

Asthma and Allergies in Rural Areas of Europe

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A large number of studies have consistently shown that growing up on a farm in various rural areas in Europe confers protection from the development of hay fever, atopic sensitization, and less consistently of asthma from childhood into young adulthood. Exposures to livestock as well as consumption of unpasteurized milk are likely to be distinct and relevant sources of protective exposures. In turn, the underlying microbial exposures have not been identified with certainty. Although environmental exposures to bacterial and fungal components have been found to be inversely related to asthma and atopy, they do not explain the "farming effect." The mechanisms conveying the protection are still poorly understood. An important role for innate immune responses is suggested by findings relating to increased expression of genes of Toll-like receptors in exposed children. How this activation of innate immunity is translated into reduced IgE-specific adaptive immune responses remains to be elucidated, but may invoke a number of distinct allergen-specific steps.

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Widespread attention has been given to the advancement of one field in allergy research that investigates the potential link between exposures to microbial sources and the development of asthma and allergic illnesses (1). The theory attempting to catch the various elements of this complex relation has been coined the "hygiene hypothesis." A large scientific and lay audience has been confronted with these ideas over the last years and in the course of numerous deliberations, new angles and aspects of the hypothesis have been proposed. At least three distinct claims on the true nature of the "hygiene hypothesis" have been brought forward. These contentions relate to the potential role of overt and inapparent infections of human subjects with viruses and bacteria; the relevance of noninvasive microbial exposures in the environment; and the influence of such exposures and infections on a subject's innate and adaptive immune responses.

Before attempting to address these various aspects of the "hygiene hypothesis," it seems justified to highlight the complex nature of the problem. The most relevant levels of complexity so far identified relate to the development of specific phenotypes, the timing of a given exposure, the environmental factors, and a subject's genetic susceptibility. Although in clinical practice manifestations of asthma and allergic illnesses sometimes appear rather uniform, a number of prospective studies have clearly shown that distinct phenotypes exist over childhood, adolescence, and adulthood. It seems therefore reasonable to assume that not a single cause but many will underlie the clinical manifestation.

Recent work has clearly demonstrated that the effect of a given exposure depends on the timing. At least throughout in-

fancy, childhood, and adolescence the human organism is in a constant process of development and maturation. It is conceivable that these predefined processes display windows of accessibility and vulnerability toward extrinsic influences at certain stages of development. Moreover, prenatal factors may play a significant role either through mechanisms acting *in utero* or as epigenetic modulation of subsequent developmental trajectories. The discovery of genes associated with asthma and allergic diseases is still in its beginnings, and so far the findings suggest that no single gene will be responsible for the clinical manifestation of asthma and allergic illnesses. Rather, alterations in many genes interacting with environmental influences at various time points during development are expected to contribute to the mechanisms underlying the various atopic conditions. There is, however, increasing interest in the regulation and fine tuning of gene expression by epigenetic processes. The relatively short time during pregnancy and early life for environmental exposures to impact on the subsequent development of asthma and allergy-related phenotypes supports the notion that epigenetic processes may be centrally involved in the biological responses of exposed subjects.

Such fine tuning of gene expression eventually results *inter alia* in distinct immune responses of exposed subjects. The immunologic mechanisms potentially underlying the protection against allergies mediated by living in a "less hygienic" environment are still being debated. One mechanism frequently associated with the "hygiene hypothesis" is the skewing of the Th1/Th2 balance away from allergy-promoting Th2 toward Th1 cells (2). The link between Th1/Th2 balance and allergic diseases is mediated in part by IgE: Th2 cells, by secreting IL-4 and IL-13, promote immunoglobulin class switch recombination to IgE.

However, there are some conflicting data which cannot be disregarded. Not only Th2-related diseases such as allergies are on the rise over the last decades, but also Th1-related inflammatory diseases such as multiple sclerosis and Crohn's disease (3). Thus, while mechanisms related to Th1/Th2 balance undoubtedly are of importance for the development of allergies, they may not suffice to explain the effects related to the "hygiene hypothesis." Mechanisms operative at other steps of immune development have to be considered when trying to understand the effects of microbial exposures. Serum levels of IgE, as of any other immunoglobulin isotype, are not only determined by class switch recombination to this isotype but also by factors regulating terminal differentiation of already switched B cells and the rate of secretion of IgE. T regulatory cells (Tregs) in interaction with dendritic cells occupy a central role in controlling immune responses, and their importance for the development of allergies has been well documented (4).

Numerous studies have addressed the "hygiene hypothesis," which has undergone numerous more or less subtle modifications by various researchers in the field of epidemiology, clinical science, and immunology. Overall, three major tracts have developed: (1) exploring the role of overt viral and bacterial infections; (2) studying the significance of environmental exposure to microbial compounds; and (3) investigating the effect of infections and environmental exposures on underlying responses of the innate and adaptive immunity. The strongest claim for a critical role of protective environmental exposures in the inception of

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asthma and allergies arises from studies investigating environmental factors in rural areas of Europe, particularly in the farming community.

THE EPIDEMIOLOGICAL EVIDENCE

Since 1999, 15 studies have been performed in rural areas in Europe, namely in Switzerland (5–7), Germany (6–8), Austria (6, 7, 9), France (10), Sweden (7, 11, 12), Denmark (13, 14), Finland (15–17), and Britain (18). Almost all studies reported a decreased prevalence of hay fever and allergic rhinoconjunctivitis, and all surveys that included objective measures of specific IgE antibodies either by skin prick tests or by serum measurements demonstrated a significantly decreased prevalence of atopic sensitization among farm children compared with non-farm children (19). Findings relating to asthma and wheeze are less consistent. While in a large survey including over 10,000 schoolchildren in Germany (8), children of farmers had a lower prevalence of asthma (odds ratio [OR], 0.65; 95% confidence interval [95% CI], 0.39–1.09) and wheeze (OR, 0.55; 95% CI, 0.36–0.86), other surveys in Switzerland (5), France (10), and Finland (15) did not find significant differences between groups. Only two studies included measures of airway hyperresponsiveness and one of these using wood and coal heating as a proxy for farming (13, 20, 21). In Denmark and Germany the prevalence of airway hyperresponsiveness was significantly reduced among farm children compared with nonfarm children (13, 20). Another Canadian survey confirmed these findings (22). To my knowledge, no study has shown an increased prevalence of airway hyperresponsiveness among children raised on farms.

Children raised on farms in Europe seem to retain their protection from allergy at least into young adulthood (10, 14, 23, 24). In a comparison of Danish farming students and, as control subjects, conscripts from the same rural areas, the prevalence of atopic sensitization to common allergens was lowest in farmers who in childhood had lived on a farm, intermediate in farmers without a farm childhood and control subjects with a farm childhood, and highest in control subjects without a farm childhood (14). Likewise, in a German study of adult farmers aged 18 to 44 years (23, 24), farm animal contact in childhood was associated with a decreased risk of atopic sensitization. Continued exposure to farm animals in adulthood further decreased the odds ratio of atopic sensitization associated with symptoms of allergic illnesses (OR, 0.2; 95% CI, 0.1–0.4). However, starting farm animal contact in adulthood increased the odds of asymptomatic atopic sensitization (OR, 2.4; 95% CI, 1.1–5.2). Whether the increased protection of continued exposure from infancy into adulthood is attributable to sustained exposure, or whether a “healthy worker effect” in part explains these findings, remains to be elucidated.

Farming practices vary between farms and between countries, and this diversity may contribute to the heterogeneity of farm effects on asthma across studies and countries. Some investigators have attempted to identify individual exposures in farm surroundings contributing to the reduction in risk of asthma and allergic diseases. Initial observations from Germany and Switzerland reported that children from full-time farmers had lower risk of atopic disease than children of part-time farmers (5, 8) suggesting a dose–response effect. Two recent studies conducted outside Europe (25, 26) seem to suggest that an important component of the farm environment is livestock exposure, since no protective effect of farming was observed among children living in a primarily crop farming region in Australia. This notion is supported by the finding of the European studies in which exposure to livestock has been identified as an important contributor to the protective farm effect (6, 8, 15, 18). Interest-

ingly, in the Austrian study children who did not live on a farm but who had regular contact with farm animals also had a lower prevalence of allergic sensitization (13.5% versus 34.8%) (9). Another source of protection has been identified in a number of studies: the consumption of unpasteurized milk (6, 18, 26). As with livestock exposure, this effect was not restricted to children living on a farm, but was also seen among nonfarm populations consuming unpasteurized milk (18). Recent work has transferred this observation into experimental studies and has shown that treatment of BALBc mice with extracts of stable dust during a conventional sensitization protocol with ovalbumin inhibits the development of airway hyperresponsiveness and eosinophilia upon ovalbumin challenge (27). These findings suggest that dust from stables of animal farms contains strong immunomodulating substances and that these as yet unknown substances suppress allergic sensitization, airway inflammation, and airway hyperresponsiveness in a murine model of allergic asthma.

Few studies have investigated the role of the timing of the various farm exposures. In both the ALEX and the PARSIFAL studies there is strong evidence pointing toward the importance of early life exposures either in the first year of life or even in pregnancy for the development of protection from atopic sensitization and asthma (6, 28). Exposures occurring after the first year of life had no or much weaker effects (6). Although these studies admittedly are cross-sectional in design and therefore the exposure has been assessed retrospectively, recall bias is unlikely to significantly confound the findings, since infantile exposures are closely linked to maternal exposures, reflecting a continuing pattern of maternal tasks on the farm.

MICROBIAL EXPOSURES

If livestock exposure is indeed associated with a decreased prevalence of asthma and atopy, then underlying exposures should be investigated. Children exposed to livestock may be exposed to more allergens, bacteria, viruses, and fungi than children without exposure to livestock. Yet only few out of the many microbial exposures have been measured in farming environments so far. Bacterial substances such as endotoxin from gram-negative species and muramic acid, a component of peptidoglycan from the cell wall of all types of bacteria, have been found to be more abundant in mattress dust from farm children than in that from nonfarm children (29). Likewise, extracellular polysaccharide (EPS) from *Penicillium* and *Aspergillus* spp. is more prevalent in farming households than in nonfarm households (29).

In the ALEX study, the multicenter study conducted in Austria, Switzerland, and Germany, endotoxin levels in samples of dust from the children’s mattresses were inversely related to the occurrence of hay fever, atopic asthma, and atopic sensitization (30). However, nonatopic wheeze was not significantly associated with the endotoxin level. In turn, independently of the endotoxin concentration, increasing mattress dust muramic acid concentrations were associated with a lower frequency of wheezing and possibly asthma among rural school children in the ALEX study (31).

The inverse association found in the ALEX study between endotoxin and atopic wheeze was confirmed in the larger multicenter PARSIFAL survey performed in Sweden, the Netherlands, Germany, Austria, and Switzerland (32). When restricting the PARSIFAL analyses to similar groups of children as in the ALEX study, farm and farm-reference children in Germany, Austria, and Switzerland, an adjusted odds ratio (aOR) of 0.56 (95% CI, 0.35–0.90) was observed. After adjustment for glucans and EPS, the effect of endotoxin was, however, no longer significant. Since exposures to endotoxin, EPS, and glucans were moderately, but significantly correlated, a firm

conclusion on the degree to which specific agents contributed to the observed effect is precluded. Moreover, these microbial agents might be markers of a much broader spectrum of microbial agents. Muramic acid was not measured in the PARSIFAL study. It is furthermore noteworthy that in this study all measured microbial compounds (endotoxin, glucans, and EPS) did not explain the protective "farming effect," suggesting that other yet unknown microbial exposures confer the protection seen in the farming environments. The results do, however, also suggest that mold components might modulate immune responses and thereby protect against allergic diseases, as previously suggested for endotoxin (33, 34). This is in line with a study on the effects of endotoxin and fungal spores on atopy and asthma in adult farmers, in which fungal spores, rather than endotoxin, were inversely related to atopic wheeze (35).

PROTECTIVE IMMUNE RESPONSES IN FARMING ENVIRONMENTS

Few studies have investigated immune responses in children raised on farms and have compared them to immune responses in children raised in rural but nonfarming environments. Since farming is associated with elevated exposure to microbes and/or microbial components, the Swiss arm of the ALEX study investigated whether growing up on a farm affects the expression of receptors for microbial compounds such as the monocytic antigen CD14 (involved in recognition of gram-positive and gram-negative products), TLR2 (a receptor for bacterial lipoproteins), and TLR4 (the receptor for lipopolysaccharide [LPS]). Peripheral blood leukocytes from children of the ALEX population living on a farm were found to display increased expression of the genes for CD14, TLR2, and TLR4 (36).

The impact of farming on the expression of innate immunity genes was then further examined by the Swiss research team in 125 children of farmers and 127 children of nonfarmers from the PARSIFAL study. RNA was extracted from EDTA-treated blood, and expression of *TLR2*, *TLR4*, and *CD14* was determined by quantitative PCR (TaqMan). The results confirmed the initial observation suggesting that environmental exposures, in particular to microbial components, affects the expression of genes encoding microbial ligand receptors (28).

The Swiss research team then assessed whether changes in gene expression correlated with prenatal or postnatal exposure to farm factors (Figure 1). After adjusting for age, sex, family history of atopy, parental education, environmental tobacco smoke, maternal smoking during pregnancy, number of older

siblings, contact with pets ever, study center, and current farm exposures, maternal exposure to stables during pregnancy (i.e., prenatal exposure of the child) significantly correlated with an increase in the expression of *TLR2* (1.44 [1.04–1.98], geometric means ratio and 95% CI), *TLR4* (1.40 [1.07–1.83]), and *CD14* (1.66 [1.18–2.33]). Interestingly, a dose–response relation was seen (Figure 2). Expression of *TLR2*, *TLR4*, and *CD14* increased with the number of different farm animal species the mother had contact with during her pregnancy (28). Overall, these findings suggest that environmental exposures very early in life may lead to epigenetic changes, which in turn may alter gene expression.

These findings also suggest a role for innate immunity in response to these environmental exposures. Pathogen-associated molecular patterns (PAMPs), evolutionarily highly conserved structural components of microbes, are recognized by similarly conserved receptors of host innate immune systems, the pattern recognition receptors (PRRs). Examples for PAMPs are the bacterial compounds cited above, LPS (endotoxin), and muramic acid, a component of peptidoglycan that is part of the cell wall of most bacteria. Examples for human PRRs are the human Toll-like receptors (TLRs) and CD14. To date, 10 functional TLRs have been described in humans. The cellular signaling cascade ensuing engagement of TLRs initiates innate host defense mechanisms (37), but it also provides signals required for initiating and modulating the adaptive immune response (38). In the context of the development of asthma and allergies, TLR4 as receptor for LPS, and TLR2 that recognizes peptidoglycan of gram-positive bacteria, have received the most attention. Polymorphisms in the genes for TLR4 and TLR2 have furthermore been shown to interact with the farm environment modulating the allergy protective effect (39).

Engagement of TLRs triggers a signaling cascade resulting in activation of host defense mechanisms, inflammatory responses, and signals for initiating adaptive immune responses. Exposure to microbial compounds may thereby skew the Th1/Th2 balance toward Th1 responses. However, in the ALEX study no skewing toward Th1 responses was observed among school-aged farm children (30). Production of both Th1 and Th2 cytokines (TNF- α , IFN- γ , IL-10, IL-12) by leucocytes stimulated with LPS was inversely related to the endotoxin level in the bedding, indicating a marked down-regulation of adaptive immune responses in exposed children. These findings point toward a phenomenon described as endotoxin tolerance, reflecting an old observation that repeated or prolonged exposure to endotoxin results in a refractory state (40). The potential role of regulatory T cells has so far not been explored in the context of farm exposures.

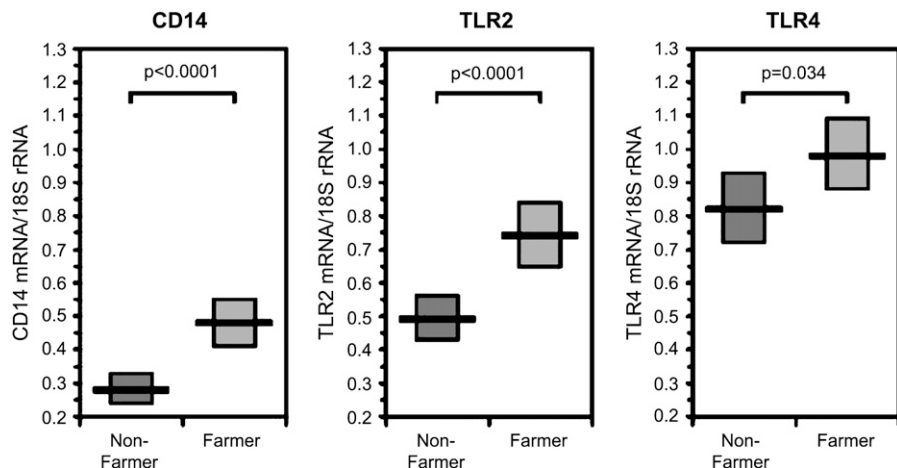


Figure 1. Innate immune receptor gene expression (GM $\pm$ CI) among farm and nonfarm children in the PARSIFAL study (data from Reference 28).

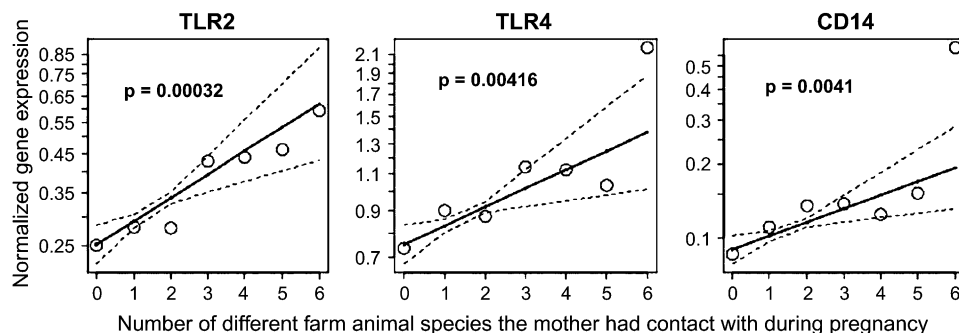


Figure 2. Adjusted geometric means of gene expression of *TLR2*, *TLR4*, and *CD14* for increasing numbers of different farm animal species the mother had contact with during pregnancy. Reprinted by permission from Reference 28.

With respect to sensitization to common aeroallergens, the underlying mechanisms may be even more complex. In the ALEX study allergen-dependent switching patterns were reconstructed *in vivo* to identify the level(s) at which farm exposure acts to protect against atopy by assessing serum IgG1 to IgG4 and IgE levels to grass, cat, and mite allergens (41). Farm exposure had complex allergen-specific effects on IgG1, IgG4, and IgE levels. Exposure protected against grass-specific responses at every step along the IgG1/IgG4/IgE switching pathway. Protection from cat responses was concentrated at the IgG1 level. For all allergens, failure to express IgG1 was associated with low prevalence of IgG4 or IgE responses. These findings suggest that the protective effects of farm exposure on atopic sensitization are allergen- and switch stage-specific. No counterbalancing, protective effect of IgG4 production as proposed in the context of urban cat exposure (42) has been seen in these farming environments.

Conflict of Interest Statement: E.v.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

- Schaub B, Lauener R, von Mutius E. The many faces of the hygiene hypothesis. *J Allergy Clin Immunol* 2006;117:969–977; quiz 978.
- Martinez FD, Holt PG. Role of microbial burden in aetiology of allergy and asthma. *Lancet* 1999;354:SI112–SI115.
- Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 2002;347:911–920.
- Akdis M, Blaser K, Akdis CA. T regulatory cells in allergy. *Chem Immunol Allergy* 2006;91:159–173.
- Braun-Fahrlander C, Gassner M, Grize L, Neu U, Sennhauser F, Varonier H, 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* 2002;57:1094–1096.
- Riedler J, Braun-Fahrlander C, Eder W, Waser M, Maisch S, Carr D, et al. Early life exposure to farming environment is essential for protection against the development of asthma and allergy: A cross-sectional survey. *Lancet* 2001;358:1129–1133.
- Alfven T, Braun-Fahrlander C, Brunekreef B, von Mutius E, Riedler J, Scheynius A, et al. Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle—the PARSIFAL study. *Allergy* 2006;61:414–421.
- Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Bohm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. *Clin Exp Allergy* 2000;30:187–193.
- 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;30:194–200.
- Leynaert B, Guilleud-Bataille M, Soussan D, Benessiano J, Guenegou A, Pin I, et al. Association between farm exposure and atopy, according to the CD14 C-159T polymorphism. *J Allergy Clin Immunol* 2006;118:658–665.
- Braback L, Hjert 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* 2004;34:38–43.
- 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* 2001;17:1151–1157.
- Omland O, Sigsgaard T, Hjort C, Pedersen OF, Miller MR. Lung status in young Danish rurals: the effect of farming exposure on asthma-like symptoms and lung function. *Eur Respir J* 1999;13:31–37.
- Portengen L, Sigsgaard T, Omland O, Hjort C, Heederik D, Doekes G. Low prevalence of atopy in young Danish farmers and farming students born and raised on a farm. *Clin Exp Allergy* 2002;32:247–253.
- Remes ST, Iivanainen K, Koskela H, Pekkanen J. Which factors explain the lower prevalence of atopy amongst farmers' children? *Clin Exp Allergy* 2003;33:427–434.
- Kilpelainen M, Terho EO, Helenius H, Koskenvuo M. Farm environment in childhood prevents the development of allergies. *Clin Exp Allergy* 2000;30:201–208.
- Kilpelainen M, Terho EO, Helenius H, Koskenvuo M. Childhood farm environment and asthma and sensitization in young adulthood. *Allergy* 2002;57:1130–1135.
- Perkin MR, Strachan DP. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? *J Allergy Clin Immunol* 2006;117:1374–1381.
- Naleway AL. Asthma and atopy in rural children: is farming protective? *Clin Med Res* 2004;2:5–12.
- von Mutius E, Illi S, Nicolai T, Martinez FD. Relation of indoor heating with asthma, allergic sensitisation, and bronchial responsiveness: survey of children in south Bavaria. *BMJ* 1996;312:1448–1450.
- Kilpelainen M, Koskenvuo M, Helenius H, Terho E. Wood stove heating, asthma and allergies. *Respir Med* 2001;95:911–916.
- Ernst P, Cormier Y. Relative scarcity of asthma and atopy among rural adolescents raised on a farm. *Am J Respir Crit Care Med* 2000;161:1563–1566.
- Radon K, Schulze A, Nowak D. Inverse association between farm animal contact and respiratory allergies in adulthood: protection, underreporting or selection? *Allergy* 2006;61:443–446.
- Radon K, Windstetter D, Eckart J, Dressel H, Leitritz L, Reichert J, et al. Farming exposure in childhood, exposure to markers of infections and the development of atopy in rural subjects. *Clin Exp Allergy* 2004;34:1178–1183.
- Downs SH, Marks GB, Mitakakis TZ, Leuppi JD, Car NG, Peat JK. Having lived on a farm and protection against allergic diseases in Australia. *Clin Exp Allergy* 2001;31:570–575.
- Wickens K, Lane JM, Fitzharris P, Siebers R, Riley G, Douwes J, et al. Farm residence and exposures and the risk of allergic diseases in New Zealand children. *Allergy* 2002;57:1171–1179.
- Peters M, Kauth M, Schwarze J, Korner-Rettberg C, Riedler J, Nowak D, et al. Inhalation of stable dust extract prevents allergen induced airway inflammation and hyperresponsiveness. *Thorax* 2006;61:134–139.
- Ege MJ, Bieli C, Frei R, van Strien RT, Riedler J, Ublagger E, 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* 2006;117:817–823.
- Schram D, Doekes G, Boeve M, Douwes J, Riedler J, Ublagger E, et al. Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children—the PARSIFAL Study. *Allergy* 2005;60:611–618.

30. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, *et al.* Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002;347:869–877.
31. van Strien RT, Engel R, Holst O, Bufe A, Eder W, Waser M, *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;113:860–867.
32. Schram-Bijkerk D, Doekes G, Douwes J, Boeve M, Riedler J, Ublagger E, *et al.* Bacterial and fungal agents in house dust and wheeze in children: the PARSIFAL study. *Clin Exp Allergy* 2005;35:1272–1278.
33. Gehring U, Bischof W, Fahlbusch B, Wichmann HE, Heinrich J. House dust endotoxin and allergic sensitization in children. *Am J Respir Crit Care Med* 2002;166:939–944.
34. Simpson A, John SL, Jury F, Niven R, Woodcock A, Ollier WE, *et al.* Endotoxin exposure, CD14, and allergic disease: an interaction between genes and the environment. *Am J Respir Crit Care Med* 2006;174:386–392.
35. Eduard W, Douwes J, Omenaas E, Heederik D. Do farming exposures cause or prevent asthma? Results from a study of adult Norwegian farmers. *Thorax* 2004;59:381–386.
36. Lauener RP, Birchler T, Adamski J, Braun-Fahrlander C, Bufe A, Herz U, *et al.* Expression of CD14 and Toll-like receptor 2 in farmers' and non-farmers' children. *Lancet* 2002;360:465–466.
37. Brightbill HD, Modlin RL. Toll-like receptors: molecular mechanisms of the mammalian immune response. *Immunology* 2000;101:1–10.
38. Takeda K, Akira S. Toll-like receptors in innate immunity. *Int Immunol* 2005;17:1–14.
39. Eder W, Klimecki WT, Yu L, von Mutius E, Riedler J, Braun-Fahrlander C, *et al.* Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J Allergy Clin Immunol* 2004;113:482–488.
40. Fan H, Cook JA. Molecular mechanisms of endotoxin tolerance. *J Endotoxin Res* 2004;10:71–84.
41. Stern DA, Riedler J, Nowak D, Braun-Fahrlander C, Swoboda I, Balic N, *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;119:351–358.
42. Erwin EA, Wickens K, Custis NJ, Siebers R, Woodfolk J, Barry D, *et al.* Cat and dust mite sensitivity and tolerance in relation to wheezing among children raised with high exposure to both allergens. *J Allergy Clin Immunol* 2005;115:74–79.