



# Environmental protection from allergic diseases: From humans to mice and back

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Allergic diseases have a strong environmental component, illustrated by the rapid rise of their prevalence in the Western world. Environmental exposures have been consistently shown to either promote or protect against allergic disease. Here we focus on protective exposures and the pathways they regulate. Traditional farming, natural environments with high biodiversity, and pets in the home (particularly dogs) have the most potent and consistent allergy-protective effects and are actively investigated to identify the environmental and host-based factors that confer allergy protection. Recent work emphasizes the critical protective role of microbial diversity and its interactions with the gut/lung and skin/lung axes — a cross-talk through which microbial exposure in the gut or skin powerfully influences immune responses in the lung.

## Addresses

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## Introduction

Allergic diseases such as asthma, atopic dermatitis and allergic rhinitis have a strong environmental component, eloquently illustrated by the rise of their prevalence in the Western world over the last few decades [1,2]. Several environmental factors have been consistently shown to increase allergic disease risk in isolation or in the context of gene–environment interactions [3,4], but remarkably other environmental exposures have been repeatedly found to confer protection against these diseases. These exposures, and the allergy-protective pathways they regulate, are the focus of this review.

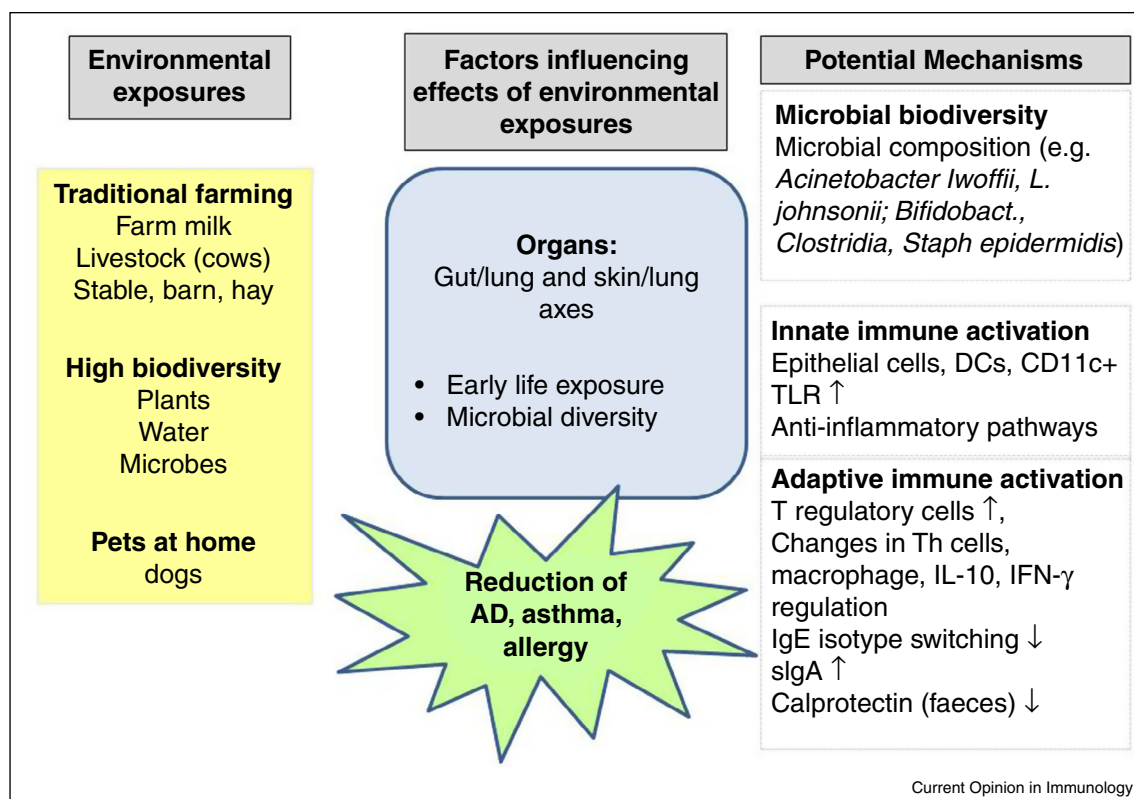
Among the exposures known to protect human populations from allergy and asthma, traditional farming [5], natural environments with high biodiversity [6•], and pets in the home (particularly dogs) [7] appear to have the most potent and consistent effects. Therefore much research in both humans and mice is currently dissecting the environmental and host-based factors that confer allergy protection under these conditions. As we will see, despite the distinct characteristics of these protective environments, a unifying theme is emerging from this research: the strong nexus between protection from allergic disease and microbiota in the environment and/or the host. In combination with population-based studies that identify the most relevant questions, mouse models are playing a critical role in shaping this novel paradigm because even with the caveats necessary when translating results across distant species, they provide a powerful tool to go beyond associations and characterize causal pathways. Seminal studies in specific pathogen-free or germ-free mice that illustrate the connection between allergic disease and microbiota have been recently reviewed [8]. Here we will discuss recent work that specifically highlights the complex relation between the environment and protection from allergic disease.

## The farm effect

A strong association between traditional farming and protection from IgE-mediated allergic diseases including asthma, hay fever, atopic dermatitis and atopic sensitization has been consistently shown throughout the world [5], particularly for prenatal exposure [9•]. Moreover, recent data from the PASTURE birth cohort enrolled in rural areas of Austria, Finland, France, Germany, and Switzerland demonstrated that not only asthma, but also rhinitis, respiratory tract infections, otitis, fever and C-reactive protein levels at 12 months were reduced by about 30% following raw milk consumption in early life [10], thus significantly extending the scope of the protection conferred by farm exposure. Interestingly, whereas the overall farm effect can be explained by specific exposures (cows, straw, and farm milk for asthma, and fodder storage rooms and manure for atopic dermatitis), the link between the farm effect and hay fever and/or atopic sensitization is still partially missing [11].

To decipher the mechanisms underpinning the farm effect, human studies have explored the impact of farm exposure on immune system maturation (Figure 1). Farm-related exposures were associated with modified expression of innate immune receptor genes in early life

Figure 1



Underlying potential mechanism of allergy protection through environmental exposure (traditional farming/high biodiversity/pets at home).

[9<sup>•</sup>] and increased T regulatory (Treg) cells at age 4 years but also already at birth [12<sup>•</sup>,13]. At age 4 years, Treg cells in part explained the asthma-protective effect of farm milk exposure [12<sup>•</sup>]. The role of diet is looming large in these studies. Indeed, a recent comparison of urban (Guangzhou) and rural (Shoaguan) China confirmed that the prevalence of self-reported asthma (6.6% vs 2.5%), rhinitis (23.2% vs 5.3%) and eczema (34.1% vs 25.9%) was significantly higher in urban subjects, and showed that the schoolchildren's diet is affected by the country's urbanization and in turn affects asthma risk. High vegetable and low milk intake protected against childhood asthma [14]. Introduction of an increasing diversity of foods within the first year of life was positively associated with increased Treg markers and protection from the development of food sensitization, food allergy and asthma up to 6 years also in children from the PASTURE/EFRAIM study. One possible protective mechanisms involving Treg cells is the inhibition of isotype switching to IgE: a decrease in C<sub>ε</sub> germline transcripts was associated with increased food diversity [15<sup>•</sup>]. Furthermore, serum IgA levels were positively associated with contact to farm animals or cats during pregnancy, and inversely associated with atopic dermatitis up to 2 years, independent of breastfeeding duration [16]. It is noteworthy that

not only the Treg but also the Th17 lineage appears to be targeted by prenatal farm exposure. Expression of Th17 cell markers in cord blood per se was not influenced by maternal farming. Yet, after stimulation of the cord blood cells with LpA, the main component of endotoxin, we found that Th17 and Treg cell markers were positively correlated only in non-farming children, potentially suggesting a stimulus-specific TH17/Treg mRNA correlated effect in children with low exposure or, alternatively, no effect in previously exposed children [17].

Several mouse studies demonstrated that inhalation of farm-derived dust extracts or selected microbes can prevent allergen-driven airway hyperresponsiveness (AHR) and lung eosinophilia [18–22]. While suggestive, those studies are difficult to interpret because the lack of true negative controls prevents determining how specific these inhibitory effects were. Significant progress may be fostered by a different, novel approach that compares and contrasts traditional and modern farm environments and populations with closely comparable genetic make-ups. Indeed, differences in asthma prevalence between farm and non-farm children are recapitulated in 2 U.S. farming populations, the Indiana Amish and the South Dakota Hutterites. Both are founder populations with

similar levels of inbreeding and patterns of linkage disequilibrium [23,24]. Moreover, the two populations share several lifestyle and dietary factors known to affect asthma and allergy susceptibility (e.g. large sibships, childhood vaccinations, diets rich in fat, salt, and raw milk, low rates of childhood obesity, long nursing durations, minimal exposure to smoking and air pollution, taboos against indoor pet). However, the Amish practice traditional farming and use animals for transportation, whereas the Hutterites embrace modern farming technologies. Strikingly, the prevalence of asthma and allergic sensitization was 5.2% and 7.2%, respectively, in Amish school children [25<sup>•</sup>] and >15% and >40%, respectively, in Hutterite school children [26,27<sup>•</sup>], with profound differences in immune regulatory profiles [27<sup>•</sup>]. Environmental factors underpin the opposite profiles of asthma and allergy prevalence among Amish and Hutterites because these profiles can be recapitulated by administering extracts of Amish or Hutterite house dust to allergen-sensitized mice [28]. Inhalation of Amish dust extracts was sufficient to strongly protect animals from AHR and lung inflammation in an ovalbumin (OVA) model of experimental asthma. In contrast, AHR was not attenuated and was even exacerbated in mice treated with Hutterite house dust extracts. Lung gene expression profiling revealed multi-pronged suppression of OVA-dependent pathways related to epithelial, dendritic and Th2 cell activation, alternative macrophage activation and airway remodeling in mice treated with Amish but not Hutterite house dust [28]. This approach is promising because comparing two farming environments, rather than a farming and a non-farming one, significantly reduces the search space and thus lends itself to a highly granular dissection of the environmental and host factors involved in allergy protection.

### The biodiversity hypothesis

The farm effect, which is intertwined with the Hygiene Hypothesis [29] both conceptually and experimentally, has been recently integrated and extended by the Biodiversity Hypothesis [6<sup>••</sup>]. The notion that reduced contact with natural environmental features and biodiversity, including environmental microbiota, may adversely affect the human commensal microbiota and their immunoregulatory properties was originally proposed by a group of researchers who compared prevalence of allergy in Finnish and Russian Karelia, two adjacent areas in northern Europe that are socio-economically distinct but geoclimatically similar. The Karelia Allergy Study was launched in 1998 to characterize the allergy profiles in the two areas. The key finding was that allergic symptoms and diseases were systematically more common in Finnish children and adults than in their Russian counterparts [30]. For example, in the early 2000s, hay fever in school children was almost non-existent in Russian Karelia, and only 2% of children were sensitized to birch pollen compared with 27% in Finnish Karelia. Among adults born in the 1940s,

sensitization to pollens and pets was at the same low level in both countries, but among the younger generation born in the late 1970s, differences were already manifold. Seropositivity to some pathogens, microbial content in house dust and drinking water seemed to confer allergy protection in Russia. Later studies of atopic sensitization in children from a small region in Eastern Finland showed that environmental biodiversity around the study subjects' homes, human microbiota and allergy are interrelated [31<sup>••</sup>]. Indeed, compared with healthy individuals, atopics had lower environmental biodiversity (amounts of forested and agricultural land) in the surroundings of their homes and significantly lower generic diversity of gammaproteobacteria on their skin. The functional link between gammaproteobacteria and atopy susceptibility was supported by the positive correlation that existed between IL-10 expression by peripheral blood mononuclear cells and the abundance of the gammaproteobacterial genus *Acinetobacter* on the skin in healthy, but not atopic, individuals [31<sup>••</sup>]. More recently, using a mouse model that seeks to recapitulate the atopic march and induces allergic lung inflammation by intradermal sensitization with OVA followed by OVA airway challenge, the same group showed that intradermal administration of *Acinetobacter lwoffii* together with OVA significantly decreased broncho-alveolar lavage eosinophilia, Th2 cytokine gene expression in lung tissue and serum levels of OVA-specific IgE upon OVA challenge through the airways. Moreover, one week after the last exposure to *A. lwoffii*, microbe-treated mice had increased levels of IL-10 and IFN- $\gamma$  in the skin at the site of injection [32<sup>•</sup>]. Overall, these results suggest that skin commensals play an important role in tuning the balance of Th1, Th2, and anti-inflammatory responses to environmental allergens both locally and more systemically.

### Exposure to indoor pets

A U.S.-based group that includes microbial ecologists, immunologists and epidemiologists is characterizing the impact that exposure to dogs has on the gut microbiome and immune system of the host. This group previously showed that dog exposure in early infancy reduces the risk of developing allergic disease in childhood [7], and dog ownership is associated with distinct microbial profiles in house dust [33]. More recently, these researchers have demonstrated that administering dog-associated house dust to mice through the gastro-intestinal tract protects against OVA or cockroach allergen-mediated airway pathology [34]. Protected animals exhibited significant reduction in the total number of airway T cells and down-regulation of Th2-related airway responses and mucin secretion. Notably, following dog-associated dust exposure, the cecal microbiome of protected animals was extensively restructured. *Lactobacillus johnsonii*, in particular, was significantly enriched in these mice, and supplementation of wild-type animals with *L. johnsonii* protected them against both airway allergen challenge

and infection with respiratory syncytial virus. At the cellular level, *L. johnsonii*-induced protection was associated with significant reductions in the total number and proportion of activated CD11c<sup>+</sup>/CD11b<sup>+</sup> and CD11c<sup>+</sup>/CD8<sup>+</sup> cells, as well as significantly reduced airway Th2 cytokine expression. These results indicate that exposing the gut to dog-associated household dust results in protection against allergen challenge through the airways and leads to a distinct gastrointestinal microbiome composition. Moreover, the study identified *L. johnsonii* as a bacterial species within the gastrointestinal tract that influences adaptive immunity at remote mucosal surfaces so as to protect against a variety of respiratory insults relevant to children's health.

### Unifying mechanisms for allergic disease protection?

Microbial diversity has been associated with asthma and allergy protection [35,36]. The data discussed above suggest that changes in the skin, intestine and lung microbiome may modulate immune responses in a way that modifies allergy risk. Microbiota may be actively selected by the host through complex interactions of different receptors with molecular patterns presented to mucosal cells, particularly during the neonatal period [37,38<sup>\*</sup>]. For instance, defined commensals have been recently shown to dominantly affect skin immunity. Colonization with *Staphylococcus epidermidis* induced IL-17A<sup>+</sup>CD8<sup>+</sup>T cells that home to the epidermis, enhance innate barrier immunity and limit pathogen invasion. Commensal-specific T cell responses resulted from the coordinated action of skin-resident dendritic cell subsets and were not associated with inflammation, demonstrating that tissue-resident cells were poised to sense and respond to alterations in microbial communities [38<sup>\*</sup>].

Several studies link microbiota to maintenance of basal immune homeostasis and propose that a reduced diversity of the gut flora is associated with the development of childhood asthma [39]. An allergy-protective effect of microbial diversity may be explained by prevention of colonization of the lower airways with harmful bacteria and in parallel stimulation of innate immune receptors that leads to adequate maturation of innate and adaptive immunity [35]. Recent airway microbiome studies demonstrated that neonatal colonization with a single strain or different combinations of *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae* is associated with increased risk for recurrent wheeze and asthma early in life [40]. Furthermore, an efficient immune surveillance of respiratory mucosal dendritic cells directed against pathogens from the nasopharyngeal microbiome was shown to be critical for resistance to respiratory viral infections and susceptibility to allergy and asthma early in life [41<sup>\*</sup>]. Caesarean section, formula feeding or early antibiotic administration affected early-life gut microbiota composition, with higher *Clostridia* and lower

*Bifidobacteria* counts, and in parallel were associated with increased risk for asthma in later childhood [42]. Furthermore, a high degree of intestinal inflammation at 2 months of age (as revealed by elevated levels of fecal calprotectin) predicted asthma and atopic dermatitis by age 6 years, and was associated with low abundance of fecal *Escherichia* and impaired IL-10 production [43]. Again, microbial profiles in the gut/skin/lung axis in early life appear to powerfully influence allergic disease risk later on.

### Conclusion: why should we care?

Several studies have established that selected bacterial species can confer protection against experimental asthma. The work discussed above, however, pushes the field forward because it emphasizes the critical role of microbial diversity and its interactions with the gut/lung and skin/lung axes — a cross-talk through which microbial exposure in the gut or skin powerfully influences immune responses in a distant organ, the lung. So far, the mechanisms underlying these distant effects are unclear, and current mouse and human data have primarily explored one microbial niche (and therefore one axis) at a time. However, it is conceivable (and in fact likely) that exposure to animals and biodiverse natural environments of high microbial complexity affects multiple niches. Therefore, models that test more than one axis simultaneously are highly desirable, especially because these data also suggest that a single commensal species (e.g. *Acinetobacter lwoffii*) may target allergen-induced lung inflammation through distinct routes (e.g. intradermal and intranasal). Therefore, future work in both humans and mice will need to decipher the pathways that connect the gut/lung/skin axes on the one hand to protective environmental exposures and on the other, to specific mechanisms that lead to allergic disease protection.

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