



The farm effect, or: when, what and how a farming environment protects from asthma and allergic disease

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Purpose of review

Multiple studies have shown that the prevalence of asthma and atopy is reduced in children raised on traditional dairy farms. This article discusses the temporal constraints for the protective farm effect, the components of a farming environment that are associated with protection, and novel mechanisms that may underlie protection from asthma and atopy in farming populations.

Recent findings

Protection from asthma and allergy is strongest when exposure occurs *in utero* or early in life, but the protective effects can persist into adulthood. Just three exposures (contact with cows and straw and consumption of unprocessed cow's milk) account for virtually all the protective farm effect for asthma but not atopy. Whey proteins appear to be critical for the protective effects of farm milk, whereas the high microbial diversity existing in a farm environment is strongly and inversely associated with asthma, but only weakly associated with atopy. Therefore, distinct mechanisms are likely to mediate protection from asthma and atopy. The biological significance of microbial diversity is still unclear, but multiple lines of evidence link the asthma-protective and allergy-protective effects of farming to immune responses and the microbiome. Work in mouse models is revealing novel cellular and molecular mechanisms through which the microbiota may modulate immune responses and allergic inflammation, and thus contribute to the farm effect. The role of the host's genetic makeup, on the contrary, remains poorly understood.

Summary

The discovery of the central role played by microbial diversity in the asthma-protective and allergy-protective effects of farming warrants metagenomic studies that concertedly and longitudinally investigate the microbiome, the genome, and the immune system of farmers and the farms they live on.

Keywords

asthma, atopy, farming, microbiome

INTRODUCTION

Over the last decade, multiple independent studies have shown that the prevalence of asthma and atopy is significantly reduced in children raised on traditional dairy farms in central Europe [1–3]. More recently, these observations have been extended to other regions of Europe and the world, including the USA and South America [4,5,6^{*}]. The consistency and robustness of the protective farm effect suggest that the characterization of the mechanisms underlying this effect may both illuminate fundamental facets of the pathogenesis of allergy and asthma and foster the development of effective preventive strategies. Here, we discuss the temporal constraints for the protective farm effect, the components of a farming environment that are associated with protection, and novel mechanisms that

may underlie protection from asthma and atopic disease in farming populations.

When: the importance of early exposure

Both early [2] and more recent [7^{**}] studies of the asthma-protective and allergy-protective effects of a farming lifestyle clearly show that protection is

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KEY POINTS

- Exposure to a farming environment prenatally or early in life protects against asthma and atopy.
- Contact with cows and straw and consumption of farm milk account for virtually all the protective farm effect for asthma but not atopy.
- The farm environment is characterized by high microbial diversity, which is strongly and inversely associated with asthma, but only weakly associated with atopy.
- Mouse models are offering clues about the cellular and molecular mechanisms that may underlie the link between the microbiome and the farm effect.

strongest when exposure occurs prenatally or within the first 2–3 years of life. These data point to a temporal window during early development that is critical to achieve protection. Which developmental processes are targeted by farm exposure during this window of opportunity is not fully understood, but the existing data suggest immune system development is likely to be a primary target. Because maturation of the immune system begins *in utero*, this process is potentially susceptible to modulation by endogenous or exogenous exposures during pregnancy. Indeed, expression of Toll-like receptor (TLR) 2, TLR4, and CD14 on blood cells is higher in farm than nonfarm children at school age [8], and exposure of the mother during pregnancy, rather than exposure of the infants after birth, appears to underpin this increase in innate immune receptor expression [9].

Other observations support an effect of prenatal farm exposure on the immune system. For instance, significantly higher levels of interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α) were secreted by mitogen-stimulated cord blood mononuclear cells from prenatally exposed farm children, compared with nonfarm children [10]. Moreover, maternal contact with different farm animal species and barns and consumption of farm-produced butter during pregnancy enhanced the production of proinflammatory cord blood cytokines [10]. Finally, cord blood CD4⁺CD25^{hi} T-regulatory cells from children born to stable-exposed mothers were both more abundant and more active. Allergen-induced levels of interleukin (IL)-5 were decreased and IL-6 levels were increased, whereas IL-17 secretion was unaffected. Most notably, maternal exposure to increasing numbers of farm animal species substantially enhanced the expression of the T-regulatory cell marker glucocorticoid-induced TNF receptor and the secretion of IFN- γ by cord blood cells in

response to allergen and peptidoglycan [11]. Collectively, these studies reinforce the idea that in-utero and neonatal exposures contribute to the farm effect by modulating several components of the immune response. It is noteworthy that the protective effects of early farm exposure can persist into adulthood. A recent study from Finland shows that contact with farm animals in early childhood reduces the risk of atopic sensitization, doctor-diagnosed asthma and allergic diseases at age 31 [12[■]], whereas a study from Sweden found a lifelong protective effect of childhood farm living on the prevalence of allergic rhinitis [13].

What: a suite of protective farm exposures

Farming is a complex lifestyle that involves multiple activities and diverse exposures. The protective effect that exposure to stables and consumption of unprocessed cow's milk independently have for asthma and atopy were identified already a decade ago [2]. However, deciphering the mechanisms underpinning protection requires further, deeper dissection of what in the farming environment confers protection. Studies of the protective effect of farming were fairly consistent with respect to hay fever and atopy, but results for asthma were quite heterogeneous, raising the possibility that not all farms are the same and specific farm characteristics may have greater effect than others. A recent study sought to disentangle the protective effects of distinct farm exposures by relying on a newly designed questionnaire focused not only on the farm's characteristics but also on the child's exposure [7[■]]. This study showed that protection was typically associated with traditional farms that engage in cattle breeding and cultivation of grain and corn for animal feeding and bedding. Moreover, and most remarkably, just three exposures (contact with cows and straw, and consumption of farm milk) were found to be sufficient to recapitulate the overall farm effect for asthma, whereas exposure to fodder storage rooms and manure explained protection for atopic dermatitis. In contrast, the protective effect of farming on hay fever and atopic sensitization was only partially explained by the exposures tested in this study, suggesting that additional, still unknown protective factors exist in a farming environment, and distinct mechanisms protect from asthma and atopy [7[■]].

If exposure to cows, straw, and unprocessed milk essentially accounts for the totality of the farm effect on asthma, what in those exposures induces protection? The notion that environmental microbes play a major role in protecting from asthma and allergy was suggested by early studies that showed association

between protection and a variety of microbial products such as endotoxin, muramic acid, and β -glucans [1,14,15]. These initial observations were confirmed and extended when the microbial diversity to which farm children are exposed was assessed. Dust samples from the mattresses and bedrooms of farm and non-farm children were screened for bacteria and fungi with culture-independent or culture-dependent techniques. Microbial (bacterial and fungal) diversity was significantly higher in farmhouse dust, and was strongly and inversely associated with the probability of an asthma diagnosis. Indeed, the microbial diversity score explained a significant proportion of the farm effect on asthma [16[■]]. In contrast, the association with atopy was weak, again suggesting that distinct mechanisms protect from atopy and asthma. The patterns of cultivable microflora in dust from the bedrooms of farm children were similar to those from animal sheds and barns, indicating that viable microorganisms are transported from animal sheds and barns into the child's house [17]. Therefore, children living on a farm are exposed both when visiting animal sheds and barns and when indoors, and the latter exposure may also contribute to the protective effect of farming. Of note, cowshed dust is rich not only in microbial products but also in plant-derived oligosaccharides, such as arabinogalactan, which protect from allergic airway inflammation in mouse models [18].

Although the farm dust studies discussed above emphasize the protective role of microbial diversity, the factors underlying the other major asthma-protective and allergy-protective exposure, unprocessed farm milk, remain partly elusive. A recent, large cross-sectional study showed that reduced risk of asthma and allergy is associated with early life consumption of unheated, unprocessed farm milk, whereas boiled milk conferred no protection [19[■]]. Contrary to expectations, though, no association was found between total viable bacterial counts (or specific subgroups) or total fat content and asthma or allergy. Moreover, a significant inverse association was found between levels of whey proteins (albumin, α -lactalbumin, β -lactoglobulin) and asthma but, again, not atopy. Because α -lactalbumin and β -lactoglobulin are major milk allergens, early life exposure to these proteins may decrease the risk of asthma by promoting early tolerance. Prospective epidemiologic and immunologic analyses and further dissection of farm milk components are required to better characterize these intriguing effects.

How: the (likely) role of the microbiome

The novel, strong association between asthma protection and high environmental microbial diversity

is perhaps the most suggestive clue that has emerged about the factors and mechanisms underpinning the farm effect [20]. Biologically, the significance of microbial diversity is by no means clear. As discussed recently [20,21], high diversity might reflect a balanced environmental colonization that promotes resilience (that is, the ability of the microbial community to withstand disturbance [22]) and prevents the overgrowth of potentially harmful bacteria, or might be a proxy for additive effects of distinct, independent exposures, or even for dose of exposure. What is puzzling in the concept of microbial diversity as a protective factor is that recognition of microbes by the innate immune system is thought to be canalized through a limited number of receptors that sense class-specific patterns rather than individual microbial species. Therefore, microbial diversity *per se* is unlikely to have a significant impact on the host's innate immune response.

Diversity of the farm microbiome, on the contrary, may well affect the host's microbiome. This possibility looms larger and larger [22,23]. The studies on the role of the microbiome in human health and disease are still in their infancy, largely because until recently, the diversity of microbes found in hosts and environments could be dissected only to a very limited extent. Next-generation technological advances now make it possible to sequence DNA from any source rapidly and in great depth. For microbiome studies, these advances imply that microbes can be identified through signature DNA sequences, bypassing the need for (and the uncertainties of) culture methods. As a result, about 100 times more microbes have become detectable in body niches and environmental samples.

Even at this early stage, a daunting complexity appears to characterize the microbiota. The human body harbors at least 100 trillion microbes that greatly outnumber human cells [24] and are extremely diverse in composition depending on body site, ethnicity, geographic locale, and other environmental influences [25]. Interestingly, inter-individual diversity over time is higher than intra-individual diversity, pointing to the existence of individual microbiota. In this novel, exciting scenario, the human body becomes a metaorganism that comprises both its own cells and the microbiota it coevolved with [26]. In most individuals, this coevolution ensures a peaceful coexistence. A complex network of host-microbe interactions ensures tolerance of commensals, protection against deleterious infections, and symbiotic metabolic interactions between the host and his/her microbiota. The characterization of a healthy, 'normal' metaorganism, with all its interindividual and temporal

variability, is a necessary prerequisite for understanding what deviations from normality mean for human disease pathogenesis.

Microbiome research is providing paradigm-shifting findings that may help explain the farm effect. Indeed, studies in mice demonstrate that commensal bacteria (particularly those residing in the gut, the most studied so far) act as gatekeepers of both immune development and immune function. For instance, polysaccharide A from the ubiquitous gut microorganism *Bacteroides fragilis* promotes systemic T-cell homeostasis, T-helper cell function, and lymphoid organogenesis [27]. These effects are directly mediated by the pattern-recognition receptor TLR2 expressed on Foxp3⁺ regulatory T cells [28²²].

Commensal bacteria are also critical for controlling allergic airway inflammation. Ovalbumin-sensitized germ-free mice exhibited exaggerated airway eosinophilia, increased local production of T helper 2 (Th2) cytokines, elevated immunoglobulin E (IgE) production, and an altered number and phenotype of dendritic cells, compared with specific pathogen-free mice [29]. This phenotype could be reversed by recolonization of germ-free mice with the complex commensal flora of specific pathogen-free mice. Interestingly, regulatory T cells were unaffected in germ-free mice, but the number of basophils was increased [29].

The intriguing link between the gut microbiota, allergic inflammation, and basophils was further highlighted by recent experiments in which commensal bacteria (particularly *Firmicutes* and *Bacteroidetes*) were deliberately altered by combined oral antibiotic treatment [30²²]. This led to elevated serum IgE concentrations, increased steady-state circulating basophil populations, and exaggerated basophil-mediated Th2 cell responses and allergic inflammation. Of note, commensal-derived signals influenced basophil development by limiting proliferation of bone marrow-resident precursor populations. This study therefore identified a previously unrecognized pathway through which commensals control basophil hematopoiesis and susceptibility to Th2 cytokine-dependent inflammation and allergic disease.

T-regulatory cells and basophils are not the only cell types affected by commensal bacteria in mouse models. Invariant natural killer T (iNKT) cells, which recognize endogenous and exogenous glycolipid antigens presented by CD1 and secrete large amounts of IL-4, IL-13, and IFN- γ upon activation, accumulate in the lung and colonic lamina propria in germ-free mice, resulting in increased susceptibility to allergic inflammation and inflammatory bowel disease [31²²]. Colonization of neonatal, but

not adult, germ-free mice with conventional microbiota protected the animals from mucosal iNKT accumulation and related disorders. These results indicate that age-sensitive contact with commensal microbes is critical for establishing mucosal iNKT cell tolerance to subsequent environmental exposures.

All of these results are tantalizing, even though it is still too soon to tell how directly relevant they are to the mechanisms underlying the farm effect in humans. The role of iNKT cells in human allergic disease remains controversial [32], and the study of human basophils is typically hindered by technical difficulties. Moreover, additional cell types (particularly, innate lymphoid cells residing at mucosal barriers [33]) are able to interact with commensal bacteria [34²¹] and may be involved in the farm effect. Nevertheless, to the extent that they reveal novel ways in which microbes may influence immune responses, these findings suggest provocative hypotheses that are worth pursuing. It is also interesting that distinct studies identify distinct cell types as targets of microbial influences. Although these results might be model specific, it is also true that farming environments harbor intense microbial diversity, with species that originate from animals, plants, and fodder. This diversity implies that multiple cell types and pathways may be responsible for modulating immune responses and conferring protection from allergy and asthma.

CONCLUSION

Multiple lines of evidence link the asthma-protective and allergy-protective effects of farming to immune responses and the microbiome. However, several critical questions need to be answered in order to better understand the farm effect. Most microbiome studies so far have focused on the gut, largely because of its accessibility. The lung is no longer considered as a sterile environment, and disordered microbial communities have been detected in asthmatic airways [35,36], but the relationship between the lung and gut microbiome and the impact of their composition on airway disease remain poorly understood. Exogenous influences, such as inhalation or ingestion of microorganisms, may modify the lung and/or gut microbiome, and farm-associated microbes may outcompete asthma-promoting microbes, thereby contributing to the farm effect. On the contrary, endogenous factors (the genetic makeup of the host first and foremost) may also play major roles in regulating microbial colonization, the immune response this elicits, and ultimately the farm effect. In this respect, a recent genome-wide association

study in the farming populations of central Europe failed to identify common genetic polymorphisms that modulate the protective effects of a farm environment on childhood asthma and atopy [37^{***}]. These negative results prompted the conclusion that the effects of farm exposure on asthma and atopy risk might be purely environmental. This is possible but in our view unlikely. As we extensively discussed elsewhere [38], gene–environment interactions are best detected when the relevant environmental exposure is clearly identified, accurately quantified, and firmly positioned within a robust biological framework. Because of the high diversity of the farm microbiome and the potential for distinct functional effects of individual microbial families, using ‘farming’ as a variable may not provide the specificity necessary for identifying true genetic associations. Therefore, these results should not be taken as conclusive evidence against the existence of gene–environment interactions in farmers but rather as an indication that making sense of the farm effect will require a new generation of metagenomic studies that concertedly and longitudinally investigate the microbiome, the genome and the immune system of farmers, and the farms they live on.

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Conflicts of interest

There are no conflicts of interest.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
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