SCIENCE AND SOCIETY

Farm living: effects on childhood asthma and allergy

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Abstract | Numerous epidemiological studies have shown that children who grow up on traditional farms are protected from asthma, hay fever and allergic sensitization. Early-life contact with livestock and their fodder, and consumption of unprocessed cow's milk have been identified as the most effective protective exposures. Studies of the immunobiology of farm living point to activation and modulation of innate and adaptive immune responses by intense microbial exposures and possibly xenogeneic signals delivered before or soon after birth.

The prevalence of asthma, hay fever, atopic dermatitis and allergic sensitization is higher in affluent, Western countries than in developing countries. A rise in the prevalence of these conditions has also occurred in the last decades of the twentieth century[1]. From a global perspective, some comparisons seem particularly informative and studies of populations with comparable ethnic backgrounds but striking differences in environmental exposures may be especially revealing. In many developing countries, westernization accompanies urbanization and thus reflects a loss of rural living conditions.

In Europe, studies comparing rates of childhood asthma and hay fever in urban and rural areas have been inconclusive[2]. However, large differences in the prevalence of childhood asthma, hay fever and atopic sensitization exist in rural areas. As we discuss here, 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. This protective 'farm effect' is seen for both the atopic and non-atopic phenotype of childhood asthma[3,4] and has been shown to be sustained into adult life. Many of the studies that primarily investigated childhood farm exposures (TABLE 1; Supplementary information S1 (table)) were carried out in Switzerland, Austria and Germany[4–8] where, traditionally, farming has been the main source of subsistence.

In these areas, most farms are involved in dairy production, but may also keep other animals, such as horses for horse-back riding, pigs for meat and poultry for egg production. In addition, some farmers raise sheep and goats. Most farmers also grow fodder material such as grass, corn and grain and many farm houses store this fodder and accommodate people and animals in close proximity under one roof. Most farms in these areas are non-industrialized and family-run. Furthermore, women are involved in stable and barn work before, during and after pregnancy and children as young as a few days are taken into stables, where mothers can look after them while working. Therefore, most farm children will have been exposed to stable and barn environments up to entry into kindergarten (at approximately 3 years of age) and many will have been exposed continuously until adolescence and beyond.

In this article, we discuss three main aspects of the farm effect. First, protective environmental exposures that are inhaled and ingested by exposed individuals have been identified. Second, the study of farm populations has pointed to the time period in which these exposures are effective for mediating the farm effect. Third, immune response studies in farm children and experiments in animal models have identified components of the protective immunobiology of farm exposures.

Allergy-protective farm exposures

Several 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, namely 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[4,6–9]. These exposures had an independent protective farm effect, which indicates that inhalation and ingestion are the two main routes of exposure. Other differences in lifestyle, such as duration of breast feeding, family and sibship size, day care, pet ownership, other dietary habits, parental education and a family history of asthma and allergies, did not account for the protective farm effect. However, the timing of this exposure was crucial, with the strongest effects observed for exposures that occurred *in utero* and during the first years of life[7,10]. Maternal contact with increasing numbers of farm animal species[10–12], work in barns[11,12] and stables[10,13], and the consumption of unprocessed cow's milk during pregnancy[12] were shown to be the relevant protective exposures. Furthermore, a study based in New Zealand showed that continued exposure to farm animals and hay or grain products from pregnancy to school age conferred the strongest protection[9].

Unprocessed cow's milk. Medical guidance in Europe is strongly prohibitive of consumption of raw cow's milk on the basis of reports of disease outbreaks from exposure to pathogenic bacteria in unpasteurized milk. Nevertheless, dairy farming families still use unprocessed milk, even for pregnant women and infants. Five studies have shown a protective effect of unprocessed milk on the development of asthma, hay fever, allergic sensitization and atopic dermatitis[4,7,12,14–16]. The cow's milk that is used for commercial purposes has been pasteurized and homogenized. In most countries, pasteurization is achieved by heating the milk for a short period (~72–75 °C for up to 30 seconds) to significantly reduce the level of microorganisms in the milk. In the homogenization process, fat globules are broken up to produce

Table 1 | **Studies primarily investigating the effect of childhood farm exposures**

Country	Age	Asthma	Wheeze	Hay fever diagnosis	Hay fever symptoms	Atopic dermatitis	Atopic sensitization	AHR	Refs
Europe									
Switzerland	6–15	↓	⇓	↓	⇓	↓	⇓	–	5
Finland	18–24	↓	–	⇓	–	–	–	–	59
Austria, Germany, the Netherlands, Sweden and Switzerland	5–13	⇓	⇓	⇓	⇓	↓	⇓	–	60
Southern Germany	5–7	↓	⇓	⇓	↓	↓	–	–	8
Sweden	7–8	⇓	–	–	⇓	↓	–	–	61
Austria	8–11	–	–	–	–	–	⇓	–	62
Austria	8–10	⇓	⇓	⇓	⇓	↔	⇓	–	6
Denmark	17–26	↓	↓	↓	–	–	⇓	⇓	63
The Netherlands	20–70	↓	–	⇓	–	–	–	–	64
Germany	18–44	↓	↓	⇓	–	–	⇓	↓	65
Finland	20–44	–	–	–	–	–	↓	–	66
UK	4–11	⇓	–	⇓	–	↓	↓	–	14
Northern Germany	18–44	⇓	–	⇓	–	↓	–	–	67
Eastern Finland	6–13	–	–	–	–	–	⇓	–	68
Sweden	17–20	⇓	–	⇓	–	↓	–	–	69
Austria, Germany and Switzerland	6–13	⇓	–	–	⇓	–	⇓	–	3
Tyrol, Austria	6–10	⇓	–	–	–	–	–	–	70
Gothenburg, Sweden	16–20	⇓	↑	–	–	–	–	–	71
West Gothia, Sweden	16–75	–	–	–	↓	–	–	–	72
Turku, Finland	18–25	⇓	–	–	–	–	–	↓	73
Belgium, France, the Netherlands, Sweden and New Zealand	20–44	↓	↓	–	⇓	–	⇓	–	74
Australasia									
Australia	7–12	↓ or ⇓	↓ or ⇓	↓	–	↓	–	–	75
New Zealand	7–10	↓	↓	↓	–	↓	↓	–	15
New Zealand	5–17	⇓	⇓	⇓	–	⇓	–	–	9
New Zealand	25–49	⇓	⇓	–	⇓	↓	–	–	24
North America									
Canada	0–11	⇓	–	–	–	–	–	–	76
British Columbia, Canada	8–20	⇓	↓	⇓	–	⇓	–	–	77
USA	20–88	⇓	–	–	–	–	–	–	78
Quebec, Canada	12–19	⇓	⇓	–	–	–	⇓	⇓	79
Wisconsin, USA	4–17	⇓	⇓	⇓	–	–	–	–	80
Iowa, USA	0–17	↓	↓	–	–	–	↓	↓	81
Iowa, USA	6–14	↓	↓	–	–	–	–	–	82

See Supplementary information S1 (table) for an extended version of this table. ↓, reduction in risk not reaching statistical significance; ⇓, reduction in risk reaching statistical significance; ↑, increase in risk not reaching statistical significance; ↔, no farm effect; –, not determined; AHR, airway hyperresponsiveness.

a standardized fat content of milk and to prevent the separation of a cream layer. Homogenization causes a reduction of fat globule size and a concurrent increase in the milk fat surface area, which alters the original milk fat globule membrane (MFGM) because the concentration of native MFGM is insufficient to cover the fat surfaces that are formed during homogenization[17]. Adsorption of new material from the milk serum at the oil–water interface occurs to cover this increase in surface area and the new MFGM consists of native MFGM plus adsorbed proteins (casein and lactoglobulins), which are the

main allergens in cow's milk. Thus, both the pasteurization and homogenization of cow's milk might abolish the asthma- and allergy-protective effects.

Microbial exposures. It is well known that in addition to plant material from grass, grain and corn, a large variety of bacteria, fungi and their compounds are abundant in animal sheds[17]. Exposure to grass pollen and other plant-derived substances, such as the water-soluble polysaccharides arabinogalactans, is extremely high in cowsheds, particularly during cattle feeding with grass and hay[18,19]. The levels 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[20]. Thus, mattress dust can be regarded as a reservoir that reflects an individual's long-term microbial exposure in indoor and outdoor environments.

Several studies have assessed the health effects of microbial exposures by measuring the markers of bacterial and fungal exposures in mattress dust. Endotoxin (that is, lipopolysaccharide) is a cell-wall component of Gram-negative bacteria, and the levels of endotoxin have been inversely related to allergic sensitization but positively related to asthma and wheeze[21]. Muramic acid is a component of peptidoglycan (a cell-wall component of all bacteria that is more abundant in Gram-positive bacteria) that has been shown to have strong inverse relationships with childhood asthma and wheeze[23]. Extracellular polysaccharides derived from *Penicillium* spp. and *Aspergillus* spp. are specific carbohydrates that are secreted or shed during growth of these fungi, and their presence has been inversely related to asthma and wheeze[4,24]. It remains unclear whether the diversity, dose or exposure only to certain microorganisms account for these protective effects. New metagenomic approaches to assess bacteria and fungi independently of culture methods will help to provide answers to these questions in the future.

Immune responses in farming populations

Innate immunity at school age. Biological studies of human environments typically seek cellular and molecular signatures of a given exposure to identify the pathways that are targeted by that exposure *in vivo*. In the case of farming, research was initially shaped 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. Early analyses in school-age children enrolled in the allergy and endotoxin (ALEX) study (BOX 1) 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[25]. Because the epidemiological evidence strongly indicated that the protective effects of farm living mostly occur prenatally and/or in early life, the association between farm living and pattern-recognition receptor (PRR) expression was re-examined in the prevention of allergy, risk factors for sensitization related to farming and anthroposophic lifestyle (PARSIFAL) study (BOX 1), in which extensive questionnaire-based information was gathered about maternal exposures during pregnancy[26]. This analysis not only confirmed the increase in *CD14* and *TLR2* expression among farm children, but also showed an increase in *TLR4* in these children and indicated that exposure of pregnant mothers to stables, rather than exposure of their infants during childhood, was associated with this enhanced PRR expression[26].

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 expression in the child's peripheral blood cells at school age[26]. Collectively, these studies indicated that prenatal and/or 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.

Adaptive immunity at school-age. The immunoregulatory effects of farming are not confined to innate immunity. A recent study explored the effect of farm exposure on allergen-induced class-switch recombination. To this purpose, IgE and IgG responses to major inhalant allergens (grass, cat hair and house dust mites) were evaluated in school children enrolled in the ALEX study[27]. This analysis revealed unexpected complexities in the effects of farm exposure on antibody production. Indeed, farm living did not affect the prevalence of IgG2 and IgG3 isotypes, but strongly protected against the development of IgG1, IgG4 and IgE antibodies (the T helper 2 (T_H2)-dependent immunoglobulin isotypes) elicited by both grass and cats. 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 allergen and immunoglobulin isotypes indicates that distinct allergenic entities trigger distinct response pathways, which differentially interact with farm-derived protective agents.

Neonatal immune responses. The immunological analyses initially performed in school-age farm children were subsequently extended to newborn babies to characterize the contribution of prenatal exposures to the asthma-protective effects of farming. The protection against allergy: study in rural environments (PASTURE) birth cohort study[28] (BOX 1) was designed to evaluate the effects of maternal farm-related exposures during pregnancy on IgE responses in the offspring. Seasonal allergen-specific IgE responses were significantly more 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_H1 cell-associated cytokine interferon-γ (IFNγ) by cord blood cells after stimulation with phorbol 12-myristate 13-acetate (PMA) plus ionophore[29].

Of note, 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_H2 cell-associated cytokine interleukin-5 (IL-5), the regulatory cytokine IL-10 and the T_H1-inducing cytokine IL-12 were

Box 1 | European studies examining the effect of farm living on asthma and allergy

The allergy and endotoxin (ALEX) study was a cross-sectional study of more than 900 school-aged farm and non-farm children living in rural areas of Switzerland, Germany and Austria that was conducted in 1999.

The prevention of allergy, risk factors for sensitization related to farming and anthroposophic lifestyle (PARSIFAL) study was a cross-sectional study that was conducted in 2000–2002 and included more than 8,000 school-aged farm and non-farm children living in rural areas of Austria, Germany, the Netherlands, Sweden and Switzerland.

The protection against allergy: study in rural environments (PASTURE) was a birth cohort study carried out in rural areas of Austria, Germany, Switzerland, France and Finland that enrolled more than 500 pregnant farm and more than 500 pregnant non-farm women in 2002. The children of these mothers were followed prospectively until 6 years of age.

unaffected[29]. Again, maternal contact with multiple animal species and barns during pregnancy enhanced this production of TNF and IFNγ by infants. Consumption of butter made from unprocessed milk during pregnancy also had striking positive effects on TNF and IFNγ production by newborns. Interpretation of the cytokine patterns detected in the PASTURE population is complex because these studies were performed on unfractionated cord blood cells treated with nonspecific stimuli. Therefore, the cellular source (or sources) of the cytokines remain undefined. 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[12].

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[11]. 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. Furthermore, allergen-induced levels of 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_{Reg} cell marker glucocorticoid-induced TNF receptor (GITR) and the secretion of IFNγ by cord blood cells in response to allergen and peptidoglycan[11]. Although the population sample size was small and the work essentially exploratory, these results reiterate 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 (see below).

Immunobiology of farming: a model

Although the studies discussed above are diverse in their design and results, they nevertheless identify several cornerstones of a working model of the immunobiology of farming (FIG. 1). The timing of exposure seems to be crucial: pregnancy and early life represent a biological window of opportunity for shaping subsequent immune reactivity. Moreover, contact with multiple animal species during pregnancy is positively associated with T_{Reg} cell activity and production of IFNγ at birth and with expression of innate immune receptors during childhood.

In mechanistic terms, 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. We propose that this rich and diverse microbial burden functions through the innate immune system and the secretion of the T_{Reg} cell-promoting cytokine TNF[30] 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. According to this model, several key effector mechanisms of allergic inflammation are inhibited by the immunoregulatory properties of farm-associated microbial exposures (FIG. 2). 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[31,32]. 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 might 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[11]. Indeed, the increase in PRR expression detected in school-age farm children testifies to the persistence of the immunological effects of early farm exposure.

IFNγ is central to our 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[33,34], and low IFNγ in the first year of life is a strong predictor of airway obstruction during childhood[35]. 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. 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 source (or sources) of neonatal IFNγ are identified, but 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[36]; 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[37]. 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[38]. Alternatively, microbial

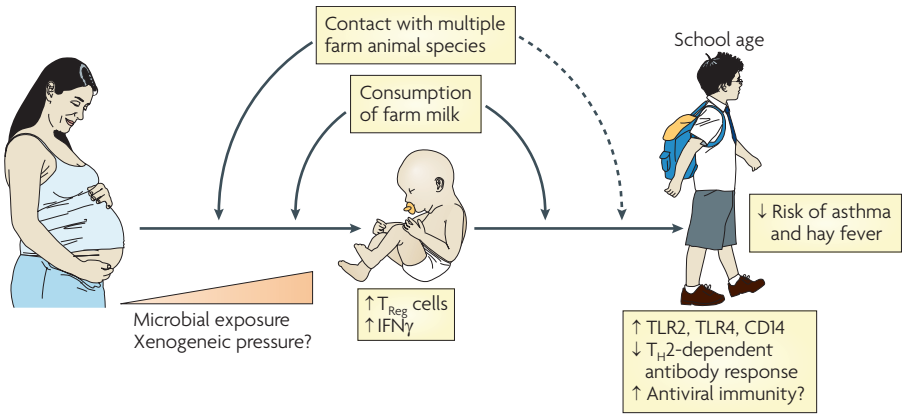

Figure 1 | **A working model of the immunobiology of farm exposure.** Contact with multiple animal species, combined with consumption of farm milk, results in strong microbial exposure of, and possibly xenogeneic pressure on, women who carry out farming duties during pregnancy. These combined exposures, 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. TLR, Toll-like receptor.

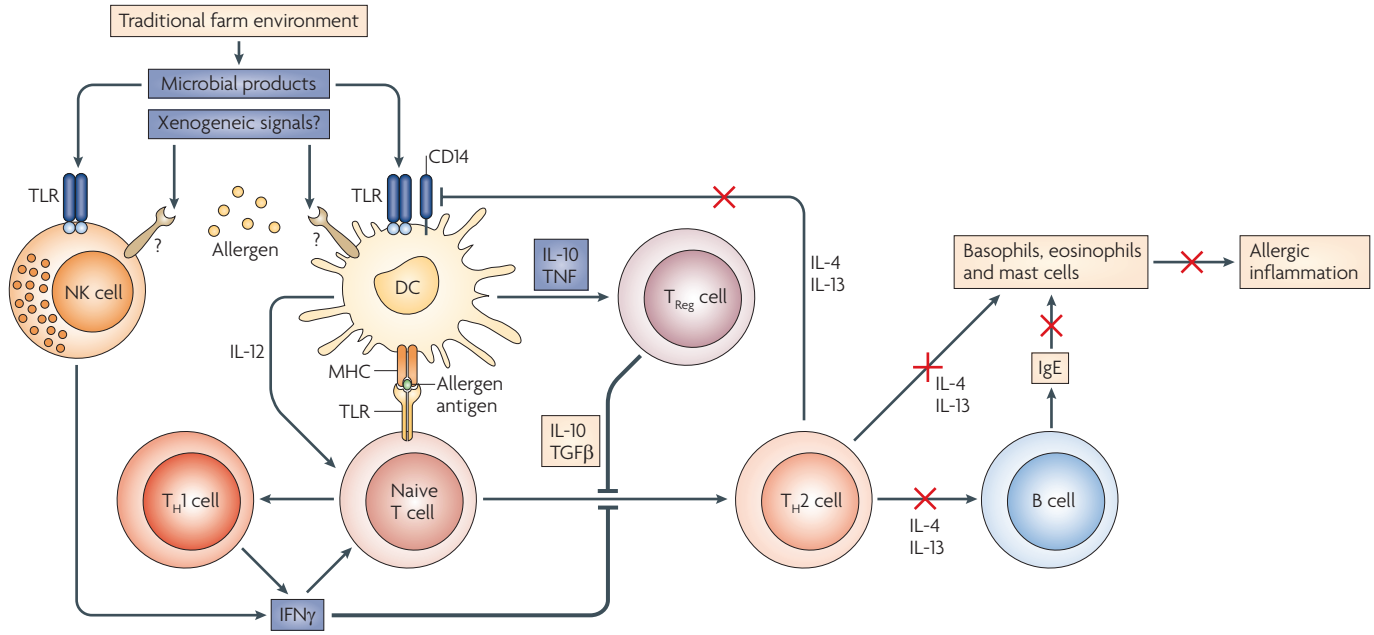

Figure 2 | **Mechanisms potentially underlying the impact of farm exposure on the human immune system.** In this model, the biological diversity of a traditional farm environment (in particular, high numbers of farm animal species) results in intense microbial pressure on the innate immune system. This in turn directs vigorous tumour necrosis factor (TNF)- and interleukin-10 (IL-10)-promoted regulatory T (T_{Reg}) cell activation, which balances adaptive immune responses and suppresses key effector mechanisms of allergic inflammation (allergen-induced T helper 2 (T_H2) cell-associated cytokine production and T_H2 cell-dependent IgE synthesis). Moreover, decreased IL-4 and IL-13 production relieves T_H2 cell-associated cytokine-dependent inhibition of CD14 expression, which leads to further enhancement of pattern-recognition receptor expression and amplification of innate immune responsiveness. Upregulation of interferon-γ (IFNγ) in children of mothers exposed to multiple farm animal species depends primarily on enhanced innate immune activation that is induced by high microbial burden through dendritic cells (DCs) and Toll-like receptor (TLR)-expressing natural killer (NK) cells, but may also be related to the constant, robust xenogeneic pressure generated by close contact with multiple animal species. Xenogeneic signals (delivered through a currently undefined mechanism) may stimulate NK cells to secrete IFNγ, which counteracts allergen-induced T_H2 cell-associated cytokine production and accelerates maturation of T_H1-type responses by activating DC-derived IL-12 production. All of these effects synergize in preventing T_H2-mediated allergic inflammation. TGFβ, transforming growth factor-β.

products may reduce DNA methylation of the *IFNG* gene in naive T cells, thereby leading to increased IFNγ expression[34].

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[39,40]. This leads us to propose that increased production of IFNγ in neonates born to mothers exposed to multiple farm animal species may rely primarily on innate immune mechanisms. 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[41]. 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[42,43], could have a pivotal role in responding to this pressure through the production of IFNγ. 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[44], thereby promoting an accelerated maturation of T_H1-type immune responses (FIG. 2). These immune responses would provide enhanced protection against intracellular pathogens, especially respiratory viruses (such as rhinovirus and respiratory syncytial virus)[45] that persistently alter immune responses and airway function in susceptible subjects and increase the risk of developing asthma, particularly in atopic children[46]. Much in the model we propose is still speculative. 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.

Mouse models of farm exposure

The interactions between farm-derived biological factors and the immune system of the host have been explored 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/or allergy protection. Each model examined distinct agents, but the results are readily comparable because 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. The farm-derived agents under study included stable dust extracts[47]; non-pathogenic Gram-negative and Gram-positive bacteria from the cowshed microflora (*Acinetobacter lwoffii* F78 and *Lactococcus lactis*)[38,48]; *Bacillus licheniformis*, which is abundant in the settled dust collected from both animal sheds and mattresses[21]; and, most recently, plant polysaccharides (arabinogalactans) derived from fodder and contained in cowshed dust extracts at high concentrations[19]. All of these 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[48] (TABLE 2).

Table 2 | **Mouse models of the farm effect on allergy and hay fever**

Farm-derived agent	Timing of agent administration	Serum	BAL	Lung	Refs
Stable dust extracts	From day 0	↓↓ IgE, ↓↓ IgG1, ↔ IgG2a	Decreased eosinophils, lymphocytes, macrophages and IgE	Reduced AHR	47
Acinetobacter lwoffii F78, *Lactococcus lactis* G121	From day –10	↔ IgE, ↓ IgG1, ↔ IgG2a	Decreased eosinophils; increased neutrophils and lymphocytes	Reduced inflammatory infiltration, mucus metaplasia and AHR	38
Acinetobacter lwoffii F78	Prenatal*	↔ IgE, ↔ IgG1	Decreased eosinophils and lymphocytes	Reduced inflammatory infiltration, mucus metaplasia and AHR	48
Bacillus licheniformis spores	From day –12	–	Decreased eosinophils and lymphocytes; increased macrophages, neutrophils, IFNγ and IL-10	Reduced inflammatory infiltration and mucus metaplasia	21
Plant arabinogalactans from cowshed dust	From day 0	–	Decreased eosinophils, IgE, IgG2a and IL-5	Reduced mucus metaplasia and AHR	19

↓, decreased; ↓↓, strongly decreased; ↔, no effect; –, not determined; AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; IFNγ, interferon-γ; IL, interleukin. *From day –11 through gestation.

Despite limitations in the experimental design (for example, short exposure to an artificial allergen through an artificial route, use of a single, T_H2-prone mouse strain and use of adult mice of limited relevance to the study of childhood asthma) and some heterogeneity in the results, these experiments showed that the products and microorganisms under study invariably provided significant protection from allergen-induced T_H2 cell-mediated immune responses, particularly those that occur locally in the lung (TABLE 2). Indeed, treatment with these agents strongly inhibited eosinophilia in bronchoalveolar lavage (BAL), inflammatory cell infiltration into the lung, mucus metaplasia and, importantly, airway hyperresponsiveness[19,21,38,47,48]. By contrast, systemic effects, including those on serum IgE levels, were less consistent and less pronounced.

The molecular and cellular pathways that sense and transduce the signals responsible for these protective effects are still largely unknown. However, it is noteworthy that the inhibition of T_H2 cell-dependent allergic inflammation mediated by farm-derived agents (with the possible exception of *Bacillus licheniformis* spores) was not accompanied by signatures of T_H1-type immune deviation (such as increased IgG2a or IFNγ in BAL), which indicates that these agents may primarily target regulatory immune processes in these mouse models. In fact, pre-treatment with dust extracts[49] or arabinogalactans[19] decreased the ability of OVA-pulsed bone-marrow-derived dendritic cells to induce T_H2 cell-mediated responses when transferred into the lungs. This inhibition was partially dependent on autocrine production of IL-10 (REFS 19,49), a classical immunoregulatory cytokine.

Perhaps more intriguing was a recent study that was specifically designed to investigate the asthma-protective effects of prenatal exposure to farm-derived microorganisms[48]. 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. Protection was dependent on intact maternal TLR signalling, 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. Microbial exposure transiently increased maternal lung and serum pro-inflammatory cytokines and upregulated TLR mRNA in the maternal lung. This mild local response was followed by systemic distribution of pro-inflammatory cytokines and downregulation of TLR mRNA and pro-inflammatory cytokine expression in the placenta[48]. These findings indicate 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 downregulation in the placenta and asthma-protective effects in the progeny are still unclear, this model may be able to address several important questions. For instance, was placental TLR mRNA downregulation directly induced by cytokines released from the maternal lung, or was it mediated by the recruitment of bone-marrow-derived myeloid dendritic cells that are mobilized by microorganism-induced signals emanating from the airway mucosa[50]? And, more generally, does this model recapitulate the essential biological signatures that are associated with farming-induced human asthma protection (increased IFNγ expression and T_{Reg} cell activity at birth, and persistent PRR upregulation later in life)? And, if so, which pathways are responsible for these events?

Future directions

Several 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. For instance, most studies focused on the microbial components of the farm environment, but the possibility that xenogeneic signals might synergize with microbial exposures is worth investigating. The protective effect of unprocessed cow's milk consumption is a recurrent epidemiological finding, but no model is yet available to explore its mechanistic implications. The importance of the exposure route (gut versus airways) is also still a matter of speculation.

The fact that all of the microbial exposures tested in animal models so far conferred strong protection from allergic inflammation is puzzling, and may reflect the extremely high cumulative doses of microorganisms used in these experiments. However, some microorganisms may be more protective than others at the concentrations at which they exist in traditional farms. The intertwining of immunological and developmental processes suggested by the pre-eminent role of prenatal exposure in protection also requires elucidation. Finally, the protective properties of farm-derived factors raise the possibility that the prevalence of other complex immune-mediated diseases may also be decreased among farm children. Indeed, recent data support this hypothesis for juvenile Crohn's

disease and ulcerative colitis[51], but not for type 1 diabetes and rheumatoid arthritis[52,53]. More generally, the mechanisms and pathways of protective farm exposure await a clearer definition. The stakes are high because few other environments have proven so effective in positively influencing the natural history of human common diseases. However, farm populations are not just peculiar populations with uncommon environmental exposures. Mankind has evolved around such exposures and most people are still exposed in rural areas of developing countries. Urbanization, which often implies loss of protective exposures related to livestock, is associated with increasing rates of allergies in many parts of the developing world[54–57]. The increasing trends of allergy prevalence, which have begun in these parts of the world[58], will be attributable to risk exposures in the absence of protection in early life.

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1. Eder, W., Ege, M. J. & von Mutius, E. The asthma epidemic. *N. Engl. J. Med.* **355**, 2226–2235 (2006).
2. Strachan, D. P., Anderson, H. R., Limb, E. S., O'Neill, A. & Wells, N. A national survey of asthma prevalence, severity, and treatment in Great Britain. *Arch. Dis. Child.* **70**, 174–178 (1994).
3. Riedler, J. *et al.* Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* **358**, 1129–1133 (2001).
4. Ege, M. J. *et al.* Not all farming environments protect against the development of asthma and wheeze in children. *J. Allergy Clin. Immunol.* **119**, 1140–1147 (2007).
5. Braun-Fahrländer, C. *et al.* Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. SCARPOL team. Swiss Study on Childhood Allergy and Respiratory Symptoms with Respect to Air Pollution. *Clin. Exp. Allergy* **29**, 28–34 (1999).
6. 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* **30**, 194–200 (2000).
7. Riedler, J. *et al.* Early life exposure to farming environment is essential for protection against the development of asthma and allergy: a cross-sectional survey. *Lancet* **358**, 1129–1133 (2001).
8. Von Ehrenstein, O. S. *et al.* Reduced risk of hay fever and asthma among children of farmers. *Clin. Exp. Allergy* **30**, 187–193 (2000).
9. Douwes, J. *et al.* Farm exposure *in utero* may protect against asthma, hay fever and eczema. *Eur. Respir. J.* **32**, 603–611 (2008).
10. Ege, M. J. *et al.* Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. *J. Allergy Clin. Immunol.* **117**, 817–823 (2006).
11. Schaub, B. *et al.* Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. *J. Allergy Clin. Immunol.* **123**, 774–782 e5 (2009).
12. Pfefferle, P. I. *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.* **125**, 108–115 e1–3 (2010).
13. Ege, M. J. *et al.* Specific IgE to allergens in cord blood is associated with maternal immunity to *Toxoplasma gondii* and rubella virus. *Allergy* **63**, 1505–1511 (2008).
14. Perkin, M. R. & Strachan, D. P. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? *J. Allergy Clin. Immunol.* **117**, 1374–1381 (2006).
15. Wickens, K. *et al.* Farm residence and exposures and the risk of allergic diseases in New Zealand children. *Allergy* **57**, 1171–1179 (2002).
16. Waser, M. *et al.* Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe. *Clin. Exp. Allergy* **37**, 661–670 (2007).
17. Perkin, M. R. Unpasteurized milk: health or hazard? *Clin. Exp. Allergy* **37**, 627–630 (2007).
18. Seedorf, J. *et al.* Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in Northern Europe. *J. Agr. Eng. Res.* **70**, 97–109 (1998).
19. Peters, M. *et al.* Arabinogalactan isolated from cowshed dust extract protects mice from allergic airway inflammation and sensitization. *J. Allergy Clin. Immunol.* **126**, 648–656 (2010).
20. Sudre, B. *et al.* High levels of grass pollen inside European dairy farms: a role for the allergy-protective effects of environment? *Allergy* **64**, 1068–1073 (2009).
21. Vogel, K. *et al.* Animal shed *Bacillus licheniformis* spores possess allergy-protective as well as inflammatory properties. *J. Allergy Clin. Immunol.* **122**, 307–312 (2008).
22. Braun-Fahrländer, C. *et al.* Environmental exposure to endotoxin and its relation to asthma in school-age children. *N. Engl. J. Med.* **347**, 869–877 (2002).
23. van Strien, R. T. *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.* **113**, 860–867 (2004).
24. Douwes, J. *et al.* Lifelong farm exposure may strongly reduce the risk of asthma in adults. *Allergy* **62**, 1158–1165 (2007).
25. Lauener, R. P. *et al.* Expression of CD14 and Toll-like receptor 2 in farmers' and non-farmers' children. *Lancet* **360**, 465–466 (2002).
26. Ege, M. J. *et al.* Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. *J. Allergy Clin. Immunol.* **117**, 817–823 (2006).
27. Stern, D. A. *et al.* Exposure to a farming environment has profound, allergen-specific effects on *in vivo* isotype switching in response to common inhalants. *J. Allergy Clin. Immunol.* **119**, 351–358 (2007).
28. von Mutius, E. & Schmid, S. The PASTURE project: EU support for the improvement of knowledge about risk factors and preventive factors for atopy in Europe. *Allergy* **61**, 407–413 (2006).
29. Pfefferle, P. I. *et al.* Cord blood allergen-specific IgE is associated with reduced IFN-γ production by cord blood cells: the Protection against Allergy-Study in Rural Environments (PASTURE) study. *J. Allergy Clin. Immunol.* **122**, 711–716 (2008).
30. Chen, X., Baumel, M., Mannel, D. N., Howard, O. M. & Oppenheim, J. J. Interaction of TNF with TNF receptor type 2 promotes expansion and function of mouse $CD4^+CD25^+$ T regulatory cells. *J. Immunol.* **179**, 154–161 (2007).
31. Lauener, R. P. L., Goyert, S. M., Geha, R. S. & Vercelli, D. Interleukin 4 down-regulates the expression of CD14 in normal human monocytes. *Eur. J. Immunol.* **20**, 2375–2381 (1990).
32. Cosentino, G. *et al.* IL-13 downregulates CD14 expression and TNF-α secretion in human monocytes. *J. Immunol.* **155**, 3145–3151 (1995).
33. Wright, A. L. The epidemiology of the atopic child: who is at risk for what? *J. Allergy Clin. Immunol.* **113**, S2–S7 (2004).
34. Vuillermin, P. J. *et al.* Microbial exposure, interferon gamma gene demethylation in naïve T-cells, and the risk of allergic disease. *Allergy* **64**, 348–353 (2009).
35. Stern, D. A., Guerra, S., Halonen, M., Wright, A. L. & Martinez, F. D. Low IFN-γ production in the first year of life as a predictor of wheeze during childhood. *J. Allergy Clin. Immunol.* **120**, 835–841 (2007).
36. Romagnani, S. Coming back to a missing immune deviation as the main explanatory mechanism for the hygiene hypothesis. *J. Allergy Clin. Immunol.* **119**, 1511–1513 (2007).
37. Amsen, D. *et al.* Instruction of distinct CD4 T helper cell fates by different notch ligands on antigen-presenting cells. *Cell* **117**, 515–526 (2004).
38. Debarry, J. *et al. Acinetobacter lwoffii* and *Lactococcus lactis* strains isolated from farm cowsheds possess strong allergy-protective properties. *J. Allergy Clin. Immunol.* **119**, 1514–1521 (2007).
39. Levy, O. Innate immunity of the newborn: basic mechanisms and clinical correlates. *Nature Rev. Immunol.* **7**, 379–390 (2007).
40. Halonen, M. *et al.* Th1/Th2 patterns and balance in cytokine production in the parents and infants of a large birth cohort. *J. Immunol.* **182**, 3285–3293 (2009).
41. Muzio, M. *et al.* Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells. *J. Immunol.* **164**, 5998–6004 (2000).
42. Bach, F. H. *et al.* Barriers to xenotransplantation. *Nature Med.* **1**, 869–873 (1995).
43. Forte, P., Lilienfeld, B. G., Baumann, B. C. & Seebach, J. D. Human NK cytotoxicity against porcine cells is triggered by NKp44 and NKG2D. *J. Immunol.* **175**, 5463–5470 (2005).
44. Snijders, A., Kalinski, P., Hilkens, C. M. & Kapsenberg, M. L. High-level IL-12 production by human dendritic cells requires two signals. *Int. Immunol.* **10**, 1593–1598 (1998).
45. Friedlander, S. L. *et al.* Viral infections, cytokine dysregulation and the origins of childhood asthma and allergic diseases. *Pediatr. Infect. Dis. J.* **24**, S170–S176 (2005).
46. Martinez, F. D. The origins of asthma and chronic obstructive pulmonary disease in early life. *Proc. Am. Thorac. Soc.* **6**, 272–277 (2009).
47. Peters, M. *et al.* Inhalation of stable dust extract prevents allergen induced airway inflammation and hyperresponsiveness. *Thorax* **61**, 134–139 (2006).
48. Conrad, M. L. *et al.* Maternal TLR signaling is required for prenatal asthma protection by the nonpathogenic microbe *Acinetobacter lwoffii* F78. *J. Exp. Med.* **206**, 2869–2877 (2009).
49. Gorelik, L. *et al.* Modulation of dendritic cell function by cowshed dust extract. *Innate Immun.* **14**, 345–355 (2008).
50. Holt, P. G. & Strickland, D. H. Soothing signals: transplacental transmission of resistance to asthma and allergy. *J. Exp. Med.* **206**, 2861–2864 (2009).
51. Radon, K. *et al.* Contact with farm animals in early life and juvenile inflammatory bowel disease: a case-control study. *Pediatrics* **120**, 354–361 (2007).
52. Radon, K. *et al.* Exposure to animals and risk of oligoarticular juvenile idiopathic arthritis: a multicenter case-control study. *BMC Musculoskelet. Disord.* **11**, 73 (2010).
53. Radon, K. *et al.* Exposure to farming environments in early life and type 1 diabetes: a case-control study. *Diabetes* **54**, 3212–3216 (2005).
54. Viinanen, A. *et al.* Prevalence of asthma, allergic rhinoconjunctivitis and allergic sensitization in Mongolia. *Allergy* **60**, 1370–1377 (2005).
55. Viinanen, A. *et al.* The protective effect of rural living against atopy in Mongolia. *Allergy* **62**, 272–280 (2007).
56. Ma, Y. *et al.* Very low prevalence of asthma and allergies in schoolchildren from rural Beijing, China. *Pediatr. Pulmonol.* **44**, 793–799 (2009).
57. Wong, G. W. Developing world: asthma in a rural setting. *Paediatr. Respir. Rev.* **7**, S119–S120 (2006).
58. Pearce, N. *et al.* Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax* **62**, 758–766 (2007).
59. Kilpelainen, M., Terho, E. O., Helenius, H. & Koskenvuo, M. Farm environment in childhood prevents the development of allergies. *Clin. Exp. Allergy* **30**, 201–208 (2000).
60. Alfven, T. *et al.* Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle – the PARSIFAL study. *Allergy* **61**, 414–421 (2006).
61. Klintberg, B., Berglund, N., Lilja, G., Wickman, M. & van Hage-Hamsten, M. Fewer allergic respiratory disorders among farmers' children in a closed birth cohort from Sweden. *Eur. Respir. J.* **17**, 1151–1157 (2001).
62. Horak, F. Jr *et al.* Parental farming protects children against atopy: longitudinal evidence involving skin prick tests. *Clin. Exp. Allergy* **32**, 1155–1159 (2002).

63. Portengen, L. *et al.* Low prevalence of atopy in young Danish farmers and farming students born and raised on a farm. *Clin. Exp. Allergy* **32**, 247–253 (2002).
64. Smit, L. A. *et al.* Hay fever and asthma symptoms in conventional and organic farmers in The Netherlands. *Occup. Environ. Med.* **64**, 101–107 (2007).
65. Schulze, A., van Strien, R. T., Praml, G., Nowak, D. & Radon, K. Characterisation of asthma among adults with and without childhood farm contact. *Eur. Respir. J.* **29**, 1169–1173 (2007).
66. Koskela, H. O., Happonen, K. K., Remes, S. T. & Pekkanen, J. Effect of farming environment on sensitisation to allergens continues after childhood. *Occup. Environ. Med.* **62**, 607–611 (2005).
67. Radon, K., Ehrenstein, V., Praml, G. & Nowak, D. Childhood visits to animal buildings and atopic diseases in adulthood: an age-dependent relationship. *Am. J. Ind. Med.* **46**, 349–356 (2004).
68. Remes, S. T., Iivanainen, K., Koskela, H. & Pekkanen, J. Which factors explain the lower prevalence of atopy amongst farmers' children? *Clin. Exp. Allergy* **33**, 427–434 (2003).
69. Braback, L., Hjern, A. & Rasmussen, F. Trends in asthma, allergic rhinitis and eczema among Swedish conscripts from farming and non-farming environments. A nationwide study over three decades. *Clin. Exp. Allergy* **34**, 38–43 (2004).
70. Kiechl-Kohlendorfer, U. *et al.* Neonatal characteristics and risk of atopic asthma in schoolchildren: results from a large prospective birth-cohort study. *Acta Paediatr.* **96**, 1606–1610 (2007).
71. Wennergren, G. *et al.* Asthma in late adolescence – farm childhood is protective and the prevalence increase has levelled off. *Pediatr. Allergy Immunol.* **21**, 806–813 (2010).
72. Eriksson, J. *et al.* Growing up on a farm leads to lifelong protection against allergic rhinitis. *Allergy* **65**, 1397–1403 (2010).
73. Kilpelainen, M., Terho, E. O., Helenius, H. & Koskenvuo, M. Childhood farm environment and asthma and sensitization in young adulthood. *Allergy* **57**, 1130–1135 (2002).
74. Leynaert, B. *et al.* Does living on a farm during childhood protect against asthma, allergic rhinitis, and atopy in adulthood? *Am. J. Respir. Crit. Care Med.* **164**, 1829–1834 (2001).
75. Downs, S. H. *et al.* Having lived on a farm and protection against allergic diseases in Australia. *Clin. Exp. Allergy* **31**, 570–575 (2001).
76. Midodzi, W. K., Rowe, B. H., Majaesic, C. M. & Senthilselvan, A. Reduced risk of physician-diagnosed asthma among children dwelling in a farming environment. *Respirology* **12**, 692–699 (2007).
77. Dimich-Ward, H., Chow, Y., Chung, J. & Trask, C. Contact with livestock – a protective effect against allergies and asthma? *Clin. Exp. Allergy* **36**, 1122–1129 (2006).
78. Hoppin, J. A. *et al.* Pesticides and atopic and nonatopic asthma among farm women in the Agricultural Health Study. *Am. J. Respir. Crit. Care Med.* **177**, 11–18 (2008).
79. Ernst, P. & Cormier, Y. Relative scarcity of asthma and atopy among rural adolescents raised on a farm. *Am. J. Respir. Crit. Care Med.* **161**, 1563–1566 (2000).
80. Adler, A., Tager, I. & Quintero, D. R. Decreased prevalence of asthma among farm-reared children compared with those who are rural but not farm-reared. *J. Allergy Clin. Immunol.* **115**, 67–73 (2005).
81. Merchant, J. A. *et al.* Asthma and farm exposures in a cohort of rural Iowa children. *Environ. Health Perspect.* **113**, 350–356 (2005).
82. Chrischilles, E. *et al.* Asthma prevalence and morbidity among rural Iowa schoolchildren. *J. Allergy Clin. Immunol.* **113**, 66–71 (2004).

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
Erika von Mutius's homepage: http://www.asthma-allergy.de
Donata Vercelli's homepage: http://www.arl.arizona.edu/index.php/divisions-mainmenu-49/az-biology-of-complex-diseases.html
'Bronchial asthma – tracing the causes' movie: http://www.visions-unlimited.com/10/1/?play

SUPPLEMENTARY INFORMATION
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SCIENCE AND SOCIETY

The challenge of immunogenicity in the quest for induced pluripotency

Paul J. Fairchild

Abstract | Few advances have been so widely acclaimed in biology as the seminal demonstration that adult somatic cells can be induced to acquire the phenotype and differentiation potential of embryonic stem cells. The capacity to produce patient-specific stem cells that are truly pluripotent has raised prospects for the treatment of many degenerative diseases through replacement of the affected cell types. In the race to the clinic, however, questions surrounding the potential immunogenicity of such cells have been largely overlooked. Here, I explore the extent of the challenges ahead and suggest that the induction of tolerance to such cells will be crucial to the future success of induced pluripotency.

A member of the audience rose in response to the chairman's invitation to ask questions: "Dr Fairchild, why do you continue to pursue strategies for the induction of tolerance for cell replacement therapies when induced pluripotent stem cells have overcome the issue of immunogenicity?" The familiarity of the question and the silent nods of approval from fellow delegates suggested that such views are widely held in the stem cell community. It is such questions, and the animated discussions that they precipitate, that indicate it might be timely to re-evaluate the extent to which induced pluripotency can circumvent the issues of immunogenicity that continue to confound the clinical use of stem cells from more conventional sources. Here, I discuss whether the limitations of such personalized approaches to medicine outweigh the advantages that an autologous source of tissues might offer, and explore whether strategies for the induction of self-tolerance might provide the most pragmatic solution to the immunological barriers encountered.

The quest for pluripotency

Pluripotency refers to the capacity of a cell to differentiate into derivatives of all three embryonic germ layers (endoderm, ectoderm and mesoderm) and, through progressive specification, to generate each of the cell types of which the human body is composed. This property, together with a propensity for indefinite self-renewal, remains the preserve of cells from the epiblast of early blastocysts and is rapidly lost after implantation. These rare cells, which were first isolated and maintained in culture in 1998 (REF. 1) as lines of human embryonic stem cells (hESCs) (FIG. 1; TIMELINE), inspired early interest in their use as a potential source of cell types and tissues to replace those damaged through ageing, injury or incipient disease, known as cell replacement therapy[2]. Although many groups remain committed to using hESCs in the clinic[3], the immunological constraints of transplanting fully allogeneic tissues from such an unconventional source pose numerous challenges, both practical and scientific.

The prospect of reawakening pluripotency in terminally differentiated cell types as a way of circumventing the immunological barriers encountered with hESCs was first formally demonstrated by somatic cell nuclear transfer (SCNT) in mice (FIG. 1). By transferring the diploid nucleus of a fully differentiated somatic cell into an enucleated recipient oocyte, nuclear transfer embryonic stem cells (ntESCs) can be generated from the resulting cloned blastocyst that are syngeneic to the nuclear donor in all but the mitochondrial genes, which remain of oocyte origin[4]. Although this is undoubtedly a significant advance in the field, the formidable logistical and ethical complexities of applying cloning technology to humans have rendered SCNT largely obsolete as a means of generating pluripotent stem cells for clinical applications. Furthermore, given that the mitochondrial genome is a source of many minor histocompatibility antigens that can be processed and presented in an MHC-restricted manner to alloreactive T cells, the potential immunogenicity of tissues differentiated from ntESCs remains uncertain[5].