

Protective effects of farming on allergies and asthma: have we learnt anything since 1873?

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“...one very curious circumstance in connection with hayfever is that the persons who are most subjected to the action of pollen belong to a class which furnishes the fewest cases of the disorder, namely, the farming class...”

In the past two decades, research on allergies and asthma has increasingly focused on factors that may ‘program’ the initial susceptibility to asthma [1]. This has also involved a shift of attention from risk factors to protective factors. Of particular relevance in this regard is the ‘hygiene hypothesis’, which postulates that growing up in an environment with moderate-to-high microbial exposures may protect against allergies and allergic asthma [2]. It has been hypothesized that the loss of these protective factors as the environment becomes more ‘hygienic’ may have contributed to the global increase in asthma prevalence [1], although the evidence is inconsistent in this regard [3].

A related issue is that a number of studies have found consistently low prevalences of allergies and asthma in farmers’ children, both in high-income countries such as Canada, the USA, Australia, New Zealand and Europe [4–20], and in low-income countries including Mongolia and Southern Africa [21,22]. These protective effects for allergies and asthma have also been observed in adult farmers [23–36], despite the increased risks of other respiratory conditions, such as chronic obstructive pulmonary disease, reduced lung function and farmers’ lung [37]. However, findings in adult farmers have been less consistent than those in children, with some studies suggesting that farming may in fact be a risk factor (rather than a protective factor) for asthma [38].

Although the reduced risk in farmers’ children was presented as a highly novel finding in 1999 in *Clinical and Experimental Allergy* [4], a lower prevalence of allergies (i.e., hayfever) among farming populations had already been reported in 1873 by Blackley:

“...One very curious circumstance in connection with hayfever is that the persons who are most subjected to the action of pollen belong to a class which furnishes the fewest cases of the disorder, namely, the farming class...” [39].

Blackley, who himself was a long-term hayfever sufferer, also predicted that allergic conditions such as hayfever would increase in the future:

“...As civilisation and education advance, the disorder will become more common than it is at the present time...” [39].

He could therefore be considered to have laid the foundation of what would later become the hygiene hypothesis.

“...a number of studies have found consistently low prevalences of allergies and asthma in farmers’ children...”

Thus, have we simply rediscovered what was already known (but had been forgotten), or have we actually learnt anything new since 1873? It could be argued that little progress has been made since Blackley’s original observations [40,41], but we think that such a pessimistic view is

unwarranted [42,43]. Recent studies on allergies and asthma in farmers may not yet have yielded a specific public health intervention for asthma, but they have made a major contribution in changing the focus from risk factors to protective factors, they have resulted in new etiological theories, inspired basic scientists to develop novel laboratory-based studies, and have provided new avenues of research with a high likelihood of eventually identifying novel prevention and treatment options. In brief, they have put asthma research 'back on track' after several decades in which the allergen hypothesis dominated the field, but produced relatively little in terms of new knowledge or effective interventions [44]. Later, we will highlight some of the most important new findings, theories and uncertainties arising from this research.

Protective exposures

The observed protective effects of farming on allergies and asthma have been particularly strong for animal contact [5,10,11,13,15,20]. Farm animals are associated with high exposures to microorganisms, in particular bacterial endotoxin [45]. For instance, endotoxin levels were significantly elevated in house dust in homes of farmers' children and in nonfarming homes where children had regular contact with farm animals [46]. In addition, an upregulation of several innate immune receptors specific for microbial products (e.g., Toll-like receptors [TLRs] and CD14; see later) has been shown in farmers' children [47], suggesting that microorganisms and microbial products may be involved. In fact, there is now a considerable body of evidence, derived both from experimental and human population studies, which suggests that repeated exposure to environmental microbial matter can influence overall immune status, and render it less susceptible to the development of asthma and allergy [48]. The evidence is strongest for bacterial endotoxin, with studies in both farming and nonfarming environments reporting a significant inverse association between indoor endotoxin levels and the prevalence of atopic sensitization [49], hayfever and atopic asthma [50] in children. Similarly, birth cohort studies in the general population have found a protective effect of endotoxin on atopy in 2-year-olds [51] and asthma symptoms in 4-year-olds [52], although an increased risk for asthma was found in 2-year-olds in another birth cohort study [53].

"...As civilisation and education advance, the disorder will become more common than it is at the present time..."

Other microbial agents have also been suggested to play a role, but the evidence is relatively limited. For example, a European study in farmers' and nonfarmers' children reported a protective effect of exposure to *N*-acetyl-muramic acid (a cell wall component of Gram-positive bacteria) on asthma symptoms independent of endotoxin exposure [54]. Other studies have provided (limited) evidence of a protective role of fungal exposures, DNA rich in non-methylated CpG dinucleotides, mycobacterial lipoglycans and fungal β (1–3) glucan [48,55].

Paradoxically, some of these same exposures (e.g., endotoxin) may, at very high levels, be risk factors for non-allergic asthma [45]; this may be related to the hypothesized U-shaped effect of endotoxin on the development of asthma (see later).

Consumption of unpasteurized farm milk in farmers' and nonfarmers' children has also been shown to be protective in several of the farmers' studies [10,14,17,56,57]. The etiological mechanisms are unclear, but probiotic bacteria or other currently unidentified non-microbial components in farm milk may play a role. Evidence from other populations with anthroposophic lifestyles, which are characterized by (among other things) diets rich in (probiotic) microbes [58], suggests that this protective effect may not be limited only to the farming environment; however, results have not always been consistent [59]. Similar to the recent 'rediscovery' of Blackley's observation of the protective farming environment, the idea that probiotic bacteria may affect the immune system and have beneficial effects on human health was first proposed more than a century ago by a contemporary of Blackley, the Russian Nobel-prize laureate Elias Metchnikoff [60]. Metchnikoff proposed that consumption of fermented milk may result in better health and an increased life span. Like Blackley's findings, Metchnikoff's hypothesis was based on observations in farmers – that is, he considered that the reason for the long life expectancy of Bulgarian farmers was their regular consumption of fermented milk.

Immunological mechanisms

It is believed that microbial exposure may – through innate immune responses – affect T lymphocytes, which have an important function in controlling adaptive immune responses. The initial interpretation was that microbial pressure may drive the response of the immune system – which is known to be skewed in an atopic Th2 direction during fetal and perinatal life – into a Th1 direction and away from its tendency to develop atopic immune responses [61]. More recently, an alternative interpretation has been offered, which involves a reduction in the capacity of T regulatory cells to effectively dampen an inappropriate immune response, resulting in a subsequent upregulation of both Th1 and Th2 immunity [62]. This hypothesis is also supported by epidemiological evidence showing an increase in some Th1-mediated diseases in parallel with Th2-mediated conditions, such as allergies and allergic asthma [63].

Programming of the antigen-specific T-cell response is controlled by innate immune responses, which are largely directed by antigen-presenting cells. Microbial exposures are believed to activate innate immune pathways through expression of TLRs and CD14 [47], suppressing Th2-cell expansion and the development of IgE-antibodies and Th2-dependent diseases, including allergic asthma, hayfever and eczema [50]. Evidence for this has been provided by several gene-association studies in farming populations. In particular, Eder *et al.* showed that genetic variation in TLR2 is a major determinant of asthma symptoms, atopic sensitization and hayfever in farmers' children [64]. This effect was not observed in nonfarmers' children. A subsequent study in farmers' children showed a significant dose–response association between maternal exposure to farm animals and

gene expression of TLR2, TLR4 and CD14 [47]. Two studies in adult farmers and young adults with a farming background showed that the *CD14/-260T* (also reported in the literature as *CD14/-159T*) allele was inversely associated with atopy [65,66], whereas the *CD14/-651T* allele was positively associated with atopy [66]. The associations were strongest in those farmers who were born on the farm or lived on a farm for the first year of life [65,66], suggesting an interaction with timing of exposure. In farmers' children, a bimodal effect of *CD14/-260* on atopy was shown, with the direction of the effect being dependent on the type and level of environmental exposures (i.e., pets, livestock and endotoxin) [64]. Indeed, it has been suggested that either genotype has the capacity to be beneficial or detrimental in terms of atopy risk, depending upon the nature of the exposure [67]. Another polymorphism in the *NOD1/CARD4* gene, which encodes an alternative intracellular receptor involved in the innate recognition of microbial components, shows a similar protective effect against allergy for T homozygosity [68]. To add a further level of complexity, a recent study has found that polymorphisms in the neuropeptide S-receptor gene can modify the relationship between allergic symptoms and farm animal contact [69]. Protective effects were particularly apparent with exposure in infancy, and diverged with subsequent exposures and different genotypes.

Thus, although not always consistent, these findings strongly suggest a key role for innate immune pathways in the protective effects of farming on allergies and asthma.

A U-shaped response?

Although the emphasis of this paper is on the protective effects of farming and microbial exposures, it is well known – as noted previously – that these same exposures can cause or exacerbate respiratory disease including non-allergic asthma, at least in adult farmers. How can this be explained?

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It may be that early microbial exposures protect against allergies and asthma, whereas exposure later in life may be a risk factor. The aforementioned study by Ege *et al.* in farmers' children showed that both atopic sensitization and gene expression of TLR2, TLR4 and CD14 were strongly associated with prenatal exposures [47]. Similarly, we showed that exposure of the mother to farm animals during pregnancy was strongly associated with a subsequent reduced prevalence of hayfever, asthma and eczema [20]. The importance of prenatal exposures has also been confirmed in animal models of asthma [70]. In addition, several studies have shown inverse associations between endotoxin exposure in the first year of life and asthma symptoms later in childhood [52]. On the other hand, there is a wealth of data showing that occupational exposures to endotoxin later in life may cause asthma [45]. These observations suggest that timing of exposure may be important, as has also been suggested by some

animal studies [71]. However, although it has been suggested that protective effects should primarily arise from exposures during the first years of life, little is known as to whether this period is critical and/or whether later exposures may also play a role. In fact, recent studies among adult farmers suggest that protection against allergy and allergic asthma may continue into adulthood [23,29,72], and that long-term continual exposure may be required to maintain optimal protection [30–32]. It has also been shown that farm exposures may reduce the risk of asthma and hayfever even in those farmers who were not exposed during childhood [32,73]. Thus, timing of exposure may play a role, but the evidence is mixed, and the assumption that protective effects only occur in early life and adverse effects only in later life may be too simplistic.

“...long-term continual exposure may be required to maintain optimal protection...”

The varying effects of different levels of exposure on different asthma phenotypes (i.e., allergic and non-allergic asthma) may also explain the U-shaped effect. In particular, the evidence for a role of endotoxin in protecting against allergies and asthma is mostly based on exposures measured in the home environment. Although exposure to endotoxin may be higher in farmers' children compared with other children [46], the levels of exposure are still generally orders of magnitude lower than are commonly observed in many occupational environments where endotoxin has been demonstrated to be a risk factor for non-allergic asthma. Thus, the protective effects of endotoxin have been demonstrated mostly at relatively low exposure levels, whereas evidence that endotoxin is a risk factor has predominantly involved relatively high exposure levels. Therefore, it may be that modest stimulation of the innate immune system facilitates protection against allergies and allergic (or eosinophilic) asthma, whereas stronger stimulation may produce an exaggerated innate immune response, thereby resulting in non-allergic (or non-eosinophilic) asthma. The protective effects on Th2-mediated atopic responses may still be evident at higher doses, but these may be masked by increased expression of Th1-mediated non-allergic asthma. In fact, it is well known that endotoxin – through TLR4 and CD14 pathways – can trigger Th1-mediated proinflammatory responses involving NF- κ B activation, chemokine production (IL-8, IL-6, IL-1 and TNF- α) and subsequent neutrophilic (non-eosinophilic) airway obstruction. As discussed previously, endotoxin may at the same time – through the same innate immune responses – inhibit IgE production by reducing the Th2 pathway of the acquired immune system (see hypothesized pathways in FIGURE 1). Some evidence for this hypothesis was found in a study of highly exposed adult farmers in Norway, where livestock farmers were less likely to have atopic asthma, but had an increased risk for non-atopic asthma [23].

Thus, both timing and level of exposure may explain the observed U-shaped response, but the specific response may partly also be due to the genotypes of the population exposed.

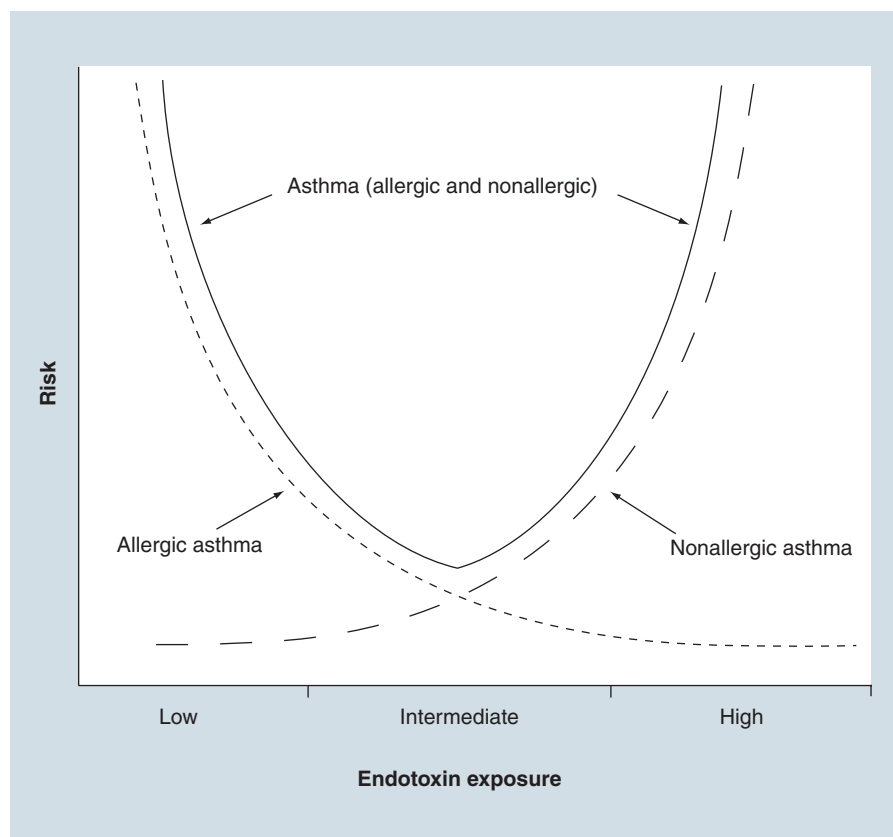


Figure 1. Endotoxin exposure and risk of asthma.

Where to from here?

So where to from here? Raising children on a farm, having them drink raw farm milk or exposing them to endotoxin to protect them from developing allergies and asthma are not feasible options for primary prevention. Nevertheless, the farm is a 'natural' environment in which a considerable proportion of the world's population currently lives, by contrast, for example, with 'allergen-free' environments, which are expensive to produce and difficult to maintain in the long term. Thus, studies of farming families have considerable potential to lead to realistic and effective interventions, even if their specific nature is unclear at this stage. The recent findings on the immunological underpinnings of the protective effects of farming on allergies and asthma, as well as the identification of potentially protective exposures, have provided considerable insights in the disease process, and have opened up new avenues for research into novel intervention strategies. Previous approaches to developing therapeutic interventions have largely been based on data derived from animal models of allergic airway disease, and have predominantly targeted Th2 cytokines such as IL-4 and IL-5. These approaches have, however, shown little capability to ameliorate airway hyper-reactivity and the late asthmatic response [74]. Studies of farmers have highlighted the importance of the innate immune system in programming the initial susceptibility to allergies and asthma, indicating that therapeutic interventions may best be targeted to aspects of the innate immune system, particularly those involving TLR and CD14 pathways. In

fact, the use of a range of TLR agonists as therapeutic agents for a number of allergic conditions is now either at the preclinical development stage, clinical trial level or is currently in use as an adjuvant in short-course immunotherapy [75,76], and we expect more studies to follow in the next few years.

Studies of farmers have also emphasized the immune-modulating characteristics of particular microbial agents, which can be used to our advantage provided we find ways to separate the protective (inhibition of Th2 immune responses) from the hazardous (over-stimulation of Th1 immune responses and direct toxic reactions) properties. However, before we can start applying this new knowledge in developing potential vaccines or other preventive measures (e.g., probiotics), we first need to further elucidate which specific microbial (and non-microbial) farm-related factors are causally associated with the protective effects. One area of particular interest is farm milk consumption. As promoted by Metchnikoff already more than a century ago, administration of milk with living microorganisms may have the potential to alter the resident intestinal microflora with

beneficial health effects. More recent studies suggest that these beneficial effects may include the prevention and/or treatment of atopic conditions [77], although the evidence for asthma is currently lacking. However, only a limited number of the components unique to the farming environment have been studied to date, and these have involved data obtained using convenient markers of environmental exposure, which do not fully reflect microbial diversity. As Blackley did with his rooftop pollen counter, we must break new ground in developing methods to more validly and completely assess the nature of the protective farming environment.

"...we must break new ground in developing methods to more validly and completely assess the nature of the protective farming environment."

Current birth cohort studies in farmers' and non-farmers' infants in Europe and New Zealand [78] focusing on pre- and post-natal exposures and early-innate (TLRs and CD14) and adaptive (Th1/Th2 and T regulatory) immune responses are ideally suited to further elucidate the complex etiology of allergies and asthma, guiding the development of effective intervention strategies. Based on the aforementioned current data, it appears that these strategies may be most effective if implemented early in life, possibly even before birth, but interventions may have to be repeated throughout life to maintain a sufficient level of protection. However, given the

complex gene–environment interactions and the diverse asthma phenotypes, successful interventions are unlikely to involve a ‘one size fits-all’ approach.

In summary, recent studies of farming families have substantially increased our understanding of the underlying etiological processes involved in the development of asthma. The current lack of feasible and effective primary prevention measures, or therapeutic interventions, is disappointing considering the considerable amount of time (more than a century) that has passed between Blackley’s and Metchnikoff’s observations and the studies described in this paper. This apparent lack of progress is, to a large degree, due to the fact that Blackley’s initial findings were largely forgotten until the 1990s, when the hygiene hypothesis was ‘rediscovered’. The first study to confirm Blackley’s finding

(despite substantial social and lifestyle changes both on and off the farm since Blackley’s observation) was conducted in the late 1990s and substantial progress has been made since. Perhaps this is an important lesson not to ignore the older literature, which may still have relevance today. Who knows what else is out there waiting to be rediscovered?

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