declined to 0.7 cm in the observational year ($P = .008$).[56]

Another study by Murray and colleagues looked at fluticasone 100 µg twice a day in 200 children (mean age, 1.2 years). Doses were reduced every 3 months if controlled, and if not undercontrolled by 3 months, open-label fluticasone was added. Children were observed until age 5 years. Lung function was measured at the end of the study and questionnaires of symptoms were given to parents of the patients. There was no difference in any measure including current wheeze, diagnosis of asthma, lung function, and airway reactivity. Similar to the study by Guilbert, patients treated with fluticasone had transient reduction of growth velocity equating to 1.4 cm at age 5 years.[57] These two studies indicated that inhaled steroids do not have a disease-modifying effect and have no role in secondary prevention of the development of asthma. However, when patients were on active therapy, they had a reduction of symptoms and exacerbation. However, both studies show a transient reduction in growth velocity, with about 1 cm loss of height. Inhaled steroids have no disease-modifying benefit, but they have a short-term benefit with some risks. In conclusion, inhaled steroids do not have a secondary preventative effect on the development or progression of asthma, but they have a tertiary protective effect with a reduction of symptoms.

Future therapies

A predisposed individual is thought to be sensitized through a defective skin barrier triggering the TSLP pathway to develop atopy. In atopic asthma, sensitization to an allergen results from activation of Th2- and B-lymphocytes, leading to the release of cytokines such as IL-4 and IL-13 and production of IgE antibodies. Immunoglobulin E antibodies bind to high-affinity (Fc**ε**RI) receptors on mast cells and basophils. Subsequent allergen exposure results in cross-linking of cell-bound IgE, leading to cell degranulation and release of proinflammatory mediators such as histamine, tryptase, leukotrienes, and prostaglandins. Future concepts to block the development of the progression of the atopic march would work on individual molecules of the pathway, including antibodies against TSLP, IgE, and its receptor, anti-IL4/13 and leukotriene modifiers.

Biologics such as omalizumab (Xolair™) block IgE and prevent many IgE-dependent activities and are approved for asthma and chronic urticaria. They have been shown to be beneficial in reducing of asthma flare-ups in adults and children.[58] Data on a disease-modifying effect are conflicting.[59] In one trial lasting 28 weeks, IgE and symptoms returned to baseline when omalizumab was stopped.[60] A review of 61 responders to omalizumab (average length, 3 years of therapy) who had discontinued therapy after a mean duration of 22.7 months showed mixed results. The loss of asthma control was documented in 34 patients (55.7%), with a median interval between discontinuation and loss of control of about 1 year. This suggests a disease-modifying effect in about half of the patients.[61] In a small trial of long-term omalizumab for 6 years in 18 subjects, 6 and 14 months after discontinuation, 13 of 18 patients' symptoms remained the same, or had improved from their symptoms during omalizumab treatment, which suggests a disease-modifying effect in long-term therapy. The use of omalizumab has not been studied for prevention of the atopic march.

Biologics against TSLP, IL-4, IL-13, and their receptors are used in various stages of clinical trials and might have benefit for preventing atopy. In particular, anti-TSLP has been shown to be critical for the development and progression of atopy in murine models. However, efficacy and safety studies in humans need to be done before clinical trials on preventing the atopic march can be formulated.

Conclusions

Prevention agents fit into one of the three general categories. For the primary prevention of developing disease, recent studies on increased use of moisturizers to treat the primary skin barrier defect are encouraging (Figure 3, Table 1). However, results are still preliminary because only early time points have been examined and patients need to be studied and observed for at least 2 years to prevent AD. In addition, the development of asthma takes 5-10 years and patients need to be examined at the later time points, because increased sensitization may occur, suggesting that the atopic march is not prevented and asthma might still develop.

Standard pharmacological measures (asthma medications, topical anti-inflammatory treating AD, or antihistamines) have not been successful for secondary preventative effects on progression of the atopic march. However, the trial of subcutaneous allergen immunotherapy showed a significant and prolonged protective effect against the development of asthma in patients with allergic rhinitis. This concept should be repeated using one of the three methods for allergy immunotherapy (subcutaneous, oral, or epicutaneous).

Table 1 **Measures to prevent the atopic march**

Type	Effectiveness
Primary prevention	
Moisturizers	+ Examined only at 6 months – Trend to increased sensitization
Diet	+/– Effective at 2 years, not at 4 years
Vitamin D	–/?
Probiotics	–
Prebiotics	+ At 1 year
Secondary prevention	
Antihistamines	–
Inhaled steroids	–
Immunotherapy	+
Topical calcineurin inhibitors	–
Tertiary prevention	
Inhaled steroids	+
Topical corticosteroids or topical calcineurin inhibitors	+

Tertiary prevention strategies reducing the severity of symptoms have been successful, because current therapies for AD and asthma have reduced the disease burden and symptoms for most patients. The ultimate goal of slowing or stopping the atopic march has not been successful using current management plans. Future biologicals might have a role in preventing the atopic march, but additional studies are needed.

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The Role of Antiviral Strategies for the Prevention of Childhood Asthma

19

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Introduction/Background

There has been a longstanding interest in the role of viral respiratory tract infections that are associated with wheezing in infancy and the subsequent development of asthma. It is unresolved whether viral infections during infancy are instrumental in causing asthma as children grow older, or whether children who are predisposed to asthma are more likely to have significant wheezing exacerbations with viral infections.

Approximately 30% of infants and toddlers will experience wheezing illnesses severe enough to require evaluation and treatment in a doctor's office or hospital during the first 2–3 years of life.[1–3] In early studies, it became clear that severe episodes of wheezing (bronchiolitis) requiring hospitalization in infancy were associated with an increased risk for recurrent wheezing into the school-age years.[4–7] Early studies also showed that infants hospitalized for bronchiolitis who also had a family history of asthma had an even greater risk for developing asthma.

In the Northern Hemisphere, episodes of bronchiolitis are most frequent during the winter months. These illnesses typically occur between November and March and have a characteristic clinical syndrome with initial symptoms of upper respiratory infection followed by wheezing and cough. After the respiratory syncytial virus (RSV) was first isolated in the late 1950s, it became clear that RSV was an important cause of bronchiolitis in infants. Since then, considerable attention has been focused on the specific role of RSV for the risk of developing asthma among young children presenting with bronchiolitis.[8–11]

The development of polymerase chain reaction (PCR)-based techniques for virus detection during the 1990s confirmed that up to 90% of wheezing attacks experienced by children during the first 3 years of life are associated with viral infections, especially with RSV and less frequently metapneumovirus, rhinovirus, and influenza during the mid-winter months in countries with temperate climates in the Northern Hemisphere.[12,13] During other months of the year, most infants hospitalized with bronchiolitis have been shown to be infected with rhinovirus.[13,14] Subsequent prospective studies have suggested that recurrent rhinovirus infections during the first 3 years of life are actually associated with a higher risk for persistent wheeze at age 6 than wheezing provoked by RSV.[15]

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Thus, whereas a number of different viral pathogens can cause bronchiolitis in young children, RSV and rhinovirus have emerged as the pathogens most closely associated with an increased risk of subsequent asthma development.

In addition to data indicating that virus-induced attacks of wheezing in early childhood increase the risk for developing asthma, it is clear that exposure to viral pathogens is not the sole factor involved. Children who attend day care or have older siblings have increased numbers of common cold illnesses during early childhood. Despite this increased exposure, these children actually have a lower risk of asthma in later years.[16] Whether other factors such as the allergen and microbiome environment, which are associated with the exposure to other children, contribute to the lower risk of developing asthma has been considered but not fully evaluated.[17] There are also predisposing genetic risk factors to consider that impart a greater risk for developing asthma. In addition to the atopic phenotype, reports indicate that children with genetic mutations at the 17q21 locus have a greater risk for wheezing illness and for developing asthma, especially young children with rhinovirus who wheeze.[18] This finding serves as an example for a greater risk for persistent wheezing as children grow older that is independent of the presence of atopy. The ability to identify these at-risk children at an early age may help treat those who are most likely to benefit from antiviral treatments.

In addition to viral respiratory tract infections that provoke wheezing, it is well-known that infants with atopic dermatitis and/or sensitization to food allergens also have an increased risk, independent of infection, for developing asthma.[19,20] Based on data from older children, it is possible that interaction between virus and allergen-induced inflammation in the airway at an early age amplifies the risk for persistent wheezing. This adds to the complexity of understanding the pathogenesis of asthma later on, but further increases the options that might be available for developing new treatment strategies to modify asthma development.

Bacterial pathogens isolated from the upper airway in neonates (*Haemophilus influenza*, *Streptococcus pneumonia*, and *Moraxella catarrhalis*) have been reported to increase the risk of developing recurrent wheezing.[21] It is not entirely clear whether these organisms are directly involved in asthma pathogenesis or whether colonization with these organisms results from an altered airway environment among children who are predisposed to asthma. A potential role for *Mycoplasma* and *Chlamydia* in asthma development has also been considered, but differentiating acute infections from prolonged carrier states with these atypical bacterial pathogens has been difficult, and their detection in the airway is generally low compared with the high prevalence of viral pathogens at the time of an acute attack.[22] Currently, considerable attention has been given to the role of commensal bacteria in the gut and airway and on the skin, in influencing the ontogeny of asthma. This will be reviewed in greater detail in other chapters.

General antiviral strategies to consider

An optimal approach to prevent or reduce the risk for developing asthma is to target critical environmental and endogenous events that occur in early life to increase the risk for asthma. The strong evidence that viral respiratory tract infections provoke

most wheezing attacks in infants and toddlers, and that more severe attacks of wheezing may be linked to asthma pathogenesis, provided the rationale for developing antiviral strategies that can be used safely in early childhood. To date, there is no evidence that influenza vaccines administered to infants or toddlers have altered the incidence of asthma, or that influenza vaccines given annually to asthmatic children have decreased the prevalence or frequency of asthma exacerbations in the school-age population. As noted, the main viruses that have been shown to impart an increased risk for developing asthma are RSV and rhinovirus. Thus, for good reason, the development of most antiviral strategies is being focused on these two pathogens.

Lessons learned from antiviral strategies focused on RSV

Several prospective studies have been designed to determine whether RSV was significantly linked to the etiology of asthma. Studies by Sigurs and colleagues focused on infants who were hospitalized for RSV-induced wheezing.[23] Follow-up revealed an ongoing association between hospitalization with RSV infection and the development of atopic asthma into the teenage years. Other studies have not been able to confirm these findings. The detection of immunoglobulin E (IgE)-specific antibody to RSV, along with evidence of histamine release in nasal secretions, from children who were hospitalized for bronchiolitis suggested that an atopic response to the virus might be involved.[24] Follow-up of these infants revealed problems with recurrent wheezing at age 7 years; however, there was no longer evidence for an association with atopy, judged by sensitization to food and environmental allergens.[25] By comparison, follow-up of children who experienced wheezing with RSV (most of whom were not hospitalized) in a cohort of children observed in Tucson revealed an association with recurrent wheeze to age 11 years, but not beyond.[26] Again, there was no association with the development of atopy. In addition, unlike the experience with rhinovirus, RSV infections do not account for a significant proportion of wheezing exacerbations after age 2–3 years, whereas rhinovirus infections continue to occur and are associated with most wheezing attacks during the school-age years.[13,27]

Antiviral agents that have been used to treat RSV infections include ribavirin, a nucleoside analogue with a broad spectrum of antiviral activity. Despite significant effort, the efficacy of ribavirin for treatment of bronchiolitis has not been clearly established because of the lack of convincing efficacy, together with the cost, the difficulty of administration, and the potential for toxicity for health-care workers. The potential for beneficial effects of ribavirin on the development of asthma is limited, aside from a few studies suggesting that treatment of bronchiolitis with ribavirin in young infants may reduce the risk of subsequent asthma.[28–30] An RSV fusion inhibitor drug, GS-5806, has been shown to reduce viral load and the severity of clinical illness in healthy adults when taken orally within the first 5 days after experimental inoculation with RSV.[31] Whether this drug might be effective or safe for infants, who lack anti-RSV immunity and may have a higher viral load compared with adults, needs to be determined.

Respigam a pooled polyclonal IVIG with high titers of antibody to RSV, and subsequently palivizumab, a humanized monoclonal antibody to the F-protein of RSV, have been used for seasonal prophylaxis against severe illness resulting from RSV infection. The costs of these medications are high and these prophylactic therapies have been recommended only for infants at the highest risk of severe disease, including those born prematurely, who have underlying chronic lung or heart disease, or who have other severe chronic illnesses such as immunodeficiency disorders.[32] Thus, this target population of high-risk infants is not large enough to determine whether these treatments can affect the overall prevalence of asthma. In addition, studies of the effect of passive immunoprophylaxis on recurrent wheezing and the development of asthma have reported mixed results.[33,34] Moreover, the administration of palivizumab to provide passive anti-RSV immunity might delay the development of acquired and persistent immunity needed to moderate severe infections caused by RSV in the future.

Efforts to develop a vaccine for RSV to stimulate an acquired immune response to this virus have met with challenges. Natural infection with RSV results in an immune response that moderates illnesses, but does not prevent infection. Furthermore, an initial attempt to produce a formalin-inactivated vaccine to RSV resulted in more severe illness associated in infants when they subsequently developed a natural infection with RSV.[35,36] This failure slowed efforts to develop a vaccine to this virus, and to date, a vaccine to RSV has not been licensed for children.

Antiviral strategies for rhinovirus

The impact of RSV infection appears to be related to severity of illness, with a greater risk for subsequent wheezing among those who are hospitalized with bronchiolitis. In contrast, the frequency of rhinovirus infections, regardless of severity, seems to be an important factor for development of subsequent wheezing.[15] The limited benefit of interventions that modify the severity of RSV infections on subsequent development of asthma suggests that antiviral interventions to prevent asthma may need to be directed at both RSV and rhinovirus. The strategy to prevent infection or moderation of illness may be different for the two viruses.

A variety of approaches to prevent or treat rhinovirus infections with antiviral agents has been explored. Although antivirals with excellent activity and favorable pharmacology have been developed, the effects of treatment begun after onset of upper respiratory symptoms have typically been modest. Similarly, efforts to prevent asthma exacerbations by initiating treatment after the onset of upper respiratory symptoms resulted in a modest benefit.[37] The fact that the onset of asthma symptoms appears to coincide with the onset of upper respiratory symptoms appears to limit the utility of this approach (Figure 1(A) and (B)).[38] In addition, the experimental rhinovirus challenge model has shown that symptoms in the lower respiratory tract do not emerge until 2–3 days after virus inoculation, when symptoms in the upper respiratory tract and viral load peak.[39] Thus, once symptoms become manifest, viral infection and replication are in progress and antiviral medications may not be optimally effective unless used prophylactically.

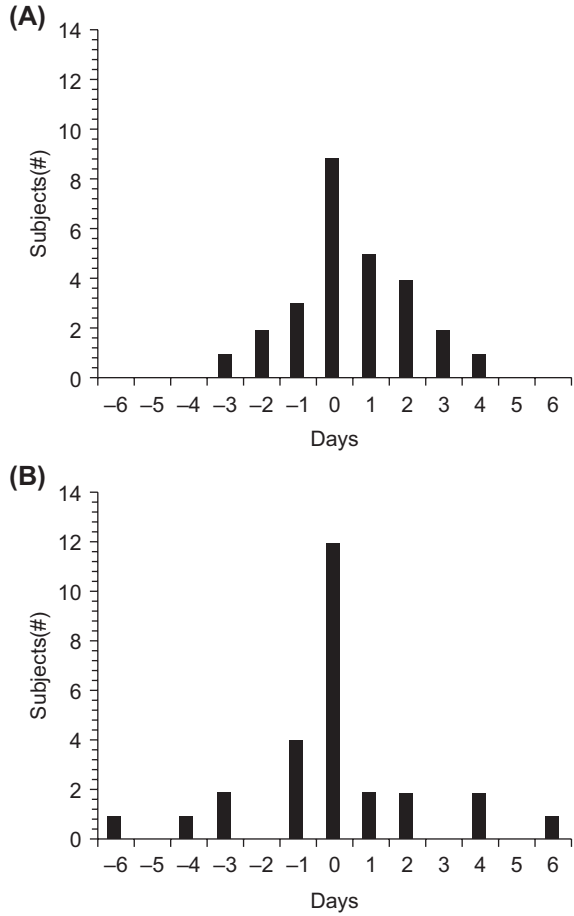


Figure 1 Timing of rhinovirus-induced asthma symptoms relative to cold symptoms. Days are numbered starting with the first day of either cold or asthma symptom onset (A), or peak symptoms (B). Positive numbers (in days) indicate when cold symptoms were noticed first. Negative numbers indicate when asthma symptoms were noted first.

The strong association between frequent rhinovirus infections in infancy and the subsequent development of later wheezing has been described above. Although it is possible that moderation of the severity of illness would be beneficial, the experience with RSV suggests that this may not be sufficient to have a meaningful effect on subsequent asthma. It is also not known whether infection of the lower versus upper respiratory tract is important in the development of asthma. Regardless, the apparent coincidence of upper and lower respiratory symptoms suggests that intervention after the onset of illness will be difficult. As a result, it seems likely that prevention of asthma with antiviral agents will be most effective if the antivirals are used to prevent rather than treat infection.

The use of antiviral agents for prophylaxis of rhinovirus infections also has challenges. In temperate climates, the viral respiratory season typically begins in the fall of the

year, with the onset of infections resulting from rhinoviruses.[40] Rhinovirus infections persist throughout the winter months but with a lower incidence before again causing another peak of illness in the spring. Targeting of antiviral prophylaxis to the fall and spring peaks of infection has been shown to be effective and could reduce the cost and complexity of providing prophylaxis.

Despite the feasibility of this approach, a strategy of seasonal prophylaxis is still less than ideal. The continuous use of an agent throughout the respiratory peak season presents issues of compliance and cost, and prolonged exposure to drug increases the potential for toxicity, side effects, and viral mutations leading to resistant strains. An alternative strategy that takes advantage of both the seasonality of the pathogen and the risk of exposure is the use of family-contact prophylaxis. Many rhinovirus infections in young infants appear to result from contact with infected school-aged siblings. The family-contact prophylaxis strategy relies on institution of the prophylactic intervention whenever a family member has symptoms of a viral respiratory infection. This approach minimizes exposure to the prophylactic agent and has been shown to be effective for prevention of rhinovirus infection and of asthma exacerbation.[41,42] Family-contact prophylaxis has the potential to reduce but not eliminate the risk of infection. Infections that occur out season or those that are acquired outside the home would not be prevented by this approach.

Specific antivirals

Interferon and interferon inducers

The innate host response initiates many of the symptoms associated with viral respiratory infections, especially those caused by rhinovirus and RSV. This suggests that modulation of the innate response might influence the development of symptoms during infection. Upregulation or stimulation of innate responses may in some circumstances prevent infection. This is the principle behind the use of the interferon inducers. Downregulation of the innate responses would be expected to moderate the symptomatic response to an acute viral infection. However, it remains to be determined whether this can be achieved without interfering with the development of the beneficial adaptive immune responses to rhinovirus as children grow older.

The discovery of interferons as natural antiviral molecules led to extensive efforts to develop preparations of interferon that could be administered as antiviral prophylaxis.[43] Initial efforts to use interferons to prevent or treat viral respiratory disease employed the use of preparations of human leukocyte interferon. Assessment of interferons as antiviral agents was facilitated by the production of these molecules using recombinant technology. Many studies were done using intranasal interferon-alfa and, to a lesser extent, interferon-beta, as treatment or prophylaxis for rhinovirus infection.[37,42] The interferons were effective when used as prophylaxis but ineffective when treatment was started after the onset of infection. When α-interferon was administered both as seasonal prophylaxis and by family contact, the incidence of rhinovirus infections during the fall months was significantly reduced.[41,42,44] However, the occurrence of significant nasal side effects soon after initiation of intranasal treatment limited the use of this interferon preparation as seasonal prophylaxis.

Limited efforts to administer agents that would induce the natural production of interferon showed some promise[45,46]; however, the effort to develop these agents was superseded by the availability of recombinant interferon. Whether this approach would produce a protective interferon response without inducing the side effects seen after the administration of exogenous intranasal interferon remains to be explored.

Vaccine development

In the decade after discovery of the rhinoviruses, there were extensive efforts to develop an effective vaccine. This effort was stymied by the poor efficacy of the inactivated vaccines and the large number of different rhinovirus serotypes.[47–50] There has been a resurgence of interest in rhinovirus vaccine prompted by the identification of conserved antigens among the various serotypes that appear to produce protective antibody responses.[51,52]

Over the past few years, there has been evidence that strains of rhinovirus may exist that might be more likely to provoke an attack of asthma. For example, there are three genetically related groups of rhinovirus (groups A, B, and C). Several studies suggest that group C rhinovirus strains are detected more frequently in nasal secretions from school-aged children during attacks of asthma requiring hospital care.[53,54] However, other studies from the United States (US) have reported that group A strains also have a significant role in causing asthma exacerbations.[55–57] Data showing that the group B strains are less likely to provoke asthma symptoms have been a consistent finding, but group B strains also account for a relatively small number of rhinovirus infections. Although the development of serotype-specific vaccines directed at asthmagenic strains would be attractive, the large number of such strains continues to present a formidable challenge. In addition, the development of an effective antirhinovirus vaccine may be further complicated by other picornavirus strains that can cause asthma attacks, including enterovirus-68.[58,59]

Other antiviral agents

Capsid-Binding Agents: Currently, no specific antiviral agents are clinically available to prevent rhinovirus infections. Several small molecules, so-called capsid-binding agents, that bind to the viral capsid and prevent attachment to the cell surface receptor and/or uncoating of the virus have been developed and have excellent activity against a broad spectrum of rhinovirus A and B serotypes.[60,61] However, the potential usefulness of these agents for prevention of asthma is limited by the fact that the rhinovirus C serotypes appear to be intrinsically resistant to the capsid-binding agents.[62]

Rhinovirus-3C Protease Inhibitors: Pharmacologic inhibition of the rhinovirus 3C protease has also been studied as an antiviral strategy for treatment of rhinovirus infections. Although the protease inhibitor ruprintrivir had excellent activity in vitro against a broad spectrum of human rhinovirus (HRV)-A and HRV-B serotypes, this agent had modest effects on human infection and illness.[63] The effectiveness of this agent against HRV-C infections is also not known.

Receptor Blockade: The observation that many rhinovirus group A and B serotypes attach to the human host cell via a common receptor, intercellular adhesion molecule-1 (ICAM-1), led to several attempts to prevent infection by blocking this receptor. Efforts

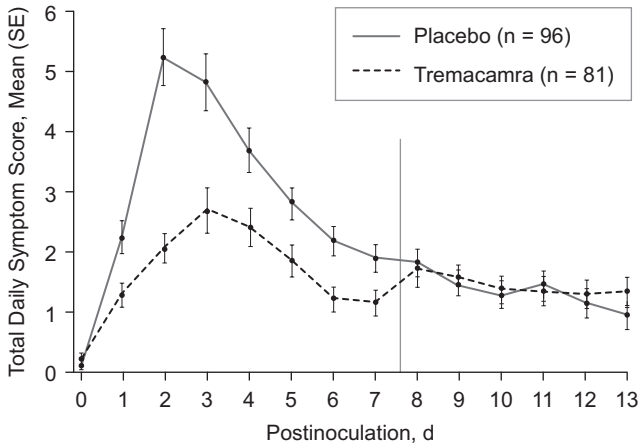


Figure 2 Mean total daily symptoms scores in tremacamra compared with placebo-treated subjects. The study drug was administered at the time of experimental inoculation with rhinovirus-type 39. The vertical line indicates the end of the treatment period.

at receptor blockade, by monoclonal antibody directed against the receptor as well as by administering a decoy soluble ICAM-1, suggested the feasibility of this approach for moderation of illness Figure 2.[64,65] Unfortunately, discovery of HRV-C viruses that do not use ICAM-1 for attachment limits the usefulness of this approach.

Small Interfering Ribonucleic Acid: Ribonucleic acid (RNA) interference appears to be an attractive technology for the suppression of virus replication. These short, double-stranded nucleotide sequences can be targeted at complementary messenger RNAs that code for essential viral proteins. These small interfering RNAs (siRNAs) are administered intranasally and are taken up by the respiratory epithelium, where they are stable and can potentially provide antiviral effects over a prolonged period of time.[66–68] Proof-of-concept studies have suggested the feasibility of this approach, but none of these siRNAs have advanced beyond early clinical trials.[69]

Probiotics: A number of studies have suggested that probiotics may moderate illness associated with viral respiratory infections.[70–73] These studies have generally not included viral diagnostics, and the role of probiotic administration for the modification of illness or prevention of infection resulting from rhinovirus or RSV is not known. Although currently available data do not support the use of probiotics as antiviral agents, the interest in probiotics has increased as a result of the speculation that probiotics may have an independent effect on atopy. Interventions that affect both infection and atopy would have obvious potential for prevention of asthma.

Asthma exacerbations provoked by rhinovirus in the atopic population

An accumulation of data, mostly from population-based and cross-sectional studies, indicates that attacks of wheezing associated with rhinovirus in the absence of allergic

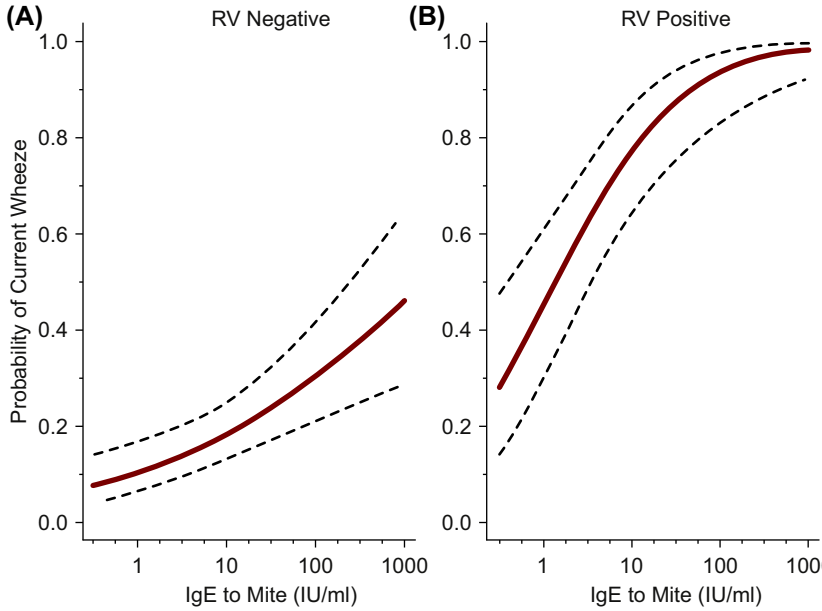


Figure 3 Probability of current wheezing based on measuring titers of IgE antibodies to *Dermatophagoides pteronyssinus* dust mite allergen in children who had negative tests for rhinovirus by PCR (A) compared with children with positive tests (B).

sensitization is uncommon among the school-aged population (7% and 14% in studies from Costa Rica and the US).[54,56] The odds for wheezing with rhinovirus at this age are also markedly increased in the atopic population (both children and adults), which suggests that synergistic interactions between allergen and virus-induced effects on airway inflammation are at play.[13,27] Data from Australia have also shown that allergy substantially increases the risk for wheezing with rhinovirus-C strains, including the risk for subsequent attacks of wheezing when children were observed over 12 months.[74] In addition, the strength of allergic sensitization, as judged by increasing titers of allergen-specific IgE antibody, has been shown to significantly increase the probability of wheezing with rhinovirus, and this relationship was true for children who tested positive for either group A or group C strains (Figure 3(A) and (B)).[54] Moreover, the administration of omalizumab, a humanized anti-IgE monoclonal antibody, significantly decreased the spring and fall seasonal peaks of asthma attacks that required urgent care among children with asthma who were enrolled in the Inner-City Asthma Study in the US.[75.] This study included a subset of children who tested positive for rhinovirus.

The observation that allergic inflammation is likely to have a critical role in the asthmatic response to rhinovirus in the school-aged population raises the questions as to how early in life the interaction between atopy and virus-induced wheezing begins, and to what extent treatments to diminish allergic inflammation might modify the effects of viral infections and lead to the development of asthma. Recent prospective data from the COAST study suggest that allergic sensitization may actually precede wheezing provoked by rhinovirus.[76] Similarly, virus-induced wheeze during the first 2 years of life (with rhinovirus more often than RSV) was associated with recurrent

wheezing at age 5 years in a prospective cohort of approximately 200 children from Australia.[77] However, this association was restricted to infants who were atopic.

Conclusions

This chapter has reviewed antiviral treatment options, especially treatments focused on infections caused by rhinovirus and RSV, which might be of interest in preventing childhood-onset asthma (Table 1). Whether these treatments and the focus on early childhood events will provide benefits and reduce the onset of asthma that begins after puberty will need to be considered and evaluated. However, treatments focused only on the pathogenicity of viral pathogens may have limitations because of the other environmental risk factors that are likely to influence the development of asthma. Many

Table 1 Summary of antiviral strategies for the prevention of childhood asthma

I. RSV[a]	A. Acute treatment 1. Ribavirin[b] 2. Fusion-inhibitor agent: GS-5806 B. Seasonal prophylaxis 1. RespiGam 2. Palivizumab[b]
II. Rhinovirus[ac]	A. Innate (e.g., type I and type III) interferons 1. Intranasal interferon-alfa (type I) 2. Inhaled interferon-beta (type I) 3. Interferon-gamma (type III)[d] 4. Interferon-inducers to stimulate IFNs endogenously B. Capsid-binding agents (pleconaril) C. Rhinovirus-3C protease inhibitors (ruprintrivir) D. Receptor blockage agents (tremacamra) E. siRNA
III. Vaccines	Developing vaccines to prevent or minimize the severity of RSV and rhinovirus infections has been challenging
IV. Probiotics	The development of probiotics is based on the hypothesis that normalizing gut microbiota during early childhood might decrease the risk for asthma
V. Treatments to reduce allergic inflammation	A. Methods to induce allergen tolerance during early childhood B. Biologics to establish: 1. an IgE blockade (omalizumab) 2. an IL4/IL-13 receptor blockade (dupilumab)

[a]Antiviral treatments have predominantly been focused on RSV and rhinovirus pathogens.
[b]Currently available for treatment, although ribavirin in not generally recommended.
[c]Evidence favors seasonal or family-contact prophylaxis for treating rhinovirus infections and possibly rhinovirus-induced asthma. Antiviral agents to treat rhinovirus infections effectively at the onset of symptoms are currently not available.
[d]The production and use of IFNλ_1 (Miller EK, Hernandez JZ, Wimmenauer V, Shepherd BE, Hijano D, Libster R, et al. A mechanistic role for type III IFNλ_1 in asthma exacerbations mediated by human rhinoviruses. *Am J Respir Crit Care Med* 2012;**185**:508–16.

antiviral treatments used currently, as well as those in development, focus on treating the acute effects of virus infection at the time of wheezing, or on prophylactic treatments such as the provision of passive immunity in high-risk infants, or the administration of drugs targeting the innate immune system.

Antiviral strategies that are currently available could modify the development of asthma if viral infections that cause wheezing in early childhood have a central role, either independently or as cofactors, in promoting asthma development. In this regard, antiviral vaccines have the advantage of stimulating a virus-specific adaptive immune response with memory to decrease the asthmatic response to the same viral pathogen later on. However, an effective vaccine for RSV without side effects has not yet been produced. Because the odds for developing asthma have been shown to be greater after early childhood wheezing with rhinovirus than with RSV, a vaccine focused on rhinovirus may actually be more beneficial yet challenging to produce because of the wide variety of serotypes that can provoke symptoms.

The importance of allergic sensitization and symptoms as risk factors for the development of asthma supports the hypothesis that strategies to reduce allergic inflammation during early childhood may also help to decrease the risk for future wheeze and reduce the adverse effects of viral infections on the lower airway. Methods to induce tolerance to environmental allergens might prove to be helpful. It is also possible that the administration of biologics to decrease T helper-2–driven pathways in early childhood, such as establishing an IgE blockade with omalizumab, or an interleukin (IL)-4/IL-13 receptor blockade with biologics in development, could become an important treatment option. However, the timing for administering these biologics is likely to present challenges. For example, how early in life these treatments can be initiated and over what period of time they should be administered are questions that will need to be considered and evaluated. Also to be determined are: (1) whether this approach will have persistent benefit if these treatments are stopped during the preschool years, and (2) whether this intervention would defer the development of allergic sensitization and symptoms that might begin after the biologic treatment is stopped.

Recent interests to normalize the microbiota in the gut, on the skin, and in the airway also deserve attention. The administration of probiotics to influence and educate the immune system, with the objective of altering the response to viral pathogens and to food and environmental allergens, may represent an additional strategy to decrease the risk for childhood-onset asthma.

Taken together, we may learn that a more comprehensive and individualized approach is needed to significantly reduce the development of asthma in at-risk children. For example, a child with genetic mutations at the 17q21 locus, which imparts a greater risk for rhinovirus-induced wheezing and asthma development, may benefit from an antirhinovirus vaccine together with a probiotic, whereas a young child with atopic risk factors may benefit from measures to reduce allergic inflammation and symptoms, combined with a probiotic and/or an antivirus vaccine. These treatment strategies will require ongoing studies to identify children who are most likely to benefit from individualized treatment plans. Of course, costs will need to be considered. Nevertheless, it is exciting to consider that a variety of treatment options may become available in the future to decrease the incidence of asthma.

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Prevention of Allergy/Asthma—New Strategies

20

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Introduction

It has been clearly established that the prevalence of allergic diseases exemplified by atopic asthma has increased markedly since the 1960s. This pattern was first recognized in first-world countries but it is now acknowledged that the pattern is repeating itself in emerging countries throughout the world[1,2] as their economies permit adoption of Western lifestyle practices, in particular those associated with diet, personal hygiene, and public health.

Starting in the 1970s, when it first became evident that asthma was associated with inflammation of the conducting airways,[3] the approach to resolving this problem, which was developed in industry and academia, has been essentially twofold: (1) attempt to identify the major molecular mechanisms driving the underlying inflammatory process(es) and then develop pan-specific anti-inflammatory medicines to suppress the inflammation (and hence the symptoms); and/or (2) attempt to identify the environmental agent(s) responsible for triggering airways inflammation and then devise ways to reduce exposure to these agents. Success with the latter approach has been at best moderate and sporadic despite multiple international trials, and as discussed further below, data on the nature of the environmental allergen exposure dose–response curve and sensitization risk seriously question the rationale for attempting this.[4] In addition, until comparatively recently, asthma drug development programs have been disappointing and have resulted in the proliferation of drugs that are only partially effective, and as a consequence require essentially continuous use before significant benefits accrue to the patients, leading to inevitable problems of compliance and cost.

The past decade has witnessed a progressive shift toward increasingly specific antiallergy/antiasthma drugs targeted more precisely at individual molecular mechanisms identified in experimental and epidemiological studies as potential hubs in disease-associated causal pathways. The archetypal example has been monoclonal antibodies against immuno globulin E (IgE),[5] which target this proinflammatory molecule with exquisite specificity and potency. However, the decade since its release for clinical testing witnessed a large series of essentially disappointing trials in a range of clinical contexts, almost always in adults, where effect sizes have frequently been equivocal. However, we[6] and others have questioned the underlying rationale for this approach to asthma control. Notably, drug development strategies have traditionally centered on candidate molecules initially identified as being hyperexpressed in experimental animal models, and subsequently verified in human clinical studies on patients with established disease. However, there is increasing

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evidence to suggest that an integral component of the asthma phenotype itself is the progressive development of hyperresponsiveness of airway tissues to inflammatory stimuli in general, as a result of long-term inflammation-driven tissue remodeling. Thus, blanketing of individual inflammatory molecules (or even pathways) at that stage of disease may not be enough to suppress symptoms in the face of ongoing exposure to environmental triggers, because the overall inflammatory response may simply default toward other (parallel) pathways that have equivalent tissue-irritant properties. We have argued that a more logical approach would be to instead focus on the life phase preceding the de novo development of the asthma phenotype, identify risk factors (especially inflammatory mechanisms/pathways) that are proximal to disease initiation, and target these for development of new treatment strategies.[6] Crucial to this new paradigm is the concept that suppression of these mechanisms/pathways in healthy at-risk children before they develop consolidated disease may provide an effective route to *primary and/or secondary prevention* of diseases such as asthma, and moreover, that blocking the early stages of the disease process may confer permanent protection.[6] The rationale underlying this approach, and progress in implementation, is summarized below.

Asthma initiation during childhood

The research approach that has been most effective in driving recent progress in elucidating the early stages of asthma pathogenesis has involved prospective birth cohort studies, tracking children through the premorbid to persistent stages of the disease. With several of these cohorts entering their third decade, an increasingly comprehensive body of evidence is building relating to the nature of the major early risk factors and associated pathogenic mechanisms, and several of these findings have spawned intervention studies that may potentially lead to asthma preventive treatments. Some of the key findings are summarized below.

Asthma genetics and environmental risk factors

Early enthusiasm for identification of *the* asthma gene has been replaced by the realization that asthma is one of a family of complex diseases in which risk is underpinned by a large number of genetic variants, all of relatively small effect size.[7,8] Importantly (and understandably), the spectrum of relevant variants differs between ethnic groups and indeed between ethnically related groups derived from geographically different regions, which underscores the importance of the *gene* × *environment* interactions that are now understood to influence pathogenesis. In addition, it is also now recognized that such interactions can exert their effects in either direction, that is, some environmental stimuli operating against particular genetic backgrounds can function as risk factors and other combinations can be protective.[9–12]

Major risk factors for atopic asthma operating during early life

Although there is still much to be learned concerning the fine details of the processes underlying the initiation of atopic asthma, significant progress has been made in this regard over the past 1–2 decades. Taking 5–6 years postnatally as an initial checkpoint,

constituting the earliest age at which a diagnosis of persistent asthma as opposed to simply wheeze can be confidently made, the major asthma risk factors identified as operative during this life phase are respiratory viral infections and allergic sensitization.[1,13,14] The crucial virus in this regard is currently believed to be rhinovirus (RhV), particularly the RhV-C subtype.[15,16] However, the impact of *respiratory syncytial virus* (RSV) is well-recognized as one of the major cause of hospitalization in the first year of life,[17] and a variety of evidence links these early events to subsequent asthma risk[18–20]; consequently, the RhV versus RSV debate in relation to asthma causality remains unresolved.[21]

Timing is crucial

The overall timing and the order in which pathogenesis-associated *gene* × *environment* interactions frequently occur are of major relevance to risk for subsequent development of allergic diseases exemplified by atopic asthma. Important issues have emerged from research findings in this regard:

- *The crucial importance of infancy* in relation to initiation of the disease process has become evident from studies on the time course of emergence of persistent asthma in the major international birth cohorts; notably, the most severe and troublesome asthma throughout the childhood years is the early-onset phenotype that manifests in the first 2 years of life (reviewed in Refs 14,22).
- The basis for this temporal relationship is thought to be that *the rate of postnatal lung growth is maximal during early life*, and perturbation of normal airway tissue differentiation programs during this crucial period by severe inflammation can lead to long-term changes owing to associated tissue repair/regeneration/remodeling; the resultant changes can be cumulative and persistent (Figure 1[22]) and as a consequence can effectively track into later life.[13]
- Notwithstanding these findings, it is also clear that progression to chronic asthma by the teen years and/or early adulthood requires further consolidation of the effects of early lung damage via environmentally triggered episodic inflammation throughout the school years.[14,22]
- The magnitude of the decline in lung function after such events is directly related to the severity of the exacerbation[23] and is reflected in the characteristic pattern of gene expression in leukocytes harvested from the blood[24] and airways[25] at that time, which is consistent with dysregulation of both innate and adaptive immune responses to the triggering agent(s).
- The most important inflammatory events in this regard are infection-associated wheezing episodes triggered by viruses, in particular in atopic children sensitized to aeroallergens (see discussion below).
- The timing and route of primary sensitization to the relevant aeroallergens remain unclear, and issues in ongoing debate in this regard include whether the process is initiated prenatally or entirely postnatally,[26,27] and the question of whether sensitization to some aeroallergens may occur via the epidermal route[28] as well as through the respiratory mucosa.

Plasticity of clinical phenotypes in early life

A complication of research in this area is that during early life, coinciding with the key events leading to programming of long-term resistance versus susceptibility to allergic diseases exemplified atopic asthma, underlying growth-associated fluctuations in relevant phenotypes continue to occur over prolonged periods. In particular, expression of

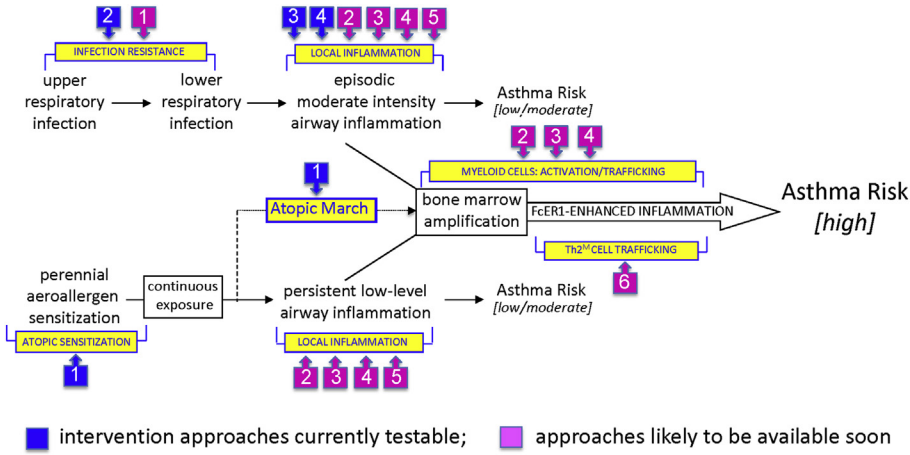


Figure 1 Causal pathways to the induction and consolidation of atopic asthma in childhood—emerging opportunities for primary and secondary prevention via early intervention.

the wheezing phenotype is common in the first 2–3 years of life, the principle trigger being respiratory infection, but in most children this remits spontaneously by the end of the preschool years, that is, the transient early wheezer phenomenon.[29] Likewise, low-level IgE production directed against both dietary and inhalant allergens in a subset of cases attaining levels above accepted clinically relevant thresholds is common during this period, but only in a minority does this consolidate into persistent sensitization into the school years.[30–32] In one closely monitored birth cohort, the critical threshold level of allergen-specific IgE during this period of cycling antibody production that predicted subsequent persistent sensitization appeared to be 0.2 kU/L,[30] a finding that has potential implications in relation to the selection of subjects for immunoprevention trials and immunotherapy.

Postnatal allergen exposure and risk for allergic disease: is more worse, or better?

The traditional view in this field, that levels of early postnatal exposure are related in a monodimensional way to sensitization risk, derived originally from studies linking birth in the pollen season to heightened susceptibility to pollen-associated allergies.[33] The first challenge to this paradigm was the finding that spring birth was also associated with increased risk for food allergies.[34] This observation was followed by a progressively accumulating series of studies (reviewed in Ref. 4) demonstrating essentially that *the dose–response curve describing sensitization risk is biphasic*, with low levels carrying increased risk for preferential priming of T helper-2 (Th2)-associated allergen-specific immunological memory, owing to inefficient stimulation of protective T-regulatory cell-mediated mucosal tolerance mechanisms. There is no dispute that once sensitization occurs, subsequent symptom expression correlates positively with aeroallergen exposure levels,[35] but in the presensitization phase the situation is

different. An elegant example is the study demonstrating that, whereas, cumulative aeroallergen exposure in the first 3 years of life correlates positively with risk for current sensitization and recurrent wheeze by that age, exposure levels during year 1 display an inverse correlation; that is, high exposures were protective.[36]

Hidden complexities relating to effects of microbial exposures on atopic disease phenotypes

It has become evident that direct exposure to the microbial environment after birth represents a double-edged sword with respect to modulation of risk for allergic diseases, and emerging research continues to reveal further unexpected complications regarding this important issue. Three of the most interesting areas of contention are as follows:

- The notion that environmental microbial exposure in the form of shared respiratory infections between siblings could somehow *protect against allergic sensitization* was first brought into prominence by David Strachan under the banner of the *Hygiene Hypothesis*[37] but was soon reinterpreted in relation to the immunostimulatory effects of normal commensal gastrointestinal (GIT) microflora.[31,39] Certain GIT viral pathogens have also been implicated in stimulation of resistance to sensitization.[39] Contaminating bacterial breakdown products in environmental (including house) dusts exemplified by lipopolysaccharide have subsequently been shown to have potentially both stimulatory and inhibitory effects on expression of allergic reactivity, depending on the precise context of the exposure.[40–43]
- The immunostimulatory effects of the microflora in this context were initially attributed to stimulation of maturation Th2-inhibitory Th1 functions,[31] which are deficient at birth in atopic family history–positive infants relative to the rest of the population[44]; however, subsequent research suggests that a much broader series of maturational defects operates in this high-risk group, including innate immune functions within the T-regulatory cell compartment[45]; these are also likely targets for the immunostimulatory effects of normal flora.
- The *chronology of microbial-mediated modulation of resistance to allergy is also unclear*, but emerging evidence suggests that the window for such effects may not be restricted to postnatal life; in particular, emerging epidemiological (reviewed in Ref. 46) and experimental[47] studies suggest that benign microbial exposure of the mother during pregnancy through the GIT and airway mucosae may promote functional maturation of immune competence in the offspring during intrauterine growth, possibly via effects on placental function.[47,48]
- Conversely, *respiratory viral infections* are now universally recognized as having important roles as *highly pathogenic allergy/asthma risk factors*[14,49]; however, *the relative impact of different types of viruses* in this context *remains contentious*, and although as noted earlier current opinion favors RhV (particularly RhV-C subtype[15,16]) as the dominant pathogen in this regard, many researchers still regard RSV as of equivalent importance, especially in the first year of life[20].
- The debate on whether opportunistic respiratory bacterial pathogens may also have key roles in asthma pathogenesis has been reignited by studies identifying distinct mixed bacterial microbiomes in the lower airways of asthmatics[50]; this has been further stimulated by findings indicating that (1) early postnatal nasopharyngeal colonization with pathogens such as *Streptococcus pneumonia* and *Haemophilus influenza* is a risk factor for early onset asthma,[51] and (2) slow postnatal development of capacity to produce the specific IgG1 antibodies, which are required for efficient clearance of these organisms, is associated with increased risk for subsequent development of both sensitization and atopic asthma.[52]

Interactions between early infections and aeroallergen sensitization in driving development of allergic disease: atopic asthma as a paradigm

As noted, there is a wide body of evidence from the major birth cohort studies that collectively argues that whereas aeroallergen sensitization and respiratory viral infections in early life can act as independent risk factors for atopic asthma development, the highest risk is associated with these two pathways to airway tissue damage operating in concert in the same individual (reviewed in Refs 4,22,52). Importantly, the relevant chronology is now at least partially understood, and it is clear from independent studies that significant levels of sensitization must be present at the time of viral infection for the synergistic interaction(s) responsible for high asthma risk to occur.[30,53] The mechanism(s) underlying these interactions include those operative specifically within the airway mucosal tissue microenvironment and within the bone marrow (reviewed in Ref. 22).

Central to the latter appears to be upregulation of expression of the high-affinity IgE receptor (FcεR1) on resident dendritic cells (DCs) in airway tissues at the site of viral infection, followed by a second and sustained wave of FcεR1 upregulation on the bone marrow precursors responsible for ongoing replenishment of this DC population, partly via upregulation of the FcεR1 gamma chain that controls surface expression of functional receptor.[22,48] These cells traffic to the airway mucosa to replace local DC, which turn over rapidly during infections, and armed with functional FcεR1 they now have markedly enhanced capacity to recruit aeroallergen-specific Th2 responses (including activation of eosinophils) into the inflammatory milieu at the infection site, contributing to overall inflammation and slowing Th1-mediated viral clearance.[22,52] These circulating DC precursors are also responsible for renewal of resident DC populations in other tissues such as GIT, skin, and nasal mucosa, thus potentially enhancing capacity for expression of Th2-associated allergic reactivity at these distal sites, and we have proposed that this process may be a key component of the *allergic march* phenomenon.[22]

Emerging evidence suggests that this pathway has a key role in driving early pathogenesis of atopic asthma during its inception stage in the preschool years and contributes to disease consolidation and progression to chronicity during the early school and teen years via amplification of the intensity of airways inflammation associated with virus-associated atopic asthma exacerbations.[14,22,24]

Causal pathways for asthma development in childhood—development of a roadmap for rational design of early intervention strategies

These mechanisms can be integrated into a model to describe the role of atopic and viral-associated inflammatory pathways in the early stages of asthma development. Figure 1 represents an updated version of a scheme proposed to explain the operation of these interlinked causal pathways,[22] focusing on potential targets for early intervention therapies. Central to this model are the epidemiological findings from multiple birth

cohort studies indicating that early viral infections and early atopic sensitization during the preschool years can function as independent risk factors in driving asthma inception, and further, that the pathways can interact synergistically (e.g., in viral-infected and -sensitized/aeroallergen-exposed children) to accelerate progression to disease persistence. The specific immunoinflammatory processes that could be targeted for primary and/or secondary preventive strategies predicted by this model are illustrated in the scheme (yellow boxes (light gray in print versions)), and corresponding therapeutic approaches that can be contemplated are discussed. We consider separately therapeutic approaches suggested by this scheme that can be undertaken on the basis of available proof-of-concept and pediatric safety data, and newer approaches likely to be available in the near-term future:

Asthma/allergy prevention trials: what can be contemplated now?

Based on this scheme, four major approaches currently appear feasible:

1. Prevention of induction and/or progression of sensitization to aeroallergens

Prevention of asthma in high-risk children by actively suppressing the allergic sensitization process is being actively investigated in a number of centers. An initial pilot study funded by the United States (US) Immune Tolerance Network focusing on atopic family history–positive children manifesting early signs of the atopic phenotype (atopic dermatitis [AD] and/or food allergy but without sensitization to aeroallergens), involving sublingual administration of mixture of mite/cat/grass allergens daily for 1 year, has established the safety of this approach,[54] but because of the low sample size it was not possible to obtain efficacy data in this trial. However, at the 2014 EAACI meeting in Copenhagen, early outcome data from a larger sublingual treatment trial employing a similar design were presented by Assad and colleagues from the University of Southampton, and this demonstrated a promising reduction in house dust mite sensitization in treated infants displaying low-level mite sensitization; to date, the trial is ongoing and future results are eagerly anticipated.

Stronger proof-of-concept data are available from older pediatric age groups, in relation to targeting the atopic march, as exemplified by progression from rhinitis to atopic asthma. Notably in the PAT study, school-aged children monosensitized to birch pollen and expressing rhinitis but not asthma symptoms were treated by subcutaneous immunotherapy (SCIT) with birch allergen for 1 year. Their rates of progression to asthma were subsequently monitored over the next 5 and subsequently 10 years,[55] and significant protection was observed. This trial supports the use of conventional immunotherapy in sensitized children for immunoprophylaxis; however, follow-up studies are required with larger group sizes and double-blind/placebo-controlled trial design.

2. Prevention of respiratory infections during early infancy

As noted, despite the increasing recognition of the importance of RhV as a respiratory pathogen in infants, RSV is still acknowledged as one of the most frequent causes of infections that result in severe lower respiratory symptoms during the first year of life

and are associated with risk for later asthma. No antiviral therapies are currently available to specifically target RhV, but monoclonal antibody (MoAb)-anti-RSV has been in clinical use for several years and has been shown to be efficacious in preventing primary RSV infection in at-risk infants and reducing subsequent recurrent wheeze.[56] This antibody could in principle be trialed as an asthma preventive, in particular, targeting high asthma–risk infants during their first RSV season.

3. Targeting atopic effector pathways for modulation of inflammatory damage associated with viral infections in school-aged atopic patients

As detailed, the progression of established atopic asthma toward long-term chronicity is driven to a large extent by repeated cycles of airways inflammation triggered by viral (especially HrV) infections, which involve FcεR1-mediated recruitment of atopy-associated effector mechanisms into the inflammatory milieu at infection sites in the airway mucosa, resulting in exacerbation events.[22] The intensity of accompanying inflammation is directly related to the degree of resultant decline in lung function after these events.[23] These interactions between atopy-associated and antiviral inflammatory pathways can be observed across the full spectrum of asthma severity in children.[22,57] Accordingly, blocking FcεR1-mediated inflammatory mechanisms by blanketing IgE with MoAb-anti-IgE exemplified by Xolair represents a logical therapeutic approach in this context. Impressive proof-of-concept is available in this regard,[58] and interest in the broader application of this type of intervention is increasing, particularly the concept of virus season—only prophylactic treatment in established early-stage asthmatic children.

4. Pan-specific approaches to protection of infants against viral-induced inflammatory damage of airway tissues

An alternative to the use of highly specific therapeutics that target individual inflammatory effector mechanisms triggered by viral infection is to focus on pan-specific anti-inflammatory mechanisms exemplified by T-regulatory (T-reg) cells. Emerging research indicates that microbial signals, particularly those derived from the flora in the GIT, have a central role in controlling T-reg function(s) throughout the body,[59,60] and the principle that this overall pathway represents a potential therapeutic target for control of inflammatory diseases is attracting increasing attention. In the context of airway inflammation, one approach already tested involves probiotics; the use of these in children to mitigate respiratory infection severity in children has met with limited success.[61] There is also increasing interest in the potential use of a related class of orally administered therapeutics derived from extracts of killed bacterial pathogens exemplified by OM-85, which in animal models have been shown to promote the functional maturation of T-regs in gut-associated lymphoid tissues[62,63]; these cells subsequently migrate to other mucosal tissues, including those in the lung and airways, thus increasing local resistance to inflammation. This therapeutic has been tested with some success in adult patients with chronic airway disease,[64] and more recently has been demonstrated to reduce the frequency and intensity of airway inflammatory episodes in infants and children with recurrent infection-associated wheeze.[65,66] Publicly funded investigator-initiated clinical trials are currently under way in the author's

institute in Australia to explore the efficacy of this agent in infants at high risk of asthma/allergy, after a winter-only treatment regime.

Possibilities for the near-term future: new therapeutics and repurposing of existing drugs for asthma prevention

Based on the emerging understanding of how the causal pathways driving early asthma pathogenesis operate (Figure 1), an increasing range of possibilities for novel preventive therapeutic approaches is opening up, and its current implementation is limited only by the availability of appropriate pediatric safety data, which in several of the cases discussed below are currently being collected. The approaches detailed below are relevant both to primary prevention of asthma development in high-risk infants/preschoolers before disease onset and to blocking progression in older children from early stage to chronic asthma. In this regard, Xolair (3., above) has already been shown to prevent virus-associated exacerbations in asthmatic children and can accordingly be contemplated for use as an inhibitor of disease progression in established asthmatic patients; however, when safety data in high-risk infants are available, its use in primary prevention can be formally trialed. Likewise, OM-85 (4., above) has been used in infants and preschoolers to mitigate infection-associated acute wheezing symptoms, and once preliminary data on high-risk (i.e., atopic family history–positive) infants are available, it can also be tested in the context of primary prevention. Additional interventions are likely to be available for testing as soon as new therapeutics become available and/or as indications for use of existing drugs expand. Approaches are likely to be available in the near/mid-term future, as illustrated within the scheme for asthma pathogenesis in Figure 1, include:

1. Topical type 1 interferon (IFN): A nasal spray delivering low doses of recombinant IFN-alpha-2b has been shown to reduce the frequency of parainfluenza, adenovirus, and influenza infections in healthy young adults in China.[67] In addition, trials in the United Kingdom with inhaled IFN-beta demonstrated modest effects in modulating asthma-associated symptoms caused by viral infections,[68] but further studies will be required to ascertain the potential of this approach.

2. A small molecule antagonist of IL-4/IL-13Rα has been trialed successfully in asthmatic adults challenged by aerosolized allergen, and shown to markedly attenuate the asthma late-phase response when delivered either in topically or systemically available forms.[69] As illustrated in Figure 1, such an agent has potential to be used in the future to ameliorate both the local and systemic components of asthma-promoting atopic responses triggered by aeroallergen alone, or aeroallergen in combination with viral infection. Comparable rationales exist for future preventive trials in a range of pediatric age groups with several new-generation (as well as established) Th2 antagonists once the requisite age-specific safety data are available, including: (1) 3. MoAb anti-IL-13 lebrikizumab, which has been demonstrated to improve lung function in poorly controlled adult asthmatic patients, in particular those manifesting the $Th2^{hi}$ phenotype[70]; (2) 4. MoAb anti-IL-4Rα dupilumab which has been reported to reduce the frequency of asthma exacerbations in moderate-to-severe adult asthmatics after withdrawal of long-acting β2-agonist-glucocorticoids and to improve lung function in treated subjects[71]; and (3) 5. MoAb anti-IL-5, which is increasingly being applied

in pediatric populations in diseases such as eosinophilic esophagitis[72] and is currently being evaluated in proof-of-concept studies relating to eosinophilic wheeze in preschoolers, in several centers.

An additional therapeutic target that is clearly relevant to the causal pathways depicted in Figure 1 involves the trafficking of effector cell populations between central lymphoid organs and sites of allergic inflammation. A promising therapeutic approach relevant to this process is 6. The CRTH2 antagonist OC000459, which blocks prostaglandin D_2-mediated activation of eosinophils and Th2 memory cells by mast cells; this agent has been demonstrated to improve lung function and reduce eosinophilic airway inflammatory symptoms in adult asthmatic patients[73] and to exert modest but significant anti-eosinophilic effects and accompanying clinical improvement in adults with active eosinophilic esophagitis.[74]

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Antiallergic Strategies: Induction of Tolerance to Food

21

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Introduction

With the recent rise in food allergy, there is much interest in better understanding the development of oral tolerance in the first year of life. This chapter explores the mechanisms of oral tolerance and how immune regulation is activated or dysregulated during the development of tolerance to food or food allergy, respectively. We discuss the different clinical phenotypes of oral tolerance and ecological factors that have been associated with deviations in the risk of developing normal oral tolerance and thus increasing the risk of food allergy development. Finally, we outline interventions that are currently under investigation, either to augment oral tolerance to prevent food allergy development or to enhance development of food tolerance in food-allergic children.

Definition of oral tolerance

Immunologically, oral tolerance is an antigen-specific suppression of cellular and humoral responses to dietary and bacterial antigens after exposure in the gastrointestinal tract. It is thought to be determined by a combination of factors including physical factors, antigen dose and timing, the microbiome, dendritic cells and other antigen-presenting cells, and T cell and antibody responses.[1] There are two types of immunological tolerance: passive and active. It is believed that long-term clinical tolerance to food is underpinned by active immune suppression, perhaps through a regulatory T cell population, although the evidence for this remains sparse. However, passive immunological tolerance always occurs on the first exposure to an antigen in the absence of differentiated antigen-specific T cells and B cells, for example, the case of encountering novel antigens not previously encountered by immune cells, such as *keyhole limpet hemocyanin* or ϕX174. There is considerable fluidity in the definition of clinical

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tolerance in the literature, especially in the case of food allergy. Clinically, oral tolerance can be defined as the ability to eat food without developing immunologically mediated symptoms irrespective of the frequency and quantity of food consumed. It is conceivable that such a stringent definition of tolerance can only occur once clonal deletion (i.e., programmed cell death of T cells) or anergy (i.e., failure to activate T cells) has happened, whereas ongoing active suppression of an immune response to food and maintenance of oral tolerance may require intermittent antigen exposure and thus regular consumption of the implicated food, but this still under debate.

Immunological mechanisms of oral tolerance

In early life, a few of the physical factors that can be protective and/or promote the development of oral tolerance are defective. For example, an immature gastrointestinal epithelial barrier can increase protein permeability[2] and low or absent secretory immunoglobulin A (IgA) in the gut mucosa[3] can facilitate the development of food sensitization and food allergy. The mucosal response to protein antigens in early life is typically T-helper 2 (Th2)-skewed and IgE to foods can develop both in atopic and nonatopic infants. In nonallergic children, this IgE response is typically transient and the Th2 response is counterbalanced by a Th1 response leading to tolerance. During weaning, exposure to a high dose of antigen, even as a single dose, via the oral route induces the absence of response (anergy) or programmed cell death (deletion) of reactive T cell clones. Whether T cell clone anergy or deletion occurs depends on whether the interaction between the T cell receptor and major histocompatibility complex class II on antigen-presenting cells occurs in the presence of Fas–Fas–ligand interaction or in the absence of costimulation. Low-dose exposure to the antigen, especially if repeated, induces T regulatory (Treg) cells such as CD4+, CD25+, and Foxp3+, naturally occurring Tregs (which exerts suppressive action via cell-to-cell contact and via the production of transforming growth factor-β [TGF-β]), and inducible or adaptive Tregs (TR1, which produce interleukin-10 [IL-10]; and Th3, which produce TGF-β). Cytokines produced by Tregs can influence B cells and lead to the production of IgG4 (induced by IL-10) and IgA (induced by TGF-β). Immunoglobulin G4 and IgA can counteract the function of IgE either by competing for binding to the allergen or by intracellular inhibitory signaling resulting from co–cross-linking of the FcεRI with immunoreceptor tyrosine-based inhibition motif–associated receptors such as FcγRIIB or FcαRI. Microbial stimulation provided by the enteric flora through innate immune receptors is critical for the development and organization of mucosal and secondary gut-associated lymphoid tissue[4] and may have an important role in modulating the immune response leading to oral tolerance.

Clinical phenotypes of oral tolerance

We recognize different phenotypes of food-tolerant children in the clinic. First, we see tolerant children with no IgE to foods (i.e., no allergic sensitization), which accounts for most individuals in the general population. For example, in the Healthnuts study, in

a population of 5300 infants, 80% had no IgE antibodies to peanuts, sesame, eggs, or cow's milk and were tolerant to all foods tested.[5] These patients have variable levels of IgG and lymphocyte proliferative response to the food. Some individuals will develop IgE to foods, but only a minority of these will develop food allergy. In other words, IgE sensitization is more common than clinical food allergy.[6,7] Thus, there are phenotypes of clinically tolerant children who paradoxically show evidence of allergic sensitization to the food in the absence of clinical reactivity. In this group, we recognize five subgroups, indicated below. It is possible that different phenotypes of allergic sensitization have different underlying immunological mechanisms and that in some phenotypes different mechanisms coexist. We speculate about some of the possible mechanisms below; further research is needed to know whether these postulates are correct.

- Antenatal sensitization

  Allergic sensitization may develop in utero and be present at birth, even in infants born from nonatopic mothers. There is also evidence that prematurity protects against allergic disease, which suggests that the risk of allergic disease increases owing to a third-trimester mechanism.[8] Furthermore, epigenetic studies suggest that the presence of risk factors before conception in both the ova and the sperm can have effects on the development of asthma in the offspring.[9] There may be similar risk factors for food allergy; if these are confirmed, food allergy prevention measures may need to be commenced earlier.

  Although studies have shown detection of IgE in cord blood to various antigens, it is unclear whether IgE persists through childhood, and there is currently no evidence that it has allergic consequences.[10] Antenatal IgE can be of maternal origin or, less commonly, of fetal origin, and is usually transient.[11,12] The clinical relevance of this prenatal IgE and a mechanism relating it to the development of allergy is not apparent.
- Stable sensitization

  In most cases, IgE develops postnatally.[10,11,13] In the case of stable sensitization, children can develop IgE for many years and never show symptoms of allergy. This is best described in respiratory allergy but has also been reported in food allergy.[14–16]

  Immunoglobulin E–sensitized patients who never develop allergic symptoms after food allergen exposure during their lifetime may have IgE antibodies of different specificity (i.e., binding to a different part of the allergen that is clinically irrelevant because it is not able to induce effector cell activation and clinical symptoms) and/or affinity (i.e., strength of antibody binding to the allergen) compared with the IgE antibodies of patients who are sensitized and develop allergic symptoms. Another possible explanation for the absence of allergic symptoms in IgE-sensitized individuals is the presence of blocking antibodies that could competitively inhibit the function of IgE, such as IgG4.[17]
- Preallergic sensitization

  Latent sensitization has been described in which IgE antibodies to foods are present in clinically nonreactive patients who subsequently become allergic to that specific food *preallergic sensitization*. A number of studies have evaluated the prospective association between asymptomatic allergen sensitization and the later development of allergic symptoms in children and adults. In the LISA birth cohort, about 10% of children sensitized to foods at age 2 years developed new-onset food allergy at age 6 years, and early food sensitization was identified as a strong risk factor for food allergy at age 6 years (odds ratio, 4.7; 95% confidence interval, 2.0–11.2).[16] In another birth cohort study, early sensitization to foods was a strong predictor of the later development of allergic disease, including allergic rhinitis and asthma.[18]

In sensitized patients who become allergic, there may be an increase in the levels and modification of the qualitative features of IgE, namely the development of additional IgE specificities, that is, the progression of IgE binding from one part of the allergen to additional parts of the allergens (so-called "epitope spreading") and an increase in the affinity of antibody for the allergens.[15]

- Postallergic sensitization

Food allergy can resolve spontaneously. Depending on the allergen, food allergy can be outgrown in childhood (e.g., cow's milk allergy, egg allergy)[19–22] or persist into adulthood (e.g., peanut and tree nut allergies).[23,24] Although acquisition of tolerance is usually preceded by a decrease in food-specific IgE levels, IgE can remain detectable after oral tolerance is established: *postallergic sensitization*. The typical decrease in food-specific IgE levels that accompanies the resolution of food allergies is used in the clinic as an indicator of when to perform oral food challenges (FCs) to assess the acquisition of tolerance.[25] The decrease in food-specific IgE is often accompanied by an increase in allergen-specific IgG antibodies, in particular of the IgG4 isotype.[26,27]

In patients who outgrow the food allergy, possible underlying mechanisms are reduced T cell proliferation and elimination of reactive T cell clones with a change in the IgE epitope recognition pattern and/or IgE affinity and the induction of regulatory T cells leading to the production of blocking antibodies such as IgG4 that are able to interfere with IgE and inhibit allergen-induced activation of basophils and mast cells. This is well described in patients who are in the process of outgrowing milk and egg allergy and tolerate extensively heated forms of these foods.[28–31]

- Desensitization

In patients submitted to allergen-specific immunotherapy, a transient increase is followed by a decrease in allergen-specific IgE levels,[32,33] "*desensitization.*" Also during immunotherapy, the decrease in allergen-specific IgE is usually accompanied by an increase in IgG4, IgG1, and IgA antibodies. This is well described in subcutaneous immunotherapy to respiratory allergens[34,35] and in studies of oral and sublingual immunotherapy (SLIT) to foods.[32,33,36–38] Unfortunately, for most patients this is transient and lasts only a few years. It is debatable whether we should call this tolerance versus transient or sustained desensitization. In egg- or peanut-allergic patients submitted to oral immunotherapy (OIT) to egg or peanut, after cessation of treatment, within weeks or months clinical improvement is often lost.[33,39,40] In contrast, in children with postallergic sensitization who have lost the allergy, it is extremely unusual for the symptoms to recur.

In patients submitted to allergen-specific immunotherapy to foods, the typical clinical outcome to date has been an increase in the threshold of reactivity with desensitization, rather than the establishment of absolute long-term tolerance. This may result from the direct effect of low-dose continuous exposure to the allergen on mast cells and basophils (which can result in the activation of inhibitory intracellular signaling molecules such as SHIP followed by progressive loss of activatory intracellular signaling molecules such as Syk and loss of the high-affinity IgE receptor FcεRI on the surface of the cells[41]) and/or from the action of blocking antibodies as a result of the induction of regulatory T cells. There is typically no evidence of changes in IgE epitope binding or affinity and some evidence of regulatory T cell changes.[40] In desensitized patients, a good response is usually maintained for as long as the food is consumed and treatment withdrawal is typically followed by a decrease in allergen threshold levels and increased clinical reactivity in most cases.[33,39,40] From an immunological point of view, reverse of the previously described mechanisms would occur with progressive restoration of the high-affinity IgE receptor FcεRI on the surface of mast cells and basophils and of the activatory component Syk and suppression of SHIP phosphorylation

inside these cells and with a progressive loss of the regulatory T cell response, possibly as a consequence of changes in the methylation patterns of transcription factors such as Fox p 3 (the master Treg switch),[40] which overall result in a consequent increase in effector cell reactivity to the allergen and the development of allergic symptoms. In true oral tolerance, the absence of clinical (and effector cell) reactivity would remain regardless of whether the food is consumed, probably because T cell clone anergy and deletion are irreversible processes.

Risk and protective factors for food allergy

The recently described rise in food allergy prevalence in developed countries[42] is likely to be driven by potentially modifiable environmental factors, because the rise is more rapid than genetic deviation would allow. At the most general level it appears that these factors are linked to the modern lifestyle, because food allergy is more common in developed than developing countries, and migrants appear to acquire the incident risk of allergy of their adopted country.[43] A whole host of factors have been examined with regard to general allergic disease, but to date, few studies have investigated factors that might increase the risk of food allergy, and more important, few have used challenge-proven food allergy as an outcome when looking for causative factors. Because most cases of food allergy occur early in an infant's life (most often when allergenic foods are first introduced into the infant's diet), causative factors are likely to reflect antenatal or early postnatal events.

Figure 1[44–58] outlines ongoing areas of investigation with regard to the rise in food allergy. The current four main hypotheses include: (1) the hygiene hypothesis (and fecal microbial diversity) and the "old friends" hypothesis (related to decreasing parasite load as countries industrialize); (2) infant feeding factors (e.g., timing of introduction of solids including allergenic solids and breast feeding); (3) other lifestyle factors that might affect the infant's immunomodulatory status, such as vitamin D, long-chain polyunsaturated fatty acids, folate, and exposure to tobacco smoke; and (4) skin barrier function and the role of eczema in increasing sensitization and potentially food allergy (the dual-allergen hypothesis) (see Figure 2 and next section). Importantly, these factors may act singly or in concert and there is likely to be at least some component of gene–environment interaction (lifestyle factors may have a differential effect depending on the genetic status of the individual).[59]

Countries with a Westernized lifestyle appear to have the highest rates of allergic disease, and allergies are less common in developing countries. To date, there has been little information on these differences or why they might be occurring. Emerging evidence suggests that changes in the environment related to a Western lifestyle and to economic development are the most important factors causing the rise in allergic disease.[42] In particular, improved hygiene, less exposure to microbial organisms, changes in diet (eating less fish and vegetables and more processed foods, and changes in cooking practices), less exposure to sunlight (reduced ultraviolet exposure and therefore reduced vitamin D), and possibly increased use of antibiotics are thought to be some of the main factors contributing to the rise in allergy in the developed world.

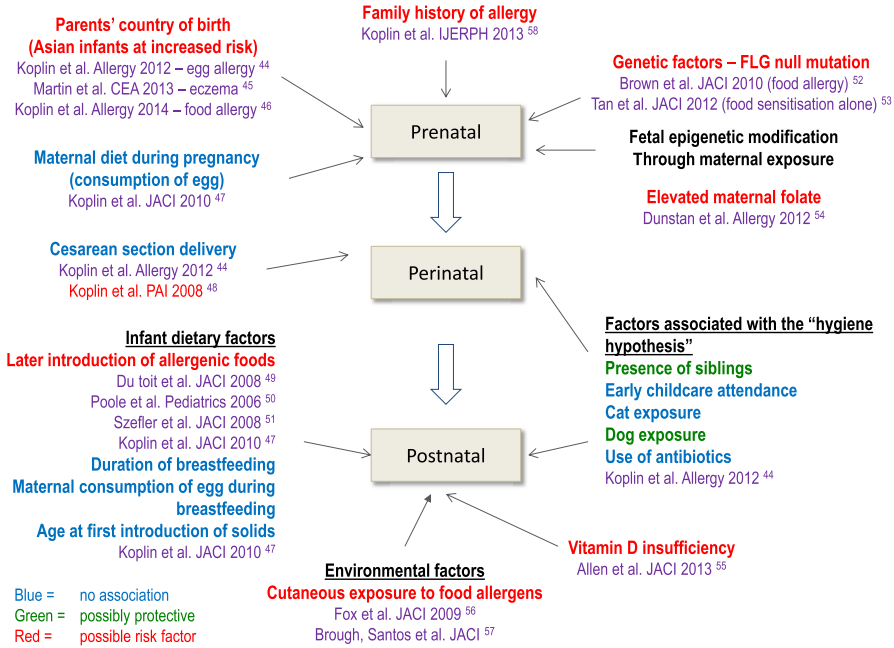


Figure 1 Lifestyle factors examined for possible association with food allergy.

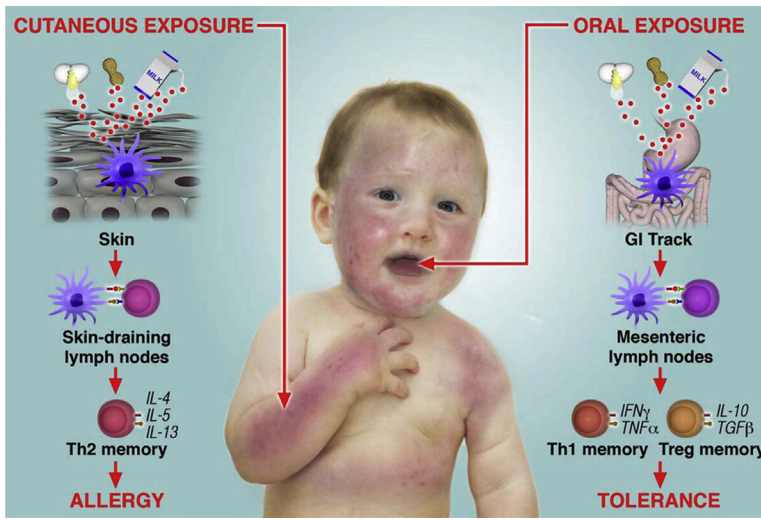


Figure 2 Dual-allergen exposure hypothesis for the pathogenesis of food allergy. Allergic sensitization results from cutaneous exposure and tolerance occurs as a result of oral exposure to food.[110]

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Associations have been found between several environmental factors and the development of food allergy. Environmental factors associated with the hygiene hypothesis (i.e., the hypothesis that early exposure to microbial antigens promote healthy immune development and reduces the risk of developing allergies) and linked to allergic outcomes such as asthma or allergic sensitization include companion animal ownership,[60] number of siblings,[61] and exposure to pets, in particular dogs.[62] Timing of introduction of solids[63] and breast-feeding regimes[64] have also been examined, with evidence that delayed timing of introduction of eggs[44] and peanuts[49] does not increase the risk of food allergy and the early introduction (between 4 and 6 months) of these foods may even be protective. As such, the results of a multitude of randomized controlled trials (RCTs) are awaited that aim to assess the impact that altered timing of introduction of allergenic solids might have on food allergy risk.

Tolerance induction in the prevention of food allergy

Early studies suggested that the delayed introduction of solids combined with maternal avoidance during pregnancy and lactation may protect against allergic disease through the prevention of sensitization. However, later follow-up of these cohorts showed that the protective effect was not maintained, which suggested that the associations may have been contaminated by confounding factors. There is emerging evidence that avoidance strategies are likely to be ineffective and may even be harmful, with a new concept of an optimal window of opportunity for the development of gastrointestinal tolerance currently being defined.[65]

Until recently, expert guidelines for infants with a family history of atopy typically recommended delaying introduction of allergenic foods (including avoiding eggs until age 2 years and nuts until 3 years), as well as delaying solid foods until after 6 months and breast feeding for at least 12 months, with the aim of reducing the risk of food allergy.[66,67] The recommendations to delay the introduction of allergenic foods have been withdrawn[68,69] because there is no evidence that it is beneficial, and some studies indicate that this practice may have contributed to the observed increase in the prevalence of food allergy.[50,70,71] However, currently there are not enough good-quality data to determine the right timing and dose of weaning foods.

Exposure to an adequate dose of antigen in early life is important to shape an appropriate immune response to food antigens. If oral exposure causes tolerance, how does food allergy occur? Observational studies indicate that primary sensitization to food allergens may occur through the skin, especially through inflamed skin. In the ALSPAC study, low-dose repeated exposure to peanut protein in the form of arachis oil applied to infants' inflamed skin was associated with an increased risk of peanut allergy at age 5 years.[13] Also, in murine studies, exposure of abraded skin mimicking eczema to food proteins led to food-specific IgE responses,[72,73] as opposed to ingestion of high doses of food protein, which promoted oral tolerance and prevented subsequent IgE sensitization and T cell proliferation.[74] Although the use of peanut oils is not widespread throughout the

world, food allergens have been measured in the home environment in quantities able to cause allergic sensitization and have been shown to be biologically active.[57,75] Household peanut exposure has indeed been shown to be a risk factor for peanut allergy.[56] Taken together, these data show that environmental epicutaneous exposure to peanut is a likely route of IgE sensitization to peanut and possibly to other foods.

The dual-allergen exposure hypothesis proposes that the development of allergic sensitization to foods and subsequent food allergy depends on the balance and timing of routes of exposure to the foods, namely oral and cutaneous exposure (Figure 2).[76] This hypothesis can be tested and formed the basis for the *LEAP study*, a large randomized trial in which 640 infants aged less than 11 months at high risk of developing peanut allergy (as defined by the presence of moderate to severe eczema and/or egg allergy) were recruited and either consumed peanut from that early age or avoided peanut until age 3 years, as per the United Kingdom Department of Health recommendations at the time.[77] The primary outcome of the study is the prevalence of peanut allergy at age 5 years, as determined by oral FC. Because a significant proportion (about 20%) of participants in the LEAP study were already IgE sensitized to peanuts when peanuts were (or not) introduced into the diet, the question was raised as to whether early peanut consumption induces transient desensitization rather than long-term tolerance. To address this question, the LEAP study was followed by the LEAP-On study, in which LEAP participants in both arms of the study, that is, consumers and avoiders, avoid peanut for 1 year and the presence of peanut allergy is assessed by oral FC at the end of this period. The results of these trials are awaited and will be known in 2015.

Other interventional studies are currently under way to investigate the role of early-life nutrition in oral tolerance induction and whether early introduction of foods in the diet can prevent the development of food allergy later in life (Table 1). These studies are being conducted in London, the United Kingdom; in Berlin, Germany; and in Sydney, Adelaide, and Perth, Australia, and include two studies on peanuts, four on hens' eggs, and one on six foods (cow's milk, eggs, wheat, peanuts, cod, and sesame). Some of these studies are on high- or moderate-risk populations and two are on populations recruited from the general population. When the outcome is the presence of allergy, it is evaluated by oral FC, the current reference standard to diagnose food allergy, in all studies. The STAR study was prematurely terminated before adequate statistical power was reached, but a trend was reported toward lower incidence of egg allergy in the consumption group compared with the control group. This study included a high-risk population defined by the presence of moderate to severe eczema and a significant proportion of highly atopic infants were already sensitized to egg at age 4 months. Raw egg powder was used in the intervention group and a substantial number of allergic reactions were observed, including one case of food protein–induced enterocolitis requiring admission to the intensive care unit and three cases of anaphylaxis, one of which was on the first exposure to the egg powder. These observations highlight the need for caution when introducing allergenic foods in the diet of high-risk infants.

Table 1 Future ongoing studies into the prevention of food allergy[101]

Name of trial	Country and institution	Allergen of interest	Population	Design and intervention	Primary outcome
LEAP (Learning Early about Peanut Allergy) http://www.leapstudy.co.uk	United Kingdom King's College London	Peanut	*N*=640 high-risk infants with moderate/severe eczema and/or egg allergy	RCT Consumption or avoidance of peanut from 4 to 10 months until age 3 years	Presence of peanut allergy at age 5 years, as defined by food challenge
LEAP-On	United Kingdom King's College London	Peanut	LEAP study population	RCT All LEAP patients avoid peanut for 1 year from the end of the LEAP study	Presence of peanut allergy at age 6 years, as defined by food challenge
EAT (Enquiring about Tolerance) http://www.eatstudy.co.uk	United Kingdom King's College London	Cow's milk, hens' eggs, peanuts, cod, sesame, wheat	*N*=1106 infants recruited prenatally from general population and enrolled from age 3 months	RCT Exclusively breast fed until age 3 months, then introduced sequentially to cows' milk, eggs, fish, wheat, sesame, and peanuts, or exclusively breast feeding until age 6 months	IgE-mediated allergy to any of the six foods between age 1 and 3 years, as defined by food challenge
PEAAD (Preventing Peanut Allergy in Atopic Dermatitis)	Germany Charité–University of Berlin	Peanut	460 high-risk children from age 5 to 30 months with atopic dermatitis	Nonrandomized, self-allocated, controlled trial. Consumption of peanut snack three times a week or peanut-free diet	IgE-mediated peanut allergy after 1 year of consumption or avoidance
HEAP (Hen's Egg Allergy Prevention Study)	Germany Charité–University of Berlin	Hens' egg	800 children recruited at birth	RPCT Children receive egg or placebo three times a week from age 4 to 6 months	Sensitization to eggs and egg allergy at age 1

Continued

Table 1 Future ongoing studies into the prevention of food allergy[101]—cont'd

Name of trial	Country and institution	Allergen of interest	Population	Design and intervention	Primary outcome
STAR (Solids Timings for Allergy Research)	Australia University of Western Australia	Hens' egg	86 high-risk infants with moderate–severe eczema enrolled at age 4–6 months	RPCT Consumption of egg or placebo until age 8 months	Development of egg allergy at age 12 months
BEAT (Beating Egg Allergy)	Australia University of Sydney	Hens' egg	290 infants with intermediate risk of developing allergy (sibling or parent with allergy) enrolled at age 4 months	RPCT Consumption of eggs or placebo until age 12 months	Sensitization to eggs at age 12 months
STEP (Starting Time for Egg Protein)	Australia University of Western Australia	Hens' egg	1512 infants with intermediate risk (atopic mothers, no eczema) enrolled at age 4–6 months	RPCT Consumption of egg or placebo until age 10 months	Development of egg allergy at age 12 months

RCT, randomized controlled trial; RPCT, randomized, placebo-controlled trial.

Allergen-specific immunotherapy in the management of food allergy

As discussed above, emerging evidence suggests that allergen avoidance for the prevention of food allergy not only is ineffective but may also paradoxically increase the risk of allergies such as eggs[44] and peanuts.[49] In parallel developments, a new cohort of studies has emerged that uses a similar mechanistic principle of allergen exposure—not for the prevention but rather the treatment of food allergy—through regular, low-dose allergen exposure or immunotherapy. The ultimate aim of these trials is to induce oral tolerance to the offending food and reach a cure, although some studies have a secondary and lesser aim of desensitizing patients to ensure a wider safety buffer against accidental ingestion reactions without aiming to achieve full oral tolerance.

Immunotherapy is a new form of treatment for food allergy that has been emerging at a rapid rate in particular over the past 5–10 years, although the first controlled trial of OIT in the modern era was described by Patriarca et al. as early as 1998.[78] Unfortunately, some of the early trials were plagued by unacceptably high rates of adverse events, and only recently have large controlled trials for food immunotherapy been mounted.

Four approaches to food allergy immunotherapy have been trialed to date: (1) Oral immunotherapy (OIT) (Table 2); (2) Sublingual immunotherapy (SLIT); (3) peptide-based immunotherapy; and (4) immunotherapy employing adjuvant therapies to increase efficacy. More recently, multiple food allergens have been incorporated into protocols for OIT as well as combined with adjuvants. Early trials were mostly uncontrolled, but there are a significant number of carefully controlled trials in progress for which the results are not yet available.

Oral immunotherapy

Eggs and cow's milk

Oral immunotherapy for eggs and cow's milk has had the highest success rates for immunotherapy, although many of the early trials were open and some did not have entry double-blind placebo-controlled (DBPC) challenges. In addition to the clear design deficits that an open trial presents because of the lack of control for a placebo effect, the absence of baseline challenges is problematic because there is a high natural rate of disease remission in infants and young children with food allergy, particularly with egg and cow's milk allergies. Some trials attempted to minimize the confounder of natural history resolution by only enrolling children who were older and therefore at a time when the disease was less likely to remit.

The most recent and successful egg OIT DBPC RCT was published in the New England Journal of Medicine.[33] Using a design of 3:2 active to placebo/avoidance, 28% showed development of tolerance at 22 months versus 0% in the placebo arm ($P < .045$). Enrolment age was greater than 5 years, which minimized the possibility that natural resolution may have accounted for the effect. However, only one case in the placebo arm would have needed to have naturally developed tolerance for the study to have found a null effect and characteristics at baseline were not matched with

Table 2 **Summary of findings of systematic reviews and meta-analyses on oral immunotherapy for food allergy**

Publication details			Characteristics of trials and participants								Outcomes reported		
Author (year)[REF]	**Journal**	**SR or MA**	**Allergen**	**No. of RCT**	**No. of other studies**	**OIT (*n*)**	**Control (*n*)**	**Total (*n*)**	**Age (years)**	**Quality of evidence**	**Main outcome**	**Outcome measurement**	**Authors' conclusions**
Nurmatov (2014)[102]	Br J Nutr	SR and MA	Milk, egg, peanut, others	14	3	270	404	674	1–21	Low to high risk of bias	Persisting food allergy	There was a substantially lower risk of reactions to the relevant food allergen in those receiving OIT (risk ratios (RR) 0.19, 95% CI 0.09, 0.37)	Given the paucity of evidence on longer-term safety, effectiveness and cost-effectiveness, OIT should not be used outside experimental conditions currently
de Silva (2014)[103]	Allergy	SR	Milk, egg, peanut, others	10	8	NA	NA	NA	Children and adults	Only 22% high-quality studies	Posttreatment tolerance and symptoms	Two SRs, nine RCT, and four nonrandomized comparisons found that OIT was associated with improved tolerability for children and adults with various food allergies. One RCT found no	OIT is currently only a research intervention, but may be promising therapeutically. AIT may

												benefit. Two SRs found mixed evidence and concluded that OIT should not be routine treatment	be disease modifying and therefore warrants further exploration
Nurmatov (2012)[79]	Cochrane Database Syst Rev	SR	Peanut	1	0	19	9	28	1–16	Low risk of bias	Posttreatment tolerance	Peanut OIT subjects ingested the maximum cumulative dose (CD) of 5000 mg (about 20 peanuts). Placebo subjects ingested a median CD of 280 mg	Larger RCTs are needed to investigate the acceptability, long-term effectiveness and cost-effectiveness of OIT
Sheikh (2012)[104]	Prim Care Respir J	SR	Peanut	0	6[b]	85	0	85	1–13[a]	Low risk of bias	Posttreatment tolerance	It is possible for many participants to increase the threshold dose for peanut exposure whilst receiving treatment	The effectiveness and cost-effectiveness of OIT (particularly over the longer-term) need to be clearly established using robust designs before its clinical use can be contemplated

Continued

Table 2 Summary of findings of systematic reviews and meta-analyses on oral immunotherapy for food allergy—cont'd

Publication details			Characteristics of trials and participants								Outcomes reported		
Author (year)[REF]	**Journal**	**SR or MA**	**Allergen**	**No. of RCT**	**No. of other studies**	**OIT (*n*)**	**Control (*n*)**	**Total (*n*)**	**Age (years)**	**Quality of evidence**	**Main outcome**	**Outcome measurement**	**Authors' conclusions**
Yeung (2012)[105]	Cochrane Database Syst Rev	SR and MA	Milk	5	0	106	90	196	2–17	Low	Full desensitization	Sixty-six patients (62%) in the OIT group were able to tolerate a full serving of milk (about 200 mL) compared with seven (8%) of the control group (RR 6.61, 95% CI, 3.51–12.44)	The current evidence shows that OIT can lead to desensitization in most individuals with IMCMA although the development of long-term tolerance has not been established
Brozek (2012)[106]	Clin Exp Allergy	SR and MA	Milk	5	5	114	104	218[d]	2–17[d]	Low	Posttreatment tolerance	OIT compared with elimination diet alone, increased the likelihood of achieving full tolerance of cow's milk (relative risk 10.0 [95% CI, 4.1–24.2])	Additional, larger RCTs measuring all patient-important outcomes are still needed

Fisher (2011)[107]	Arch Dis Child	SR and MA	Milk, eggs	3	0	68	57	125	0.6–17	1+ and 1++ (NIHCE)	Posttreatment tolerance	Lower RR of allergy after SOTI, but this was not statistically significant (0.61; 95% CI, 0.32–1.16)	Further research is needed using large, high-quality RCT that investigate a variety of food allergens and assesses the long-term efficacy, safety, and cost-effectiveness of SOTI
Chafen (2010)[108]	JAMA	SR	Milk, eggs	3	2[c]	117	98	215[d]	1–17	Fair to good	Posttreatment tolerance	Tolerance was achieved more often in OIT group compared with control group	Immunotherapy is promising but data are insufficient to recommend use
Calvani (2010)[109]	Eur Ann Allergy Clin Immunol	SR	Milk, eggs	4	0	95	87	182	1–17	Sufficient	Posttreatment tolerance	Tolerance in the OIT group was between 30 and 90% whilst in control group was 0–60%	Additional studies are needed to clarify which is the best treatment and protocol to follow in order to reduce the adverse events frequency and to increase the success rate

OIT, Oral immunotherapy; AIT, Allergen specific immunotherapy; SR, Systematic review; MA, Meta-analysis; IMCMA, IgE-mediated cow milk allergy; NA, Not available.
[a]Not detailed in some included studies.
[b]Case series.
[c]Two studies involved the sublingual administration of AIT.
[d]Included in RCT.

regard to IgE titer at baseline, which suggested that there may have been a differential recruitment into each arm. Importantly, egg-allergic patients were not characterized at baseline by their ability to tolerate baked eggs in small amounts. This could be problematic if there is a differential in recruitment of baked egg–tolerant patients into each arm because we have shown that baked egg–tolerant children are five times more likely to develop tolerance than those who are baked egg–allergic.[5] However, Burks et al.[33] demonstrated immune changes associated with tolerance including reduced Th2 responses, increased Th1 and Treg responses, and increased sIgG4 and reduced sIgE.

Peanuts and tree nuts

In 2009, the first trials looking at peanut OIT emerged.[32] Since then, other studies[36,39,79] have shown that peanut OIT is effective; most treated patients are able to pass an oral FC, with a 20- to 100-fold increase in the threshold of clinical reactivity. In these studies, high-maintenance doses are usually reached; however, a considerable rate of systemic reactions has been observed, with up to 25% of participants requiring intramuscular epinephrine, including during home dosing.

Sublingual immunotherapy

Sublingual immunotherapy is an increasingly accepted treatment for desensitization to pollen and other aeroallergen allergies. Compared with subcutaneous therapies, it has a better acceptability profile because of patient comfort factors, although it appears to be a less effective route of administration and requires long-term daily dosing.[80] Based on the growing acceptance of SLIT for other allergic conditions interest has turned to its use in food allergy treatment. Enrique et al.[81] were the first to describe the use of SLIT in 2005 to treat hazelnut allergy. Twenty-three patients were enrolled in a well designed, DBPC trial of hazelnut SLIT versus placebo with updosing to occur so that the maximum dose was achieved at 4 days. Systemic reactions were observed in only 0.2% of the total doses administered. Mean hazelnut quantity provoking objective symptoms at study completion increased from 2.29 to 11.56 g ($P = 0.02$; active group) versus 3.49–4.14 g (placebo; not significant). Moreover, almost 50% of patients who underwent active treatment reached the highest dose of hazelnut (20 g), but only 9% in the placebo. The active groups also demonstrated an increase in IgG_4 and IL-10 levels after immunotherapy, suggesting good tolerance to this treatment.

A more novel approach was described by Keet et al.,[82] in which SLIT was first used followed by OIT for cow's milk. Thirty patients (aged 6–17 years) with challenge-proven cow's milk allergy were randomized to cow's milk SLIT alone versus SLIT followed by one or two doses of milk OIT. Sublingual immunotherapy escalation to 7 mg daily in both arms was followed by OIT to 1000 mg for the OIT B group or OIT to 2000 mg for the OIT A group. After maintenance, subjects were challenged with 8 g milk protein; if they passed the 60-week challenge, therapy was withdrawn and challenges were repeated at 1 and 6 weeks. After therapy, 1 of 10 subjects

in the SLIT group, 6 of 10 subjects in the SLIT/OIT B group, and 8 of 10 subjects in the OIT A group passed the 8-g challenge ($P=0.002$ SLIT vs OIT). After avoidance, 6 of 15 subjects regained reactivity, 2 after only 1 week. The authors concluded that OIT was more effective for desensitization to cow's milk than SLIT alone, but it had more systemic side effects.

Immunotherapy with multiple food allergens

Among children with food allergy, up to 30.4% are allergic to more than one food[83] and the prevalence is potentially even higher in atopic children.[84] So far, most studies on OIT for FA have been conducted using single food allergens, because this approach has been reported to be relatively safe. A study by Bégin et al.[85] assessed the safety of a modified OIT protocol by administering multiple foods simultaneously. Forty subjects aged 4–46 years met inclusion criteria on DBPCFCs that included peanut followed by tree nuts, sesame, dairy, or eggs. Fifteen participants were allergic to peanuts only and were assigned single OIT with peanuts, whereas the 25 remaining had additional allergies and received multiple OIT including a mixture with up to five foods (range, 2–5 foods). Reaction rates per dose did not differ significantly between groups (median reaction rate, 3.4% [multiple OIT] vs 3.7% [single OIT]; $P=.031$). In both groups, most reactions were mild, but two severe reactions requiring adrenaline occurred in each group after home dosing. The time to reach equivalent doses per food was significantly faster in the single OIT group than for those on multiallergen OIT. Although further evaluations in the context of RCTs are required to assess the safety and efficacy of OIT with multiple allergens, preliminary data show that this approach can be feasible and relatively safe.

Peptide immunotherapy

The use of linear peptide sequences representing fragments of the native allergen that target allergen-specific CD4+ T cells but do not bind IgE constitutes an option to reduce the allergenicity of immunotherapy while maintaining immunogenicity. Studies using peptides to treat allergic diseases have been conducted mainly for cat, ragweed, and bee venom allergies.[86] Recent studies, however, have explored the potential role of peptide immunotherapy in treating food allergy. Rupa et al.[87] assessed the effects of OIT using T cell epitope peptides of the dominant egg white allergen ovomucoid (Ovm) in a murine model of egg allergy. Mice were orally sensitized to Ovm and subsequently received single or multiple Ovm T cell epitopes peptides or phosphate-buffered saline only as control group; then, mice underwent an oral challenge with Ovm. Signs associated with the challenge were more frequently observed in the control group compared with those in the peptide-treated groups ($P\leq0.05$). Mice in the treated group had lower levels of specific IgE and an increased percentage of circulating Treg cells. Prickett et al.[88] conducted a study to identify T cell epitope-based peptides as candidates for peanut-specific immunotherapy. Multiple CD4+ T cell lines specific for the major peanut allergen Ara h 2 were generated from PBMCs of 16 human leukocyte antigen (HLA)-diverse subjects with peanut allergy. Five immune-dominant

CD4+ T cell epitopes of Ara h 2 that collectively show diverse HLA-II restriction were identified. Cysteine-free variants of these epitopes were incorporated into a combinatorial panel of three short (≤17 aa), HLA-degenerate peptides that can target pathogenic T cells in HLA-diverse individuals with allergy without binding peanut-reactive serum IgE. The same group of researchers also identified 10 CD4+ T cell epitopes of Ara h 1 that collectively show diverse HLA class II restriction. These epitopes were incorporated into a panel of seven short (≤20 aa), HLA-degenerate peptides.[89] Although further evaluations are required, the combination of these Ara h 1 peptides with the three T cell epitope-based Ara h 2 peptides described previously shows considerable promise as a potential candidate for a peptide-based treatment of peanut allergy.

Adjuvant/combination therapies

There is evidence that although RCTs of egg, milk, and peanut OIT can consistently induce desensitization (i.e., the transient ability to tolerate a food that is lost once OIT is stopped), the ability to induce tolerance (or sustained unresponsiveness—the persistence ability to tolerate a food after food allergen OIT has been stopped) remains uncertain. A new class of immune response modifiers or adjuvants is emerging and it is hoped that it will increase the efficacy and the safety of immunotherapy, either OIT or SLIT.

Probiotics

Probiotics are live non-pathogenic microorganisms that benefit the health of the host when administered in adequate amounts.[90] Probiotic bacteria have potent documented immune modulatory effects that are species- and strain-specific. *Lactobacillus rhamnosus* GG has been demonstrated to induce Treg activity in vivo,[91] increase Th1 responses,[92] and promote IgA responses.[93] Tang et al. enrolled 62 patients into a DBPCRCT of probiotic and peanut OIT (PPOIT) versus probiotic alone. The average age was 6 years with a mean SPT at baseline of 17 mm that was not significantly different between active and control groups. This study was unique because it tested for sustained unresponsive as a primary outcome although only 2 weeks after the conclusion of the trial, when more than a 4-week time frame would have been more valuable. A total of 82% of those who received PPOIT sustained unresponsiveness versus 3.6% ($P<0.001$). There was also a significant difference in desensitization status as well as a lower peanut SPT at 18 and 21 months in those who received the active arm versus placebo. These outcomes were associated with appropriate sIgE and sIgG4 responses.[94]

Anti-immunoglobulin E Monoclonal Antibodies

Diverse studies have been conducted to investigate the effects of anti-IgE monoclonal antibodies treatment in subjects with food allergy. In a RCT, Leung et al.[95] compared the effects of three doses of TNX/901 versus placebo given subcutaneously every 4 weeks for four doses in 84 patients with a history of immediate hypersensitivity to peanut. The primary outcome was the change from baseline in the threshold dose that induced hypersensitivity to peanut flour, as assessed by an oral FC. Participants on the highest dose of TNX/901 (450 mg) had a significant increase in the threshold of sensitivity to peanut

(from 178 to 2805 mg; $P < .001$). In another study, Sampson et al.[96] assessed the efficacy of omalizumab in reducing the risk of peanut-induced allergic reactions. Twenty-six subjects were randomized to receive omalizumab every 2–4 weeks ($n = 17$; minimum 0.016 mg/kg/IgE [IU/mL]) or placebo ($n = 9$). After 24 weeks, 14 subjects completed their post-therapy OFC. Four of nine omalizumab-treated subjects (44.4%) versus one of five placebo-treated subjects (20%) could tolerate ≥1000 mg peanut flour ($P = .324$). When comparing the change from baseline in maximum tolerable peanut dose after treatment, there was a greater shift in peanut tolerability in subjects treated with omalizumab ($P = .054$). Because anti-IgE monoclonal antibodies are quickly cleared in vivo, this approach represents a long-term treatment involving frequent injections without a disease-modifying effect.

Several studies have shown that omalizumab increases the threshold for adverse reactions in subjects undergoing a food allergen challenge.[97] These observations have led to the hypothesis that in patients receiving OIT with food allergens, the concomitant administration of anti-IgE therapy can potentially increase the allergen dose tolerability and therefore speed up the treatment progression.

Nadeau et al.[98] conducted a phase I study in cow's milk–allergic children aged 7–17 years. Nine weeks after the start of omalizumab, oral desensitization with cow's milk was conducted initially after a rush OIT protocol (nine of 10 reached the 1000-mg dose in the first day). Desensitization with daily doses of milk continued in the 10 subjects, with weekly increases in the dose of milk over the next 7–11 weeks. Omalizumab was then discontinued at week 16. Nine of 10 subjects passed the final DBPCFC, tolerating a cumulative dose of 7250 mg milk protein (about 220 mL of milk). In patients with peanut allergy, Schneider et al.[99] performed oral desensitization in 13 peanut-allergic children (median age, 10 years) at high risk for developing significant reactions. Omalizumab was administered 4 months before and during oral peanut desensitization. Twelve subjects then reached the maximum maintenance dose of peanut flour per day (4000 mg) in a median time of 8 weeks, when omalizumab was discontinued. All 12 subjects continued to receive 4000 mg peanut flour/day and subsequently tolerated a challenge with 8000 mg peanut flour (about 20 peanuts), a dose approximately 160–400 times the dose tolerated before desensitization.

Bégin et al.[100] evaluated the safety and dose tolerability of a phase I OIT protocol using omalizumab in patients who were desensitized with multiple foods simultaneously (up to five). Twenty-five participants (median age, 7 years) were enrolled in the study. Omalizumab was administered 8 weeks before and 8 weeks after the initiation of a rush multiple OIT schedule. After pretreatment with omalizumab, 19 participants reached up to 1250 mg of combined food proteins on day 1 (in 1:1:1:1:1 proportions), requiring minimal or no rescue therapy. The remaining six were started on their highest tolerated dose as their initial daily home doses. Participants reported 401 reactions per 7530 home doses (5.3%). Most (94%) allergic reactions were mild and included mainly abdominal pain, pruritus, and local ear, nose, and throat symptoms. There was one severe reaction. Participants reached their maintenance dose of 4000 mg protein per allergen at a median of 18 weeks (range, 7–36 weeks). All participants reached a dose equivalent to a 10-fold increase of all of their allergens by 2 months of treatment. Although these approaches seem promising, further evaluations in the context of phase II RCTs are required.

Although the rapid growth in publications outlining at least some success from various immunotherapy protocols offers an exciting development and real hope for patients, a note

of extreme caution needs to be sounded because of the risks inherent to study participants, such as anaphylaxis from experimental immunotherapy protocols as well as the risk that patients themselves might attempt initiation of immunotherapy protocols at home without appropriate medical supervision. Of further concern are the wide variation in protocol methodologies and the lack of standardization of outcome measures. The persistent heterogeneity of study design and quality will ultimately curtail the ability to formally assess the overall effectiveness of OIT protocols across multiple centers, which is a regulatory requirement before such therapies could be safely considered for routine clinical use.

Conclusion

Different types of tolerance have been identified and may have different underlying mechanisms. The realization that antigen exposure may drive tolerance is being explored in prophylactic and therapeutic trials for food allergy. It is too early to say whether food allergy can be prevented early in life through oral tolerance induction. Clinical trials demonstrate that food allergen–specific immunotherapy will markedly reduce clinical reactivity to food, but thus far the evidence points more to a phenomenon of transient desensitization rather than long-term tolerance. It is possible that many years of OIT may be required to induce long-term tolerance in patients with food allergy. In addition, the combination of an adjuvant approach together with gastrointestinal exposure to food antigens is being explored to assess whether they can induce long-term tolerance.

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