

Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children

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Background: There is increasing evidence that environmental exposures determining childhood illnesses operate early in life. Prenatal exposure to a farming environment through the mother might also play an important role.

Objective: We sought to investigate the role of maternal exposures to environments rich in microbial compounds for the development of atopic sensitization, asthma, and corresponding alterations in the innate immune system in offspring.

Methods: In the children of the cross-sectional Prevention of Allergy Risk Factors for Sensitization in Children Related to Farming and Anthroposophic Life Style study, asthma and atopy were assessed by means of standardized questionnaires (n = 8263) and serum IgE measurements (n = 2086). In a subsample (n = 322) gene expression of Toll-like receptors (TLR2 and TLR4) and CD14 was assessed. Maternal exposures were defined through questionnaire information.

Results: Both atopic sensitization (adjusted odds ratio, 0.58; 95% CI, 0.39-0.86) and the gene expression of receptors of innate immunity were strongly determined by maternal exposure to stables during pregnancy, whereas current exposures had much weaker or no effects. A dose-response relation was found between the extent of upregulation of these genes and the number of different farm animal species the mother had encountered in her pregnancy. Each additional farm animal species increased the expression of TLR2, TLR4, and CD14 by a factor of 1.16 (95% CI, 1.07-1.26), 1.12 (95% CI, 1.04-1.2), and 1.10 (95% CI, 1.03-1.23), respectively.

Conclusion: Maternal exposure to an environment rich in microbial compounds might protect against the development of atopic sensitization and lead to upregulation of receptors of the innate immune system. The underlying mechanisms potentially operating through the intrauterine milieu or epigenetic inheritance await further elucidation.

Clinical implications: When assessing risk factors of allergies in an infant's medical history, attention must also be paid to environmental exposures affecting the mother. (J Allergy Clin Immunol 2006;117:817-23.)

Key words: Asthma, allergy, atopic sensitization, gene expression, Toll-like receptors, CD14, farming, maternal exposure, microbial exposure

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Childhood asthma and allergies manifest in the first years of life.^{1,2} Environmental exposures implicated in the inception of these illnesses must therefore occur even earlier (ie, before the manifestation of the first symptoms). In recent years, a number of studies have shown that

Abbreviations used

EPS: Extracellular polysaccharide

OR: Odds ratio

PARSIFAL: Prevention of Allergy Risk Factors for
Sensitization in Children Related to Farming
and Anthroposophic Life Style

TLR: Toll-like receptor

exposures in the first 12 to 24 months of life might indeed determine the development of childhood asthma and allergies.^{1,3-5} The temporal sequence of events might, however, be traced back to prenatal exposures, namely exposures of the mother. Active smoking during pregnancy⁶; exposure of the pregnant mother to antibiotics,⁷ paracetamol,⁸ and allergens^{9,10}; and the effect of such exposure on the fetus have been investigated in some detail. Still, little is known about the effects of maternal exposure to microbial compounds in the environment on the development of atopy and asthma in offspring.

A deviated humoral immune response resulting in the production of IgE rather than IgG antibodies is the common feature of atopic diseases. The regulation of the humoral response by B cells depends on T effector cells. In turn, T-cell function is regulated by mediators of the innate immune system. Although it has been shown that adaptive immune responses can be shaped *in utero*,¹¹ little is known about potential prenatal determinants of the innate immune response and its relation to the development of atopy and asthma.

The aim of the present study was to investigate the role of exposures of the mother and the child, respectively, to environments rich in microbial burden for the development of atopy and asthma, as well as the gene expression of receptors of the innate immune response in the offspring.

METHODS

Population and study areas

The cross-sectional Prevention of Allergy Risk Factors for Sensitization in Children Related to Farming and Anthroposophic Life Style (PARSIFAL) study aimed at studying the determinants of childhood asthma and allergies in farming and anthroposophic populations, as described previously.¹² A child who lived on a farm and whose family ran the farm was considered a *farm child*. Other children were termed *farm reference children*. The present analyses focus on 2823 farm and 5440 farm reference children aged 5 to 13 years from rural areas of Austria, Germany, the Netherlands, Sweden, and Switzerland. In Sweden farmers with children were identified from the Farming Registry at the National Bureau of Statistics, and farm reference children were randomly selected from the population registry among children living in the same area. In Austria all farm children of a given school class were chosen by teachers who had a good knowledge of the region, and reference children were randomly selected from the same schools by the local study group. In the other countries children were randomly selected from schools in areas known to have a high percentage of farmers.

The study was approved by the ethical boards of the 5 study centers, and written informed consent was obtained from the

children's parents or guardians for questionnaires, blood sampling, and genetic analyses.

Questionnaires

The questionnaire comprised questions on sociodemographic background, parents' atopic diseases, family, and housing characteristics. In addition, information on the child's farm activities, as well as the mother's farm exposures during pregnancy, were available. Questions related to the child's contact with different farm animals (during the first year of life or later), consumption of farm milk (during the first year of life or current), and regular stable or barn visits and helping with haying at present. Activities occurring at least weekly were defined as occurring regularly. Maternal exposure included contact to different farm animal species during pregnancy and regularly working in stables (sheep shelters, hog, cow, and chicken houses) during pregnancy and lactation. The questionnaire asked for contact with 6 different farm animal species (cows, pigs, sheep, horses, goats, and poultry), and these were summed.

Questions on health outcomes and farm exposures were derived from the internationally validated International Study of Asthma and Allergies in Childhood II¹³ questionnaire and the Allergy and Endotoxin study,¹⁴ respectively. Children were considered to have current rhinoconjunctivitis symptoms if sneezing, runny nose, stuffy nose, and itchy eyes were reported in the last 12 months without the child having a cold at the same time. Current wheezing was defined as at least one episode of wheezing during the last 12 months. Children reported to have had symptoms of seasonal rhinoconjunctivitis in the last 12 months were defined as symptomatic, whereas children with a report of a physician's diagnosis of hay fever were considered to have a physician's diagnosis of seasonal rhinoconjunctivitis. Children with a report of a physician's diagnosis of asthma or of obstructive bronchitis more than once in their lifetime were considered to have a physician's diagnosis of asthma.

Measurement of allergen-specific serum IgE levels

In Austria, the Netherlands, and Sweden all children whose parents had consented to blood sampling were invited to a physical examination with blood sampling. In Germany and Switzerland only a random sample of those who consented were invited because of the comparatively large number of children included in these countries.¹² This subsample of children with blood analysis ($n = 2086$) did not significantly differ from the whole sample in regard to disease frequencies or family history of atopic disease (data not shown).

Allergen-specific IgE for common inhalant (Phadiatop) and food allergens (fx5; Pharmacia CAP System; Pharmacia Diagnostics AB, Uppsala, Sweden) was measured in serum. Two cut-off values for atopic sensitization were used: IgE values of 0.35 kU/L or greater and 3.5 kU/L or greater for either inhalant or food allergens.

Expression of Toll-like receptors

For the Swiss branch of the PARSIFAL study, RNA samples were collected from 195 farm and 127 reference children (95.3% of children who provided blood samples) to analyze gene expression of innate immunity receptors. Children with available RNA samples did not differ significantly from the total Swiss PARSIFAL population with respect to farm exposures and health outcomes (data not shown).

The total RNA was isolated with the QIAmp RNA Blood Mini Kit (Qiagen, Hilden, Germany) supplemented with RNase-free DNase (Qiagen). Quantitative real-time PCR (TaqMan; Applied Biosystems, Foster City, Calif) was performed, as described elsewhere.¹⁵ In brief, primers and probes were designed by using the primer design software Primer Express (Applied Biosystems).

Optimal concentrations for primers and probes were determined according to the manufacturer's instructions. The reactions for the target and the endogenous control were performed in separate tubes. All PCR reactions were analyzed on an ABI Prism 7700 Sequence Detection System (Applied Biosystems). Experiments assessing background signals were performed for every assay by running the reaction without templates ("no template controls"). No amplification was observed in any of these "no template controls," indicating that there was neither contamination nor unspecific fluorescence (see Fig E1 in the Online Repository at www.jacionline.org). The data are presented as normalized values (the amount of mRNA of the target molecule divided by the amount of mRNA of the endogenous control [18s rRNA]).

Measurement of endotoxin and extracellular polysaccharide in dust samples

Endotoxin and fungal extracellular polysaccharide (EPS) were measured in mattress dust samples of 83.9% of children with complete gene expression data ($n = 270$). Sampling and detection methods are described elsewhere.¹⁶ In brief, endotoxin was measured with the kinetic chromogenic Limulus Amebocyte Lysate test (Bio Whittaker, Walkersville, Md) and EPS with a specific sandwich enzyme immunoassay for EPS of *Aspergillus* and *Penicillium* species.¹⁷

Statistical analysis

Statistical analysis was performed with SAS 9.1.3 (The SAS Institute, Cary, NC) and Stata/SE 8.2 (StataCorp LP, College Station, Tex) software. Odds ratios (ORs) or geometric means ratios and 95% CIs were calculated in multiple logistic regression analysis.

In preliminary logistic analyses the associations of atopic sensitization, atopic symptoms, or diseases and exposure to farm characteristics were explored and adjusted for predefined covariates (age, sex, parental education, maternal and paternal atopy, number of older siblings, exposure to pets, and study center). We included farm characteristics that best predicted being a farm child, namely the child's current exposure to a farming lifestyle (regular stable or barn visits or helping with haying), the child's exposure to farm animals (ever), predominant consumption of farm milk (ever), and regular maternal work in stables during pregnancy. In the final regression model all variables were mutually adjusted. The independent effects of maternal work in stables during pregnancy and lactation were investigated to evaluate the effect of timing of the exposure.

Because the distribution of gene expression levels of the CD14, Toll-like receptor (TLR) 2, and TLR4 genes was skewed, these variables were log-transformed, resulting in a good approximation to the normal distribution (data not shown). For farm and farm reference children, these log-transformed values were compared by using the t test, and crude geometric means with their 95% CIs were computed. Multiple regression models adjusting for the same covariates as mentioned above were used to explore the associations between gene expressions and farm-related exposure measures. Results were expressed as adjusted geometric means ratios. In addition, adjusted geometric means of normalized gene expression were calculated for different levels of specific exposure variables (eg, the number of farm animal species the mother was exposed to during pregnancy).

In further analyses the effect of additional exposures during intervening years (5-13 years) that might influence atopic sensitization or gene expression were tested. In turn, the following variables were included in the regression models: worm infestations ever, respiratory infections during the first 2 years of life, day-care attendance, prescription of antibiotics or antipyretics before or after the first year of life, and history of measles, rubella, mumps, pertussis, or mononucleosis.

RESULTS

Of the 11,969 invited farm and farm reference children, 8402 (70%) returned the questionnaires. A total of 139 children were excluded because of missing values for sex and age or because they did not meet the age criteria of 5 to 13 years.

The prevalence of all outcomes (atopic sensitization, rhinoconjunctivitis symptoms and physician's diagnosis of seasonal rhinoconjunctivitis, and current wheezing and physician's diagnosis of asthma) was significantly lower in farm children compared with in nonfarm children (see Fig E2 in the Online Repository at www.jacionline.org),¹² despite some heterogeneity across the countries. The most pronounced differences between farm and nonfarm children were seen in Germany.

Factors mediating the farm effect

Determinants of the protective farm effect were assessed in multiple regression models (Table I). Predominant farm milk consumption was inversely related to the symptoms ($P = .079$) and diagnosis of seasonal rhinoconjunctivitis ($P = .022$), asthma ($P = .038$), and wheezing ($P = .065$). Additionally, regular contact with farm animals contributed to the protective effect on the physician's diagnosis of seasonal rhinoconjunctivitis ($P = .049$). Rhinoconjunctivitis symptoms in the last 12 months were also inversely related to frequent stable or barn visits or help with haying ($P = .007$). The strongest protective effect, however, was found for maternal stable work during pregnancy on atopic sensitization (OR, 0.58; 95% CI, 0.39-0.86; $P = .007$). Moreover, this association remained significant after additional adjustment for being a farm child or correction for multiple testing ($P_{Bonferroni} = .035$).

Maternal stable exposure during pregnancy versus lactation

Because 20% of all mothers working in the stable during pregnancy ($n = 2184$) stopped working in the stable after giving birth to the child, it was possible to disentangle the contributions of pregnancy and lactation, respectively, to the maternal effect. The effect of maternal stable work during lactation tended to be less pronounced (OR, 0.66; 95% CI, 0.45-0.96). It was no longer statistically significant when adjusted for maternal exposure in pregnancy (OR, 0.92; 95% CI, 0.52-1.61). When assessing the effect of timing of first exposure to farm animals on atopic sensitization, adjusted ORs for first contact in pregnancy, first contact in first year of life, first contact between first year and current, and current first contact were 0.36 (95% CI, 0.25-0.51), 0.54 (95% CI, 0.32-0.92), 0.77 (95% CI, 0.49-1.22), and 0.71 (95% CI, 0.43-1.20), respectively.

Variation of the effect on atopic sensitization across countries

We furthermore assessed the effect of maternal stable work during pregnancy on atopic sensitization for each

TABLE I. Mutually adjusted ORs for associations of farm-related exposures with health outcomes

	Atopic sensitization (≥ 3.5 kU/L) (n = 285/2086)	Rhinoconjunctivitis symptoms (n = 507/8174)	Physician's diagnosis of rhinoconjunctivitis (n = 343/8130)	Wheezing (n = 552/8169)	Diagnosis of asthma (n = 656/8080)
Current farm exposure*	0.96 (0.63-1.46), $P = .854$	0.63 (0.45-0.88), $P = .007$	0.66 (0.41-1.07), $P = .090$	0.88 (0.65-1.19), $P = .403$	0.82 (0.62-1.09), $P = .172$
Regular contact with farm animals ever	0.76 (0.51-1.15) $P = .194$	0.87 (0.67-1.14), $P = .321$	0.69 (0.47-1.00), $P = .049$	0.97 (0.75-1.26), $P = .822$	0.94 (0.75-1.19), $P = .629$
Farm milk consumption ever	0.76 (0.52-1.11), $P = .162$	0.77 (0.58-1.03), $P = .079$	0.63 (0.42-0.93), $P = .022$	0.77 (0.58-1.02), $P = .065$	0.76 (0.59-0.99), $P = .038$
Stable exposure in pregnancy†	0.58 (0.39-0.86), $P = .007$	0.74 (0.50-1.09), $P = .126$	0.77 (0.44-1.36), $P = .371$	0.76 (0.54-1.07), $P = .120$	0.86 (0.63-1.16), $P = .325$

ORs are given with 95% CIs in parentheses and P values. The models are adjusted for age, sex, family history of atopy, parental education, environmental tobacco smoking, maternal smoking during pregnancy, number of older siblings, contact with pets ever, study center, and the other exposure variables presented in the table.

*Current regular exposure to stable or barn or regular participation in haying.

†Mother worked regularly in stable during pregnancy.

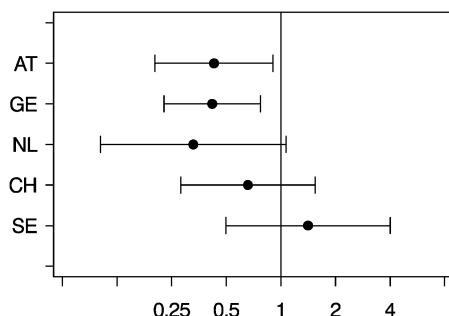


FIG 1. Adjusted ORs for maternal work in stables during pregnancy on atopic sensitization across countries. As a cut-off point for atopic sensitization, a value of 3.5 kU/L or greater was used. The ORs are given separately for the following countries: Austria (AT), Germany (GE), the Netherlands (NL), Switzerland (CH), and Sweden (SE).

country separately (Fig 1). The protective maternal effect was found in all countries but Sweden (OR, 1.41; 95% CI, 0.50-4.01). We investigated different cut-off definitions in the 4 countries showing a protective maternal effect. Using the detection limit as cut-off (≥ 0.35 kU/L) yielded a somewhat weaker but still significant effect (OR, 0.73; 95% CI, 0.54-0.995) compared with a cut-off level of 3.5 kU/L or greater (OR, 0.53; 95% CI, 0.35-0.80).

Association of farm-related exposure with gene expression

Because environmental factors have been shown to correlate with the expression of receptors of the innate immune system, we next investigated the expression of the genes for TLR2, TLR4, and CD14 in the Swiss subpopulation. Geometric means of the expression of these genes were significantly higher among farm children than among farm reference children (TLR2: 0.74 [95% CI, 0.65-0.84] vs 0.49 [95% CI, 0.43-0.56], $P < .0001$; TLR4: 0.98 [95% CI, 0.88-1.09] vs 0.82 [95% CI, 0.72-0.93], $P = .034$; CD14: 0.48 [95% CI, 0.41-0.55] vs 0.28 [95% CI, 0.24-0.31], $P < .0001$), thereby confirming

previous data from another farming population.¹⁸ It was, however, not the current exposure to several farm characteristics but rather maternal exposure during pregnancy that was associated with increased gene expression (Table II). Likewise, current levels of endotoxin and EPS in mattress dust were not correlated with gene expression of their respective receptors (data not shown and Fig E3 in the Online Repository at www.jacionline.org). Day-care attendance, reported childhood infections, worm infestation, and use of antibiotics or antipyretics during intervening years were not associated with gene expression of TLR/CD14 and atopy and did not affect reported point estimates. However, a dose-response relation was found when relating the number of different farm animal species the mother had contact with during pregnancy to gene expression (Fig 2).

Each additional species of farm animal the mother was exposed to during pregnancy increased the expression of TLR2, TLR4, and CD14 by a factor of 1.16 (95% CI, 1.07-1.26), 1.11 (95% CI, 1.03-1.19), and 1.14 (95% CI, 1.04-1.24), respectively. Neither restricting the analysis to farm children only nor omitting the highest category of contact to farm animal species during pregnancy materially changed the results. In a model including exposure to different animal species during pregnancy and during the first year of life simultaneously, stronger effects were observed for exposure during pregnancy (TLR2, 1.11 [95% CI, 1.01-1.21]; TLR4, 1.05 [95% CI, 0.97-1.14]; CD14, 1.09 [95% CI, 0.99-1.21]) than during the first year of life (TLR2, 1.03 [95% CI, 0.94-1.12]; TLR4: 1.02 [95% CI, 0.95-1.10]; CD14: 1.00 [95% CI, 0.91-1.10]), respectively. The correlation between these 2 variables was moderate ($r = 0.69$).

DISCUSSION

The present study showed a clear inverse association of a farming environment (farm milk consumption, stable/barn visits, and contact with farm animals) with

TABLE II. Mutually adjusted geometric means ratios for associations of farm-related exposures with the expression of the TLR2, TLR4, and CD14 genes (n = 322)

	TLR2	TLR4	CD14
Current farm exposure*	1.04 (0.69-1.55), <i>P</i> = .851	0.93 (0.66-1.3), <i>P</i> = .671	1.01 (0.66-1.54), <i>P</i> = .964
Regular contact with farm animals ever	1.09 (0.75-1.58), <i>P</i> = .650	0.92 (0.67-1.25), <i>P</i> = .577	0.97 (0.65-1.43), <i>P</i> = .866
Farm milk consumption ever	1.04 (0.77-1.42), <i>P</i> = .813	1.06 (0.81-1.4), <i>P</i> = .656	1.16 (0.83-1.64), <i>P</i> = .385
Stable exposure in pregnancy†	1.44 (1.04-1.98), <i>P</i> = .027	1.4 (1.07-1.83), <i>P</i> = .015	1.66 (1.18-2.33), <i>P</i> = .003

Geometric means ratios are given with 95% CIs in parentheses and *P* values. The models are adjusted for age, sex, family history of atopy, parental education, environmental tobacco smoking, maternal smoking during pregnancy, number of older siblings, contact with pets ever, and the other exposure variables presented in the table.

*Current regular exposure to stable or barn or regular participation in haying.

†Mother worked regularly in stable during pregnancy.

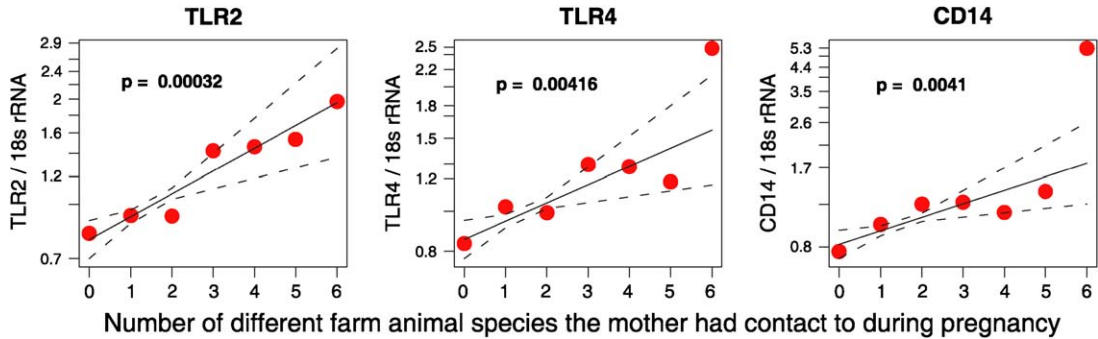


FIG 2. The x-axis shows increasing numbers of different farm animal species the mother had contact with during pregnancy (n = 322). The y-axis shows geometric means (dots) of gene expression of TLR2, TLR4, and CD14, respectively, which were normalized by dividing by gene expression of 18s rRNA. In addition, the linear fit of the association (solid line), along with the corresponding 95% CI (dashed line), is displayed. All analyses were adjusted for age, sex, family history of atopy, parental education, environmental tobacco smoking, maternal smoking during pregnancy, number of older siblings, contact with pets ever, child's current exposure to a farming lifestyle, child's exposure to farm animals, and predominant farm milk consumption of the child.

the prevalence of seasonal rhinoconjunctivitis, symptoms of seasonal rhinoconjunctivitis, and asthma. Maternal exposure to stables in pregnancy was associated with atopic sensitization and the expression of genes for receptors of the innate immune system (ie, TLR2, TLR4, and CD14). A dose-response relation was found between the extent of the upregulation of these genes and the number of different farm animal species the mother had contact with during her pregnancy. However, only longitudinal studies will eventually establish a causal relationship.

The identification of farm milk and stable animal contact as being protective for asthma and seasonal rhinoconjunctivitis is in accordance with the results of the Allergy and Endotoxin study.¹⁴ The much larger size of the PARSIFAL population allowed, however, a more detailed investigation of the relevant time windows of exposure for each outcome. A stronger effect of prenatal (ie, maternal) exposure was seen for atopic sensitization. Moreover, this effect was observed for gene expression of innate immunity receptors. Stable exposure during pregnancy was more relevant than exposure while the mother was breast-feeding, thereby shifting the focus to prenatal exposure. The cross-sectional design of the study might have resulted in recall bias. However, this is

unlikely because the outcomes (IgE levels and gene expression) were laboratory parameters not known to the mothers when answering the questionnaires. The maternal effect was independent of the mother's history of atopy and consistent over geographic areas, with the exception of Sweden. In Sweden none of the stables were connected to farmhouses in contrast to the other countries, potentially indicating a different pattern and intensity of exposure. Moreover, the levels of microbial compounds in house dust differ between Sweden and the other countries.¹⁶

Exposure to farm animals and stables has been found to be associated with increased exposure to various microbial products.^{19,20} TLR2 is a receptor for various microbial compounds from gram-positive bacteria and fungi, such as lipopeptides, lipoarabinomannan, lipoteichoic acid, and glucans, whereas TLR4 recognizes LPSs mostly from gram-negative bacteria. CD14 in turn is involved in the recognition of LPSs and other bacterial wall components.²¹ It seems therefore likely that the upregulation of these genes with an increasing number of farm animal species the mother was exposed to during pregnancy is attributable to increasing levels, diversified levels, or both of microbial exposure associated with contact with these animals.

By recognizing microbial compounds, TLRs are part of innate immunity, the first line of defense. The innate immune response communicates with the effector cells of the adaptive immune response, which includes the T helper cell populations.²² T_H1 cells favor the production of IgG antibodies, whereas T_H2 cells promote the production of IgE antibodies and thereby the development of atopic diseases. In experimental studies it has been shown that stimulation of the innate immune response might skew the balance toward antiallergic T_H1 responses.^{23,24} However, in children of farming environments with high endotoxin exposure, a decreased secretion of both T_H1 and T_H2 cytokines was observed on restimulation of leukocytes through the TLR4 ligand LPS.²⁵ This finding suggests that mechanisms other than a T_H1/T_H2 imbalance might be operational in these environments. T regulatory cells or mechanisms inherent to innate immune signaling, such as LPS tolerance, might play a role.^{18,26,27}

In the present study current exposures to either stables and barns or to measured levels of microbial products indoors were not related to expression of these innate immunity genes. Yet other microbial exposures than the ones assessed might have been of greater importance. However, endotoxin and EPS are specific ligands for TLR4 and TLR2, respectively,²¹ which makes it improbable that we have missed major environmental signals.

In contrast, maternal contact with increasing numbers of farm animal species, which is likely to parallel microbial exposures, was significantly related to a long-lasting upregulation of the expression of these innate immunity genes, which was still detectable at school age. Similarly, maternal work in stables during pregnancy was associated with decreased production of allergen-specific IgE antibodies, which was again detectable at school age. Thus prenatal environmental influences might affect the long-term development of innate and adaptive immune responses. In turn, the manifestation of organ-specific atopic conditions of the upper and lower respiratory tract might depend on the interplay between the growing and maturing child and his or her environment.

It is unknown how the maternal environment exerts its effect on the offspring. One might speculate that the fetal immune system interacts with a certain cytokine pattern prevailing in the maternal organism²⁸ or with the antigens themselves,²⁹ implying the establishment of immunologic tolerance. Another attracting explanation might consist in epigenetic inheritance. Differential patterns of gene expression arise during development *in utero* and are subsequently retained through mitosis. Stable alterations are heritable but do not involve mutations of the DNA itself.^{30,31} Recent data suggest that environmental factors might alter methylation processes, a key element of epigenetic inheritance.³⁰ Furthermore, there is some evidence suggesting that endotoxin has a role in gene silencing because LPS is a potent inflammogen that stimulates acetylation of histones.³²

The PARSIFAL study does not provide information on the maternal exposure before pregnancy, and therefore we cannot exclude a preconceptional effect. In other words,

the mother's earlier exposure to a farming environment might have determined a configuration of her immune system, which she then passed on to her child. If indeed preconceptional exposures were important, environmental determinants of disease inception would also operate in the parental generation. Recent observations demonstrating a plateau of the prevalences of asthma, seasonal rhinoconjunctivitis, and atopic sensitization after 3 decades,³³⁻³⁵ which approximates a generation, might indirectly support the notion of a generational effect. Furthermore, a transgenerational association of a grandmother's smoking with her grandchildren's risk for asthma has recently been reported.³⁶ Of note, in our rural population the effect of maternal work in stables during pregnancy on the child's atopy risk was independent from the mother's smoking habits. The lack of insight into the determinants of the increase in the prevalence of asthma and allergies over time might therefore in part be explained by the neglect of the potential effect of preconceptional exposures of the parents.

In conclusion, our results suggest that maternal exposures to environments rich in microbial exposures determine the priming of a child's immune response. For organ-specific manifestation of allergic diseases, additional factors, continuing exposure, or both effective later in life seem to be important. Our findings stress the importance of the identification of the appropriate time window leading to the modulation of immune responses and disease manifestation, respectively. Longitudinal studies will eventually deepen our understanding of the relevant temporal sequences.

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