

EDITORIAL

Farming and protective agents against allergy and asthma

Since the first publication of papers in *Clinical and Experimental Allergy* on the reduced risk of hayfever, asthma and the expression of atopy in children from farming families compared with their peers from non-farming families [1–3], the interest in the farm environment as a valuable model to study environmental determinants of the development of asthma and allergies is still on the rise.

Growing up on a farm – more specifically having contact with farm animals – has been shown to be associated with a substantial decrease in risk for allergic diseases [2, 3].

The results of the ALEX (ALlergy and EndotoXin) Study, a cross-sectional study of more than 800 children from Germany, Austria and Switzerland, further suggest that the timing of exposure to a farm environment during or before the first year of life, and the amount and duration of exposure from the first year to the fifth year of life, were crucial for the protective effect on asthma and allergies at school age [4]. It has been speculated that this protective 'farm effect' may result from elevated exposure to bacterial compounds encountered in the microbial environment of stables where cattle are kept. Elevated levels of endotoxin, an intrinsic part of the outer membrane of gram-negative bacteria have indeed been found in homes of farmer's children and children with regular contact to livestock as compared to non-farm children without animal contact [5].

More recently the relation between exposure to endotoxin measured in mattress dust of the ALEX children at school age and the occurrence of asthma and allergies has been investigated [6]. In addition, the association between endotoxin levels in mattresses and the cytokine-production profile of peripheral-blood leucocytes after activation of the immune system by stimulation with lipopolysaccharide and staphylococcal enterotoxin B was assessed. A strong inverse association was found between endotoxin exposure and the risk of hayfever, atopic sensitization, atopic asthma, and atopic wheeze but not for non-atopic wheeze and asthma. Interestingly, the protective effect of endotoxin exposure on atopic outcomes was not restricted to farming households but was equally strong in children from non-farming homes indicating that even lower levels of endotoxin as encountered in non-farming environments may favourably influence the risk of atopic diseases in childhood.

Some recent studies of infants and children from non-farming environments support the notion of a protective effect of endotoxin exposures on atopic sensitization [7–9] or atopic eczema during the first 6 months of life [10]. However, the risk for wheezing during the first year of life which often occurs with viral respiratory illness was reported to increase with elevated endotoxin levels in house dust in two ongoing birth cohorts [10, 11]. The recent longitudinal analysis of childhood wheezing in a cohort of children from allergic or asthmatic parents over a 4-year period found an increased risk for wheeze associated with high levels of house dust endotoxin early in life which rapidly decreased over time suggesting that exposure to endotoxin might protect against further episodes of wheezing as the children get older [12]. As wheezing episodes of older children

are more often associated with an atopic phenotype the results might also be interpreted as pointing to a protective effect of endotoxin on atopic wheeze as suggested by the ALEX results.

It has been speculated that the observed inverse relation between pet keeping early in life and the development of atopic sensitization, hayfever and asthma [13–15] might be explained by the higher endotoxin levels found in homes where cats and dogs are kept [16, 17]. However, the recent US [12] cohort study found exposure to high levels of cat allergen and having a dog in the home to be associated with decreased risks for wheezing, independent of the effect of endotoxin in house dust, suggesting that these exposures might be working through different mechanisms.

The observations that increased levels of endotoxin in the environment are paralleled by a decreased risk of developing allergies in children suggests that in some way microbes and/or microbial components may influence the immune system protecting against allergies. However, only recently a direct impact of microbial structures on the immune system in these children could be made evident. Interestingly, such an impact was found at the level of the innate immune system: expression of the genes for Toll-like receptor (TLR)2 and CD14 was found to be increased in leucocytes from farmers' children when compared to non-farmers' children [18]. TLRs are a family of highly conserved, germ-line encoded receptors recognizing conserved microbial patterns. TLR4, together with CD14, serves as receptor for lipopolysaccharide (LPS; endotoxin), while TLR2 is a receptor for components of gram-positive bacteria. An increased expression of CD14 as well as TLR2 is observed in human leucocytes following treatment with LPS *in vitro* [19], suggesting that the differences found *in vivo* between farmers' and non-farmers' children are mirroring different degrees of exposure to such microbes and/or microbial components in the environment. It is important to note that no conclusions should be drawn on the nature of the microbial compound responsible for the observed differences: gram-positive as well as gram-negative micro-organisms have been reported to induce similar changes in the expression of these receptors [20]. Thus, it also remains open whether LPS, or endotoxin, is of particular importance in triggering these changes (and, by implication, in conferring protection against the development of allergies) or whether other microbes/microbial compounds present in the farming environment will have similar effects.

While currently available data suggest that microbes or microbial structures present in the environment do have an impact on the immune system, especially on the innate immune system, the mechanisms by which this should lead to protection against the development of allergies remains a topic of current investigations.

The production of IgE is a hallmark of allergic diseases. Isotype switching of B cells is regulated by T cells; Th2 cells, characterized by their production of IL-4, IL-5, and IL-13, induce B cells to produce antibodies of the IgE isotype, whereas Th1 cells, typically producing INF- γ , lead to the production of

IgG antibodies. The question then arises what governs polarization of Th cells into either Th1 or Th2 cells. Differential stimulation by dendritic cells (DC) plays a crucial role in this step (for review, see [21]). One important factor determining the outcome of priming of naïve T cells by DCs is IL-12: DCs producing IL-12 promote Th1 differentiation. It has been proposed that different DC cell subsets prime naïve T cells for either Th1 or Th2 differentiation. Alternatively, one could imagine that some triggers induce DC to produce IL-12 (and thereby favour a Th1 response) whereas other triggers would fail to induce DC to secrete such factors (thereby favouring Th2-type responses) [22, 23]. Independently of this, the question remains: what determines whether DCs will promote T cell differentiation towards Th1 or Th2-type responses?

Recognition of microbial compounds by innate immune receptors may be the answer [24]. Indeed, activation of TLR2 on human dendritic cells has been shown to trigger induction of IL-12 [25]. Mice unable to respond to stimulation through TLRs, because the signalling pathway activated through TLRs has been interrupted by deleting the gene for the TLR-associated molecule MyD88, show a profound defect in Th1, but not Th2-type immune responses [26]. Furthermore, microbial compounds have been shown to selectively induce Th1 cell-promoting or Th2 cell-promoting dendritic cells [27].

This leads us back to the observation that children exposed to an increased microbial load benefit from a protection against the development of allergies. Microbial components such as endotoxin may, by binding to TLRs, activate DCs to produce IL-12 and other factors favouring Th1 cell differentiation and, ultimately, resulting in lower levels of IgE-mediated sensitization. It is tempting to postulate that the protection against the development of allergies from which farmers' children benefit can be fully explained by exposure to microbes resulting in preferential Th1 cell differentiation. However, while Th1/Th2-related mechanisms undoubtedly account for many of the results observed in farmers' children, other observations remain unclear. Particularly intriguing is the observation that, upon restimulation with LPS as well as with Staphylococcal enterotoxin B (SEB), leucocytes from farmers' children produce less TNF- α , IL-12, and IL-10 when compared to their peers not growing up on a farm [6], an observation hard to explain by the Th1/Th2 dogma. Reduced responsiveness upon repeated stimulation with LPS is a phenomenon previously described in the literature as LPS-tolerance [28]. In addition to mechanisms of the adaptive immune system, accounting for some of the observations made in farmers' children, mechanisms rooted in the innate immune response may contribute to the observation that farmers' children benefit from some protection against the development of allergies.

Although recent studies suggest an important role for microbial exposures in explaining the inverse association between farming and childhood allergy there might be additional factors related to a farming lifestyle contributing to the observed protective effect. Dietary habits such as consumption of fatty acids, anti-oxidants including vitamin C and E, sodium and magnesium are among those lifestyle factors that might differ between farming and non-farming families and have been associated with asthma and allergy risk [29].

In the present issue of the journal, Remes et al. [30] explore the role of diet and other lifestyle factors in explaining the effect of farming on childhood allergy in a cross-sectional study of 366

farmers' and 344 non-farmers' school-aged children in eastern Finland. Information on exposure and background characteristics were obtained by a parental questionnaire, skin prick testing was used to assess atopic sensitization. Although current and first year exposure to farm animals and pets again showed a strong inverse association with atopic sensitization, it did not entirely explain the farming effect. Dietary habits differed considerably between farming and non-farming families, the former consuming significantly more butter and less margarine and drinking full milk or farm milk more often. However, in contrast to previous reports of the pivotal role of fat-derived inflammatory substances on the development of atopic diseases in children [31, 32] and the protective role of farm milk consumption during the first year of life [4], none of these dietary factors was associated with atopic sensitization. Yet, consumption of fresh vegetables, although not different between farming and non-farming families was inversely related to atopy. The effect was stronger for vegetables grown in the family's garden or in the nearby farm as compared to vegetables brought from the grocery store leading to the speculation that the soil-derived microbial content may be important. The cross-sectional design of the study limits the explanatory power of the study but the issue of diet as an additional aspect of the farming lifestyle deserves further exploration.

Over the past years the hypothesis that acquisition of certain infections or exposure to naturally occurring microbial exposures as encountered in the farming environment confer protection of asthma and allergies has gained considerable scientific support. In this context it is interesting to note that the generational increase in atopy and allergic rhinitis in western countries was not observed in subjects who were exposed to a farming environment in childhood or had serologic markers of acquisition of food-borne and orofecal infections as recently evidenced by analyses of the European Community Respiratory Health Survey [33] and the Third National Health and Nutrition Examination Survey in the USA [34].

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