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Are Children Who Grow Up on Traditional Farms Protected From Asthma, Allergic Rhinitis and Allergic Sensitization?

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Authors' contributions

This work was carried out in collaboration between both authors. Author FK conceptualized the idea, supervised the research and drafted the final manuscript. Author TRD managed the literature searches and produced the initial draft. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Epidemiological studies have revealed that children who grow up on traditional farms are protected from asthma, hay fever and allergic sensitization. It has been speculated that allergic diseases may replace infectious diseases in developing countries and should be prioritized and treated as a major public health problem. Exposure to farming materials at early life such livestock, mostly cattle, pigs and poultry; contact with animal feed such as hay, grain, straw and silage; and the consumption of unprocessed cow's milk have been identified as most farm protective elements. This review discusses the evidences supporting the claim that children from rural areas who grow up on farms are at a significantly lower risk of developing these conditions than children who live in the same rural area but do not grow up on farms.

Keywords: Atopic dermatitis; asthma; hay fever; allergic sensitization; endotoxin.

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1. INTRODUCTION

Asthma, hay fever, atopic dermatitis and allergic sensitization are more prevalent in the developed countries than in developing ones. Literature available suggests that the prevalence of these conditions has increased over the last decades [1]. It is estimated that 4-12% of the world's population suffers from these allergic diseases [2]. Approximately 20% of the population in developing nations is battling with these diseases of which 18% are asthmatic [2]. Reports from European countries comparing rates of childhood asthma and hay fever in urban and rural areas have been inconclusive [2], however, significant differences in the prevalence of childhood asthma, hay fever and atopic sensitization has been recorded in rural settings. Children living in rural areas and growing up on farms are at a lower risk of developing these conditions than children who live in the same rural area but not growing up on farms [3]. This form of protective farm effect is observed for both the atopic and non-atopic phenotype of childhood asthma and has been shown to be sustained into adult life [3]. In addition, farmers may also grow grass, corn and grain and may also store the fodder whilst housing people and animals in close proximity under one roof. Most farms in these areas are non-industrialized and family-run. It is worth noting that women in these communities are involved in stable and barn work before, during and after pregnancy. Subsequently, their children as young as a few days are taken into stables so that their mothers can take care of them while working. Hence, most farm children will have been exposed to stable and barn environments up to entry into lower primary school levels and many will have been exposed continuously until early adolescence and beyond.

Although researchers, scientists and clinicians are studying the role of genetics and environmental factors of these allergic diseases, the etiology of these diseases remains unknown. Allergic diseases are now a public health concern both in developing and developed countries. Environmental factors associated with allergic diseases, difficulties in diagnosis, challenges of complex treatments requirements and improved quality of life require further investigation and understanding to reduce the morbidity and burden of illness for children and their families afflicted with allergic disease. The review takes a critical look at some of the evidences that support the claim that children who grow up on traditional farms are protected from asthma, hay fever and allergic sensitization.

2. FARM EXPOSURES CONTRIBUTE TO ALLERGY RESISTANCE

Studies that investigated childhood farm exposures have been done in Austria (Tables 1 and 2) and Germany where, traditionally, farming has been the major source of livelihood. Here, the farms focus in dairy production and may also keep other animals such as horses, pigs and poultry. The two findings from Austria (Tables 1 and 2) are in agreement, depicting on a lower prevalence of hay fever and allergic sensitization by skin prick test in children whose parents were famers. Similarly, in a study from Germany, 5-6-year-old farmers' children had a lower risk to hay fever and asthma than peers from non-farming families (data not shown).

Studies have identified some of the exposures associated with a farming lifestyle that contribute to the reduced risk of asthma and allergies in farm children. These include contact with livestock, mostly cattle, pigs and poultry; contact with animal feed such as hay, grain, straw and silage; and the consumption of unprocessed cow's milk [4].

These exposures to a large extent had an independent protective farm effect, thereby implicating inhalation and ingestion as two main routes of exposure. Also, variations in lifestyle such as duration of breast feeding, family and sibship size, day care, pet ownership, dietary habits, parental education and a family history of asthma and allergies, did not account for the protective farm effect [5]. The timing of this exposure remains critical, having the strongest effects observed for exposures that occurred *in utero* and during the first few years of life [5]. Maternal contact with animals and their continuous engagement in barns and stables and the consumption of unprocessed cow's milk during pregnancy offered the most relevant protective exposures [6]. In New Zealand for instance, a study showed that the continuous exposure to farm animals and hay and other cereals from pregnancy to school age produced the strongest protection [6].

2.1 Unprocessed Cow's Milk

In Europe for instance, it is strongly discouraged to consume raw cow's milk since there has been

reports of disease outbreaks from exposure to pathogenic bacteria in unpasteurized milk. Despite this prohibition, dairy farming families still use unprocessed milk, including expectant mothers and infants. Several studies (Table 3) have shown a protective effect of unprocessed milk on the development of asthma, hay fever, allergic sensitization and atopic dermatitis [7].

In most instances, the cow's milk used for commercial purposes are pasteurized and homogenized. Consequently, pasteurization is achieved by heating for a short period to significantly reduce the level of microorganisms in the milk.

It is important to note that homogenization reduces the fat globule size which in turn increases the milk fat surface area and consequently alters the original milk fat globule membrane (MFGM) since the MFGM is insufficient to cover the fat surfaces [9]. In the process, there is adsorption of casein and lactoglobulins, which are the main allergens in cow's milk. Hence, both the pasteurization and homogenization of cow's milk might abolish the asthma- and allergy-protective effects.

2.2 Microbial Exposures

In addition to plant material from grass, grain and corn, a variety of bacteria, fungi and their compounds are also common in animal shed [9]. Research has shown that the exposure to grass pollen and water-soluble polysaccharides and arabinogalactans, is concentrated in cowsheds, when cattle is being fed with grass and hay. It has been established that the levels of these pollen and polysaccharides exceed outdoor concentrations and children are exposed continuously. Children also bring their microbial exposures into the indoor environment, where microorganisms and their compounds settle in floor and mattress dust [10]. Hence, mattress dust can be regarded as a reservoir that reflects an individual's long-term microbial exposure in indoor and outdoor environments.

Table 1. Prevalence of hay fever, asthma and eczema in children living on a farm compared with children from non farming-environment (Adapted from Reidler et al. [3])

	Prevalence (%)		P-value
	Living on a farm (n=282)	Not living on a farm (n=1710)	
Hay fever (ever)	3.1 (8/261)	10.3 (166/1614)	0.0002
Runny nose and itchy eyes last 12months	4.1 (11/268)	7.8 (131/1677)	0.03
Asthma (ever)	1.1 (3/278)	3.9 (66/1693)	0.017
Asthmatic, obstructive, spastic bronchitis	10.3 (29/282)	15.2 (260/1710)	0.029
Wheeze last 12months	4.7 (13/278)	7.5 (128/1701)	0.087
More than 4 asthma attacks last 12 months	0.7 (2/278)	0.8 (13/1697)	0.377
Eczema (ever)	11.4 (31/273)	10.9 (183/1678)	0.826
Itchy rash (ever)	9.1 (25/276)	11.0 (186/1693)	0.337

Table 2. Prevalence of allergic sensitization (Skin prick test) to 7 common local allergens in children living on a farm compared with children from a non-farming environment (Adapted from Reidler et al. [3])

	Prevalence (%)		P-value
	Living on a farm (n=138)	Not living on a farm (n=868)	
At least one positive reaction	18.8	32.7	0.001
Timothy grass	7.2	21.4	0.00009
Birch pollen	0.7	8.3	0.001
Dermatophagoides pteronyssinus	12.3	15.8	0.294
Dermatophagoides farina	8.0	10.6	0.344
Cat fur	9.4	12.2	0.345
Alternaria tenuis	0	0.1	0.689
Cladosporium herbarum	0	0.3	0.489

Table 3. Overview of epidemiological studies assessing the effect of farm milk consumption on asthma and allergic disease (Adapted from Braun and von Mutious, [8])

Authors	Study population	Countries	Exposure	Main results - OR (CI95)
Riedler et al. 2001	Rural farm and non-farm children (n=812), aged 6-12 years (ALEX study)	Austria, Germany, Switzerland	Milk directly produced or purchased on a farm	Consumption of farm milk during first year of life significantly inversely associated with asthma, hay fever, and atopy, independent of other farm exposures
Waser et al. 2007 [7]	Rural farm and non-farm, Steiner Schools', and peri-urban children (n = 14 893) aged 5–13 years, (PARSIFAL-Study)	Sweden, Netherlands, Austria, Germany, Switzerland	Milk directly produced or purchased on a farm	Adj. OR and (95% CI) of farm milk consumption ever in life and asthma: 0.47 (0.61–0.88), rhinoconjunctivitis: 0.56 (0.43–0.73), sensitization to pollen: 0.67 (0.47–0.96), and food mix: 0.42 (0.19–0.92). Association observed in all subgroups, independent of farm-related co-exposures
Bieli et al. 2007 [7]	ALEX (n = 576) and PARSIFAL (n = 1478) children with available DNA samples	Sweden, Netherlands, Austria, Germany, Switzerland	Milk directly produced or purchased on a farm	Association between farm milk and asthma varied between genotypes of CD14/-1721. Adj. OR (95%CI) AA: 0.81 (0.07–0.47); AG: 0.47 (0.26–0.86); and GG: 0.98(0.46–2.08). Similar patterns for symptoms of hay fever and pollen sensitization.
Perkin and Strachan, 2006 [9]	Rural farm and non-farm children (n=4767), subsample (n = 879) with skin prick test	England	Unpasteurized milk (based on food frequency questionnaire)	Current unpasteurized milk consumption associated with less eczema adj. OR and (95% CI): 0.59 (0.40–0.87) and atopy: 0.42 (0.10–0.53), and higher production of whole blood stimulated IFN-g. Effect independent of farming status. No effect on asthma
Barnes et al. 2001	Rural farm and non-farm and urban children aged 11-19 years (n=929)	Crete (Greece)	Unpasteurized milk products	Adj. OR and (95% CI) of atopy and unpasteurized farm milk consumption with and without simultaneous farm animal contact: 0.32 (0.13–0.78) and 0.58 (0.34–0.98), respectively
Radon et al. 2004	Rural farm and non-farm young adults aged 18-44 years (n=321)	Northern Germany	Raw, unboiled farm milk	Raw milk consumption and atopy adj. OR and (95% CI): 0.65 (0.36–1.18). In those visiting animal houses before age 7 years raw milk consumption and atopy: 0.35 (0.17–0.74)5
Wickens et al. 2002	Children living on farms or in small towns aged 7-10 years (n=293)	New Zealand	Unpasteurized milk ever, yogurt at least weekly before age 2 years	Adj. OR and (95% CI) for early yogurt consumption and hay fever 0.30 (0.1– 0.7); any unpasteurized milk and atopic eczema: 0.2 (0.1–0.8). No significant association between unpasteurized milk consumption and asthma or atopy
Remes et al. 2003	Rural farm and non-farm children aged 6-15 years (n=710)	Finland	Farm milk in infancy	Farm milk consumption not associated with atopy. No other allergic health outcomes reported
Ege et al. 2008 [1]	922 farm and non-farm children, followed since pregnancy (PASTURE study)	Finland, France, Austria, Germany, Switzerland	Maternal consumption of boiled and unboiled farm milk during pregnancy	Maternal consumption of farm milk during pregnancy not related to IgE to seasonal allergens in cord blood of neonates. Boiled farm milk consumption during pregnancy positively associated with specific IgE to cow's milk: adj. OR and (95% CI): 1.78 (1.08–2.93)
Pfefferle et al. 2010 [6]	PASTURE study	Finland, France, Austria, Germany, Switzerland	Skimmed and unskimmed farm milk, farm produced butter and yogurt during pregnancy	Maternal consumption of farm produced butter during pregnancy associated with increased IFN-g and TNF-a production in cord blood, farm produced yogurt inversely associated with these cytokines

A number of studies have attempted to address the health effects of microbial exposures by measuring the markers of bacterial and fungal exposures in mattress dust. Information available suggest that endotoxin levels, which is a component of Gram negative bacteria cell wall, have been inversely related to allergic sensitization but positively related to asthma and wheeze [11]. Again, muramic acid which is a cell-wall component of all bacteria has been shown to have strong inverse relationships with childhood asthma and wheeze [12]. Extracellular polysaccharides derived from *Penicillium* spp. and *Aspergillus* spp. are secreted during growth of these fungi and their presence has been observed to be inversely related to asthma and wheeze [4]. It has not been established as to whether the diversity, dose and exposure only to certain microorganisms account for these protective effects. It is suggested that new metagenomics approaches to assess bacteria and fungi independently of culture methods will help clarify these questions in the future.

The molecular mechanisms by which endotoxin exposure may protect against the development of atopic immune responses and diseases are not fully understood [13]. The time a child starts school, environmental exposure to endotoxin levels might have been at its peak and consequently might have resulted in a marked suppression of the capacity for cytokine production in response to activation of the innate immune system [13]. Since lipopolysaccharide stimulation triggers an innate immune response by activating mainly antigen-presenting cells, staphylococcal enterotoxin B also activates T cells thereby resulting in a varied pattern of cytokine production [14]. The reduction in responsiveness to stimulation with lipopolysaccharide after previous stimulation with lipopolysaccharide is a phenomenon referred to as lipopolysaccharide tolerance [15]. Other findings suggest that such a down-regulation occurs *in vivo* as a consequence of long-term exposure to environmental endotoxin.

Natural immune response has an instructive role in adaptive immunity [16], Variations in expression of lipopolysaccharide receptors in children from farming and non-farming households have recently been reported, [15] an indication that the innate immune system responds to the high microbial burden of the farming environment. In a recent study, although only current endotoxin exposure was recorded, the levels are an indicative of long-term exposure; hence long-term, high-level environmental exposure may favor a state of tolerance, [14] which may prevent the development of allergic immune responses. It has been demonstrated that exposure during the first year of life to stables and farming activities that are likely to reflect exposure to microbial products have a strong protective effect against the occurrence of asthma and atopy at school age [15]. However, independent of and in addition to this effect, endotoxin exposure at school age was associated with a markedly decreased risk of atopic outcomes. This protective effect has been observed in children with no exposure to farming whose mattress endotoxin levels are similar to levels found in urban homes in the Netherlands [16] and urban areas in the United States [17]. This shows that the exposure to ubiquitous microbial products strongly affects the development of atopy and childhood asthma.

The protective effect as a result of endotoxin exposure at school age was observed for both atopic wheeze and asthma and not for non-atopic wheeze [17]. Childhood asthma may be characterized by multiple symptoms. These include wheezing that develop during the infant, toddler, school-age, and adolescent years in a survey that monitored children from birth to adolescence and adulthood [18]. Although, in many cases, asthma is associated with atopic sensitization to a variety of allergens, illnesses involving wheezing also occur in the absence of increased IgE responses. Differences in genetics, environmental factors, and the interplay among them are likely to account for the varying clinical presentations of wheeze. In studies of human exposure [19] and in studies of animals [20], endotoxin has been shown to induce airway hyper-responsiveness in healthy, non-atopic subjects but to decrease airway responsiveness in sensitized animals, supporting the notion that the effect is modified by atopy.

Park et al. [21] measured the endotoxin levels in mattress dust, since children come into close contact with the microbial environment of their beds while sleeping and since the reproducibility of repeated endotoxin measurements is greater for dust from the bed than for dust from the floor. Endotoxin measurements in dust from the bed have been reported to show little variation over time, with non-significant differences over a six-month period. Environmental endotoxin levels are therefore likely to reflect longer-term exposure to microbial compounds [21].

Other bacterial components, such as non-methylated cytidine phosphate guanosine dinucleotides specific for prokaryotic DNA (CpG motifs) or cell-wall components from a typical mycobacteria or Gram-positive bacteria such as lipoteichoic acid are known to affect immune responses in ways similar to that of endotoxin [22]. Mechanisms relating to the recognition of these microbial compounds by the innate immune system and the regulation of the resulting inflammatory responses through adaptive immunity are likely to be of key importance for the development of atopic illnesses such as hay fever and childhood asthma and wheeze. These revelations may enhance the generation of novel strategies aimed at the prevention of these diseases.

2.3 Innate Immunity at School Age

Study on human environments may yield cellular and molecular characteristics of a given exposure that may specify the pathways that are targeted by that exposure *in vivo*. Regarding farming, research was initially guided by the hypothesis that the innate immune system senses the signals delivered by the high microbial burden associated with farming and transmits these signals to the adaptive immune system. An analysis in school children enrolled in the allergy and endotoxin (ALEX) study showed that peripheral blood cells from farm children expressed significantly higher levels of *CD14* and Toll-like receptor 2 (*TLR2*) mRNA than cells from non-farm children [23].

The epidemiological evidence strongly indicate that the protective effects of farm living occur prenatally and in early life and the association between farm-living and pattern-recognition receptor (PRR) expression. This evidence was re-evaluated in the prevention of allergy, risk factors for sensitization related to farming and anthroposophic lifestyle [1]. The outcome did not only confirm the increase in *CD14* and *TLR2* expression among farm children, but also showed an increase in Toll-like receptor 4 (*TLR4*) in these children and indicated that exposure of pregnant mothers to stables, rather than exposure of their infants during childhood, was associated with elevated pattern recognition receptor (PRR) expression. It is interesting to note that perhaps even more suggestive was the detection of a dose-response relationship in the association between the number of farm animal species encountered by the mother during pregnancy and the levels of *TLR2*, *TLR4* and *CD14* mRNA expressions in the child's peripheral blood cells at school age [1]. In totality, these studies indicated that early life exposure to the rich microbial environment of traditional farms induces an upregulation of innate immunity receptors that is both robust and long-lasting.

2.4 Adaptive Immunity at School Age

The immunoregulatory effects of farming are not confined to innate immunity. A recent study investigated the effect of farm exposure on allergen-induced class-switch recombination. Immunoglobulin E (IgE) and Immunoglobulin G (IgG) response to major inhalant allergens such as grass, cat hair and house dust mites were evaluated in school children enrolled in the ALEX study [24]. The study revealed unexpected complexities in the effects of farm exposure on antibody production. However, the prevalence of IgE specific for house dust mites was slightly, but significantly, increased among farm children. The mechanisms underlying these responses need further clarification, but the finding that the protective effects of farm exposure are specific to certain allergens and immunoglobulin isotypes indicates that distinct allergenic entities trigger distinct response pathways, which differentially interact with farm-derived protective agents [24].

2.5 Neonatal Immune Responses

The immunological analyses initially performed in school-age farm children have been extended to newborn babies to explore the contribution of prenatal exposures to the asthma-protective effects of farming. The protection against allergy: study in rural environments (PASTURE) birth cohort study was designed to evaluate the effects of maternal farm-related exposures during pregnancy on IgE responses in the offspring [6]. It has been revealed that seasonal allergen-specific IgE responses are prevalent in cord blood from infants whose mothers had not been exposed to animal sheds and grass, and were strongly associated with reduced production of the T helper 1 (T_H1) cell-associated cytokine interferon-γ (IFNγ) by cord blood cells after stimulation with phorbol 12-myristate 13-acetate (PMA) plus ionophore [6]. Here, significantly higher levels of IFNγ and tumour necrosis factor (TNF) were secreted by cord blood mononuclear cells from farm infants compared with non-farm infants, whereas the T helper 2 (T_H2) cell associated cytokine interleukin-5 (IL-5), the regulatory cytokine interleukin-10 (IL-10) and the T_H1-inducing cytokine interleukin-12 (IL-12) were

unaffected [6]. Clearly, maternal association with multiple animal species and barns during pregnancy enhanced the production of TNF and IFNγ by infants and the consumption of butter made from unprocessed milk during pregnancy also had striking positive effects on TNF and IFNγ production by newborns [6]. However, these results confirmed that maternal exposure to farming activities during pregnancy has a profound effect on the cytokine-producing capacity of the offspring at birth.

Recent immunological analyses of an additional birth cohort confirmed and extended these findings by exploring the hypothesis that the allergy-protective effects seen in children of mothers exposed to a farm environment during pregnancy may involve regulatory T (T_{Reg}) cell activation. Indeed, cord blood $CD4^{+}CD25^{hi}$ T_{Reg} cells from children born to stable-exposed mothers were both more numerous and more efficient in suppressing T cell proliferation [25]. In addition, allergen-induced levels of IL-5 were decreased and IL-6 levels were increased, whereas IL-17 secretion was unaffected [6]. Arguably, maternal exposure to increasing numbers of farm animal species substantially enhanced the expression of the T_{Reg} cell marker glucocorticoid- induced TNF receptor (GITR) and the secretion of IFNγ by cord blood cells in response to allergen and peptidoglycan [25]. Although the population sample size was small and the work essentially exploratory, these observations confirm the intriguing relationship between immunomodulation and number of animal species to which mothers are exposed during pregnancy, and highlight the potential role of IFNγ as a key mediator of the farm effect [25].

3. HYPOTHETICAL MODELS FOR IMMUNOBIOLOGY OF FARMING

Even though the studies discussed above are diverse in their execution, they lack a prescribed working model of the immunobiology of farming. It is however important to note that in such models, the timing of exposure is crucial. Literature available suggests that pregnancy and early life represent a biological window of opportunity for shaping subsequent immune reactivity and contact with multiple animal species during pregnancy is positively associated with T_{Reg} cell activity and production IFNγ at birth and with expression of innate immune receptors during childhood [26]. Here, the extreme biological diversity of a traditional farm environment and particularly the elevated numbers of animal species that typically live on those farms are likely to result in a microbial pressure that may have few equals in the western world. Rich and diverse microbial burden function through the innate immune system and the secretion of the T_{Reg} cell-promoting cytokine TNF [26] to direct vigorous T_{Reg} cell activation and expansion. These in turn, balance adaptive immune responses and dampen allergen induced, T_H2 cell-associated cytokine production and T_H2 cell-dependent IgE production. In this model, several key effector mechanisms of allergic inflammation are inhibited by the immunoregulatory properties of farm-associated microbial exposures (Fig. 1). It has been shown that a decrease in IL-4 and IL-13 expression levels decreases IgE class switching and relieves the T_H2 cytokine-dependent inhibition of CD14 expression in one study [27]. This leads to further enhancement of PRR expression and amplification of innate immune responsiveness, which in turn favours non-T_H2-type immune responses. Therefore, maternal exposure to farm animals represent a model of natural immunotherapy in which delivery of a strong innate immune stimulation at the time of initial allergen exposure activates regulatory networks that confer a long-lasting balance to the child's immune responsiveness [25].

It is interesting to note that an increase in PRR expression detected in school age farm children testifies to the persistence of the immunological effect of early farm exposure. Evidence available shows that IFNγ is central to this model because this cytokine functions as a master regulator of allergy and asthma risk. Low IFNγ expression levels at birth are known to be associated with an increased risk for the later development of allergic symptoms and atopic disease [28] and low IFNγ in the first year of life is a strong predictor of airway obstruction during childhood.

Therefore, the ability of maternal farm exposure to increase IFNγ expression during the critical time at which a child's immune system is programmed may be essential for the allergy-protective effects of farming later in life [28]. This raises an important question: what are the mechanisms underlying IFNγ upregulation in newborns of mothers exposed to multiple farm animal species? This question cannot be definitively answered until the cellular sources of neonatal IFNγ are identified although the existing data are compatible with several hypotheses. For example, the association between farming and IFNγ upregulation has been proposed to reflect

the restoration of a missing immune deviation; that is, the shifting of allergen specific responses from the T_H2 to the T_H1 phenotype owing to microorganism-dependent induction of a delta–Notch-mediated T_H1 cell-polarizing programme in dendritic cells [28]. Indeed, incubation of human adult monocyte- derived dendritic cells with cowshed-derived bacteria (*Acinetobacter lwoffii* F78 or *Lactococcus lactis*) enhanced the secretion of the T_H1-inducing cytokine IL-12 and *Delta4* mRNA expression [30]. Alternatively, microbial products may reduce DNA methylation of the *IFNG* gene in naive T cells, thereby leading to increased IFNγ expression [28]. Studies have shown that human adaptive immune responses (of both the T_H1 and the T_H2 type) are typically immature and suppressed at birth. This led to the proposal that increased production of IFNγ in neonates born to mothers exposed to multiple farm animal species may rely primarily on innate immune mechanisms [31]. In this respect, we are intrigued by the possibility that the unusual abundance of microbial products resulting from contact with several animal species may trigger TLR-expressing natural killer (NK) cells to release IFNγ. Moreover, contact with multiple animal species may generate an intense biological diversity that leads to a constant, robust xenogeneic pressure on pregnant mothers exposed to a farm environment. NK cells, an essential barrier to xenogeneic influences, could have a pivotal role in responding to this pressure through the production of IFNγ [32].

Regardless of its cellular source and mechanisms of induction, high levels of IFNγ at birth can directly counteract allergen induced T_H2 cell differentiation and activate high levels of IL-12 production by dendritic cells, thereby promoting an accelerated maturation of T_H1-type immune responses [32]. These immune responses would provide enhanced protection against intracellular pathogens, especially respiratory viruses that persistently alter immune responses and airway function in susceptible subjects and increase the risk of developing asthma, particularly in atopic children. Future analyses of the cellular, genetic and epigenetic mechanisms of IFNγ regulation at birth and in early life will clarify these fundamental aspects of the immunobiology of farm exposure [32].

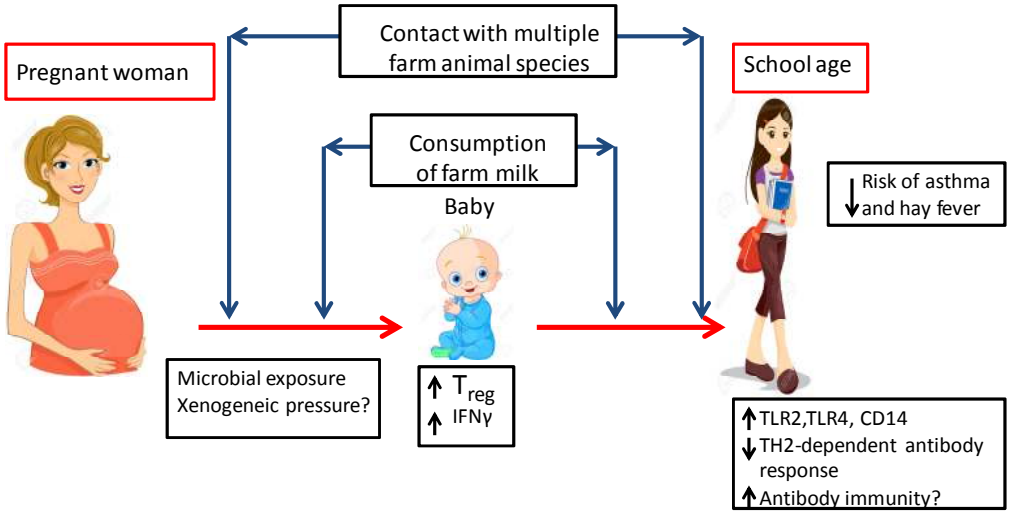

Fig 1. Model depicting the Immunobiology of farming. Contact with multiple animal species, combined with consumption of farm milk, results in strong microbial exposure of, and possibly xenogeneic pressure on, women who carry out farming duties during pregnancy. these combined exposures, which occur at a crucial time for programming immune responses, upregulate regulatory T (T_{Reg}) cell function and interferon-γ (IFNγ) production at birth, which in turn enhance innate immune responses (through increased expression of pattern-recognition receptors), and dampen T helper 2 (T_H2) cell dependent allergic inflammation in early childhood. Exposure to animals and farm milk in early life reinforces the protective effects of prenatal exposures. The ability to produce high levels of IFNγ at birth may also ensure effective responses to respiratory viral infections in early life, thereby counteracting the contribution of these infections to increased asthma susceptibility. (Adapted from von Mutius and Vercelli, [29])

3.1 Mouse Models of Farm Exposures

The link between farm-derived biological factors and the immune system of the host have been studied extensively in several mouse models. The primary goal of these studies was to dissect the biological complexity of farm exposure and identify the components that are most relevant to asthma and allergy protection. In these models, distinct agents were critically examined but the results are readily comparable since all experiments relied on one mouse strain (BALB/c) and one allergen sensitization protocol: intraperitoneal administration of ovalbumin (OVA) with an adjuvant (alum), followed by OVA aerosol challenge [30]. The farm-derived agents under study included stable dust extracts; non-pathogenic Gram-negative and Gram-positive bacteria from the cowshed microflora (*Acinetobacter lwoffii* F78 and *Lactococcus lactis*) [30]; *Bacillus licheniformis*, which is abundant in the settled dust collected from both animal sheds and mattresses; and, most recently, plant polysaccharides (arabinogalactans) derived from fodder and contained in cowshed dust extracts at high concentrations [10]. Here, all of the agents were administered intranasally to adult mice before and/or during allergen sensitization, except for the experiments in which exposure to *Acinetobacter lwoffii* F78 occurred prenatally [33].

Although the experimental design were limited to some extent, the experiments showed that the products and microorganisms under study invariably provided significant protection from allergen-induced T_H2 cell-mediated immune responses even in those that occured locally in the lung. The study further revealed that, treatment with the agents strongly inhibited eosinophilia in bronchoalveolar lavage (BAL), inflammatory cell infiltration into the lung, mucus metaplasia and, importantly, airway hyperresponsiveness [10,30,33]. Conversely, systemic effects, including those on serum IgE levels, were less consistent and less pronounced [33]. Although the molecular and cellular mechanism that channel signals for these protective effects are still not well explored, it is interesting to note that the inhibition of cell dependent allergic inflammation mediated by farm-derived agents was not accompanied by signatures of T_H1-type immune deviation (such as increased IgG2a or IFNγ in BAL), which indicate that these agents may primarily target regulatory immune processes in these mouse models [34]. Studies have shown that pre-treatment with dust extracts [35] or arabinogalactans decreased the ability of OVA-pulsed bone marrow-derived dendritic cells to induce T_H2 cell-mediated responses when transferred into the lungs.

Perhaps more intriguing was a recent study that was specifically designed to investigate the asthma-protective effects of prenatal exposure to farm-derived microorganisms. Intranasal exposure of female mice to *Acinetobacter lwoffii* F78 before and during pregnancy protected the progeny from experimental asthma development in response to OVA sensitization and challenge, even though IgE levels were only marginally affected [33]. Protection was dependent on intact maternal *TLR* signaling, because heterozygous *TLR*-sufficient offspring of *Acinetobacter lwoffii*-exposed female mice lacking *TLR2*, *TLR3*, *TLR4*, *TLR7* and *TLR9* developed OVA-induced allergic inflammation as readily as the offspring of non-exposed mothers [33].

Evidence available implicates microbial exposure in increasing maternal lung and serum pro-inflammatory cytokines as well as upregulates *TLR* mRNA in the maternal lung [33]. This mild local response was followed by systemic distribution of pro-inflammatory cytokines and down regulation of *TLR* mRNA and pro-inflammatory cytokine expression in the placenta, hence an indication that the fetal immune system can be transplacentally programmed by maternal innate immune responses to mucosal microbial stimulation during pregnancy. Although the mechanisms that link *TLR* mRNA upregulation in the maternal lung, *TLR* mRNA down regulation in the placenta and asthma-protective effects in the progeny are still unclear, this model may be able to address several important questions [33].

4. CONCLUSION

This review has discussed in detail some of the important arguments that support the claim that children who grow up on traditional farms are protected from asthma, hay fever and allergic sensitization. It is concluded that possible explanations for the lower prevalence of asthma, hay fever and allergic sensitization in children living on a farm might be the development of immunotolerance or the stimulation of T_H1 cells and suppression of T_H2 cells by increased exposure of farm children to microbial antigens in the stables or farm houses. The fact that all of the microbial exposures tested in animal models

so far conferred strong protection from allergic inflammation, it may reflect the extremely high cumulative doses of microorganisms used in those experiments. Further fundamental questions concerning the immunobiology of traditional farming need to be answered before the biological impact of this complex environment on its inhabitants can be fully appreciated.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ege MJ, Herzum J, Buchele G, et al. Specific IgE to allergens in cord blood is associated with maternal immunity to *Toxoplasma gondii* and rubella virus. Allergy. 2008;63(11):1505-1511.
2. Strachan DP. Is allergic disease programmed in early life? Clin. Exp. Allergy. 1994;124(7):603-605.
3. Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. Clin. Exp. Allergy. 2000; 30(2):194-200.
4. Douwes J, Cheng S, Travier N, et al. Farm exposure *in utero* may protect against asthma, hay fever and eczema. Eur. Respir. J. 2008;32(3):603-611.
5. von Ehrenstein OS, von Mutius E, Illi S, et al. Reduced risk of hay fever and asthma among children of farmers. Clin. Exp. Allergy. 2000;30(2):187-193.
6. Pfefferle PI, Büchele G, Blümer N, et al. Cord blood cytokines are modulated by maternal farming activities and consumption of farm dairy products during pregnancy: The pasture study. J. Allergy Clin. Immunol. 2010;125(1):108-115.
7. Waser M, Michels KB, Bieli C, et al. Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe. Clin. Exp. Allergy. 2007;37(5):661-670.
8. Braun-Fahrlander C, von Mutius E. Can farm milk consumption prevent allergic diseases? Clin. Exp. Allergy. 2011;41(1): 29-35.
9. Perkin MR, Strachan DP. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? J. Allergy Clin. Immunol. 2006;117(6):1374-1381.
10. Sudre B, Vacheyrou M, Braun-Fahrländer C, et al. High levels of grass pollen inside European dairy farms: A role for the allergy-protective effects of environment? Allergy. 2009;64(6):1068-1073.
11. Vogel K, Blümer N, Korthals M, et al. Animal shed *Bacillus licheniformis* spores possess allergy-protective as well as inflammatory properties. J. Allergy Clin. Immunol. 2008;122(2):307-312.
12. van Strien RT, Engel R, Holst O, et al. Microbial exposure of rural school children, as assessed by levels of N-acetyl-muramic acid in mattress dust, and its association with respiratory health. J. Allergy Clin. Immunol. 2004;113(5):860-867.
13. Braun-Fahrlander C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. N. Engl. J. Med. 2002;347(12): 869-877.
14. Schade U, Schlegel J, Hofmann K, Brade H, Flach R. Endotoxin tolerant mice produce an inhibitor of tumor necrosis factor synthesis. J. Endotoxin Res. 1996;3(6):455-462.
15. Douwes J, McLean D, van der Maarl E, Heederik D, Pearce N. Worker exposures to airborne dust, endotoxin and beta (1,3)-glucan in two New Zealand sawmills. Am. J. Indutrial Med. 2000;38(4):426-430.
16. Lauener RP, Birchler T, Adamski J, et al. Expression of CD14 and Toll-like receptor 2 in farmers' and non-farmers' children. Lancet. 2002;360(9331):465-466.
17. Gereda JE, Leung DYM, Liu H. Levels of environmental endotoxin and prevalence of atopic disease. J. Am. Med. Assoc. 2000;284(13):1652-1653.
18. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. N. Engl. J. Med. 1995;332(3):133-138.
19. Rylander R, Bake B, Fischer JJ, Helander IM. Pulmonary function and symptoms

after inhalation of endotoxin. Am. Rev. Respir. Dis. 1998;140(4):981-986.
20. Tulic MK, Wale JL, Holt PG, Sly PD. Modification of the inflammatory response to allergen challenge after exposure to bacterial lipopolysaccharide. Am. J. Respir. Cell Mol. Biol. 2000;22(5):604-612.
21. Park JH, Spiegelman DL, Burge HA, et al. Longitudinal study of dust and airborne endotoxin in the home. Environ. Health Perspect. 2000;108(11):1023-1028.
22. Abou-Zeid C, Gares MP, Inwald J, et al. Induction of a type 1 immune response to a recombinant antigen from *Mycobacterium tuberculosis* in *Mycobacterium vaccae*. Infect. Immun. 1997;65(5):1856-1862.
23. Lauener RPL, Goyert SM, Geha RS. Interleukin-4 down regulates the expression of CD14 in normal human monocytes. Eur. J. Immunol. 1990;20(11): 2375-2381.
24. Stern DA, Riedler J, Nowak D, et al. Exposure to a farming environment has allergen-specific protective effects on T(H)2-dependent isotype switching in response to common inhalants. J. Allergy Clin. Immunol. 2006;119(2):351-358.
25. Schaub B, Liu J, Hoppler S, et al. Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. J. Allergy Clin. Immunol. 2009;123(4):774-782.
26. Chen X, Baumel M, Mannel DN, Howard OM, Oppenheim JJ. Interaction of TNF with TNF receptor type 2 promotes expansion and function of mouse CD4+CD25+ T regulatory cells. J. Immunol. 2007;179(1):154-161.
27. Cosentino G, Soprana E, Thienes CP, Siccardi AG, Viale G, Vercelli D. IL-13 downregulates CD14 expression and TNF-α secretion in human monocytes. J. Immunol. 1995;155(6):3145-3151.
28. Vuillermin PJ, Ponsonby AL, Saffery R, et al. Microbial exposure, interferon gamma gene demethylation in naive T-cells, and the risk of allergic disease. Allergy. 2009;64(3):348-353.
29. von Mutius E, Vercelli D. Farm living: Effects on childhood asthma and allergy. Nat. Rev. Immunol. 2010;10(12):861-868.
30. Debarry J, Garn H, Hanuszkiewicz A, et al. *Acinetobacter lwoffii* and *Lactococcus lactis* strains isolated from farm cowsheds possess strong allergy-protective properties. J. Allergy Clin. Immunol. 2007;119(6):1514-1521.
31. Levy O. Innate immunity of the newborn: Basic mechanisms and clinical correlates. Nature Rev. Immunol. 2007;7(5):379-390.
32. Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. N. Engl. J. Med. 2002;347(12): 911-920.
33. Conrad ML, Ferstl R, Teich R, et al. Maternal TLR signaling is required for prenatal asthma protection by the nonpathogenic microbe *Acinetobacter lwoffii* F78. J. Exp. Med. 20009;206(13): 2869-2877.
34. Peters M, Kauth M, Scherner O, et al. Arabinogalactan isolated from cowshed dust extract protects mice from allergic airway inflammation and sensitization. J. Allergy Clin. Immunol. 2010;126(3):648-656.
35. Gorelik L, Kauth M, Gehlhar K, et al. Modulation of dendritic cell function by cowshed dust extract. Innate Immunity. 2008;14(6):345-355.

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